Karch's

Pathology of Drug Abuse

Fifth Edition

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As always, for my wife Donna for her constant support. For Hardwin and Roger who, more than anyone else, allowed the process to go forward. For my parents, who died far too young to have seen any of the editions, and for kindred spirit Richard Ullman of Princeton, who also died much too young. My special thanks to Olaf Drummer, who helped me make sense of chemistry too arcane for a simple pathologist to understand! Thanks also to Ruth Swan, who managed to convert some of my meanderings into sensible English. And, most of all, to those of you who have come to rely on my book.

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Preface

It has been almost a quarter of a century since the first edition of this book was published, and that book was written almost without any real planning. I had no more room on my desk, or anywhere else, to put the piles of papers that were accumulating on the floor and everywhere else in my office. I only wanted to organize all this information so I could find it when I needed it. The process of organizing large amounts of information almost forces one to synthesize and understand the material before organizing it. Only when I had finished the organizing process did it occur to me that I had the makings for a book. It turned out that many more people than I had ever imagined felt a need for the same kind of book: a source describing both the big picture and the small, vitally important details. Now, it seems to me that the need is even greater than when I wrote the first edition.

When I wrote the preface to the fourth edition in 2008, I was not even sure there would be another edition. New discoveries in molecular biology and genetics were coming so fast that I could barely keep up with them. Learning about these discoveries and incorporating them into my work were harder than I could have imagined. New signaling cascades, new genes, and new interrelations are being announced almost daily. The faster the new genetic discoveries were announced and made available to pathologists, the faster that many age-old conundrums and puzzles could be explained. Why do low doses of methadone kill some and not others? This is because of hERG polymorphisms, and this was known long before the fourth edition was ever completed. How is it possible for someone to die suddenly and yet have an entirely negative autopsy? Geneticists know the answer, but genomics still remains a blind spot for too many forensic toxicologists and pathologists. We have known for years that the diagnoses of poisoning couldn't be made solely on the basis of isolated drug concentration measurements. Now, we know genetic variation is often the cause.

It is with much misgiving that I leave the scene just when things are getting more interesting, and I envy those who follow me, specifically Dr. Christopher Milroy of the University of Ottawa, who will work on the sixth edition, but don't expect to see it any time soon! Not so many years ago, while testifying at a murder trial, I was criticized for being an overly "enthusiastic proponent of testing for genetic (DNA) and chemical markers for heart disease…." It turns out I wasn't enthusiastic enough. As I wrote in the preface to the fourth edition, anyone who chooses to read the next edition "is going to need to know as much about DNA technology, molecular biology than they do now." That remains the case.

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Authors

Dr. Steven B. Karch received his undergraduate degree from Brown University in Providence, Rhode Island. He attended graduate school in anatomy and cell biology at Stanford University. He received his medical degree from Tulane University in New Orleans and did postgraduate training in neuropathology at the Royal London Hospital and in cardiac pathology at Stanford University. Dr. Karch is a fellow of the Faculty of Forensic and Legal Medicine of the Royal College of Physicians of London and also a fellow of the Forensic Science Society. He served as a cardiac pathologist at the office of the San Francisco Medical Examiner, publishing a number of books and papers when he was serving there.

Dr. Karch is the author of nearly 100 papers and book chapters, most having to do with the effects of drug abuse on the heart. He has published 12 books and is at work on several more, including a novel on the medical legal fallout of Hurricane Katrina. He was a forensic science editor for Humana Press and continues as an associate editor for the *Journal of Cardiovascular Toxicology*.

Dr. Karch was elected as a Wellcome fellow to the Royal Society of Medicine in 1972 and has remained active in the organization ever since. He is best known in the United Kingdom as one of the Crown pathologists who helped build the case against Dr. Harold Shipman and testified against him at trial. He also played a key part in the defense of Dr. David Moor who had been wrongfully charged with the death of one of his patients.

Olaf H. Drummer is deputy director (academic programs) and professor at the Victorian Institute of Forensic Medicine and is head of the Department of Forensic Medicine, Monash University. He has held an executive position at the institute since 1989.

He is a forensic pharmacologist and a toxicologist and has been involved in the analysis of drugs and poisons and in the interpretation of their biological effects for almost 40 years. He lectures widely on this subject and has given evidence in court in well over 250 cases around Australia and in New Zealand and has provided expert medicolegal reports in other parts of the world. He is gazetted as an approved expert under the Victorian Road Safety Act (1986).

He has published more than 200 peer-reviewed scientific papers in journals and has published many other reports, book chapters, and encyclopedia articles and is the main author of *The Forensic Pharmacology of Drugs of Abuse* (Arnold, 2001) and coeditor of the e-book *Forensic Analysis of Drugs*. He is the editor for drugs and toxicology submissions

for the scientific journal *Forensic Science International* and holds editorial board positions for a number of other scientific journals.

His formal qualifications include a bachelor of applied science in chemistry from Royal Melbourne Institute of Technology (1974) and doctor of philosophy in medicine and pharmacology from Melbourne University (1980). He is a member of a number of professional societies, including the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists, the Australian and New Zealand Forensic Science Society, the German Society for Toxicology and Forensic Chemistry, and the Royal Australian Chemical Institute (as chartered chemist) and an honorary member of the Italian Forensic Toxicology Society.

He was president of the International Association of Forensic Toxicologists from 2008 to 2011 and has served as the inaugural president of the Forensic and Clinical Toxicology Association of Australia and New Zealand since 2010.

He is an honorary fellow of the Royal College of Pathologists of Australasia and a founding fellow of the Faculty of Science of the Royal College of Pathologists of Australasia.

Cocaine 1

1.1 Prevalence of Abuse

According to United Nations (UN) estimates, two million cocaine users live in the United States alone, and the number of users elsewhere in the world is growing rapidly (Figure 1.1). The prevalence of cocaine abuse within the United States seems to have been stable for several years and may now be declining (Figure 1.2). A 2010 U.S. survey, released by the Office of National Drug Control Policy (ONDCP) (Substance Abuse and Mental Health Services Administration, 2010) found that a 9% decline in cocaine usage had occurred from the previous year. Recent evidence suggests that this decline may be just an artifact, due largely to government underestimation of the magnitude of worldwide cocaine production. In fact, the results of one recent study suggest that Colombian production alone may exceed 1000 tons/year (Leoncini and Rentocchini, 2012). That would explain why cocaine is still widely abused within the United States.

The use of cocaine is growing at alarming rates within the United Kingdom and the European Union (EU). Many countries do not even bother to track the true extent of the problem. In particular, little or no information is available from Africa, the Middle East, or much of Eastern Europe, and the situation is not much better in South America. Even in countries where systematic tracking is attempted, patterns of use and distribution evolve so rapidly that government and international surveys are usually out of date before they are published.

Most of what is known about the prevalence of drug abuse in the United States comes from annually conducted surveys such as the National Household Survey on Drug Abuse (NHSDA). The latest figures from NHSDA, from 2008 to 2009, indicated there were 1.9 million current cocaine users aged 12 or older, comprising 0.7% of the total U.S. population. These estimates were similar to the number and rates estimated for 2007 (2.1 million or 0.8%) but lower than the estimates for 2006 (2.4 million or 1.0%) (Substance Abuse and Mental Health Services Administration, 2009). Another way to gauge the size of the problem is the number of emergency room visits mentioned in the U.S. Drug Abuse Warning Network (DAWN) Report (Table 1.1). It showed there were nearly one million visits for illicit drugs, nearly double the number for alcohol. Another perspective is to be had by considering the types of drugs responsible for emergency room visits more than a decade ago (Table 1.2).

The second largest proportion of cocaine production goes to Europe. The 27 countries of the EU and the 4 countries of the European Free Trade Association are home to 90% of Europe's 4.5 million cocaine users. The single largest cocaine market within Europe is the United Kingdom, followed by Spain, Italy, Germany, and France in descending order. The European Monitoring Centre for Drugs and Drug Addiction (EMCDDA)

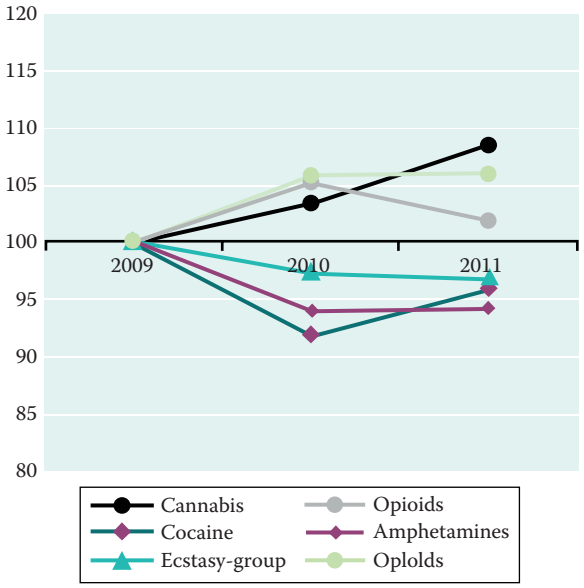


Figure 1.1 UN estimate of worldwide trends in the prevalence of abused drugs in 2009–2011. (Reproduced from the United Nations Office on Drugs and Crime, World Drug Report 2012, United Nations, New York, 2012.)

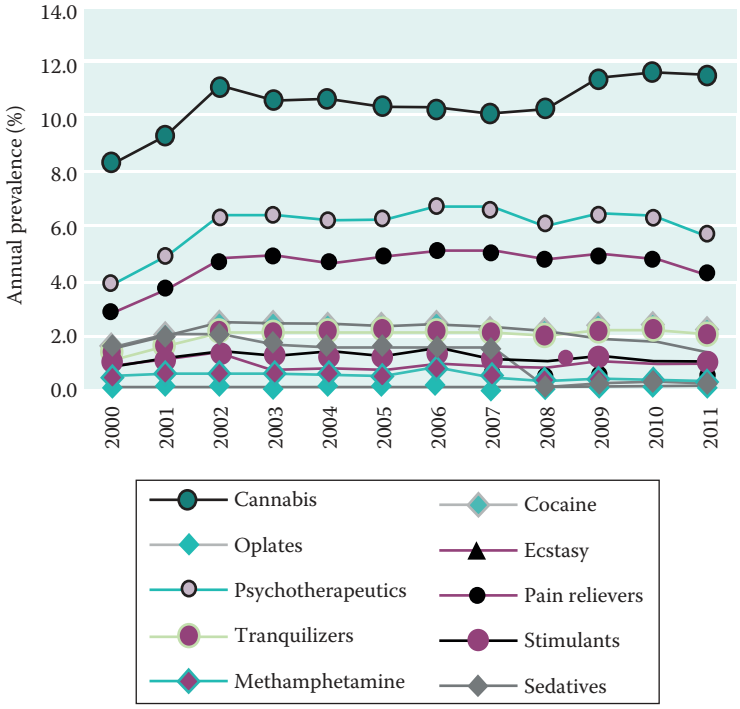


Figure 1.2 Trends in annual prevalence of drug use among the population 12 years and older in the United States, 2000–2011. (From United States Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-44, HHS Publication No. (SMA) 12-473, Office of Applied Studies, Rockville, MD, 2012.)

Table 1.1 U.S. Statistics Showing Estimated Numbers of Emergency Room Visits by Drug Type, 2009

Drugs	Number of ED Visits	Number of ED Visits per 100,000 Population
Alcohol in combination with other drugs[a]	519,650	169.3
Underage drinking[b]	199,429	227.2
Illicit drugs	973,591	317.1
Cocaine	422,896	137.7
Marijuana	376,467	122.6
Heroin	213,118	69.4
Pharmaceuticals	1,244,679	405.4
Pain relievers	595,551	194.0
Narcotic pain relievers	397,160	129.4
Oxycodone products	175,949	57.3
Hydrocodone products	104,490	34.0
Drugs to treat insomnia and anxiety	433,600	141.2
Benzodiazepines	373,328	121.6
Antidepressants	104,940	34.2

Source: Reproduced from the 2010 DAWN Report. Substance Abuse and Mental Health Services Administration, Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of National Findings, NSDUH Series H-38A, Office of Applied Studies, Rockville, MD, 2010.

[a] Use of alcohol in combination with other drugs is recorded by DAWN for patients of all ages.

[b] Underage drinking includes both use of alcohol in combination with other drugs and use of alcohol only for persons aged 20 or younger.

estimates that 13 million adults (3.9% of the population) have tried cocaine on at least one occasion, that roughly one-third of these individuals are lifetime users, and that 1.5 million Europeans had used cocaine within the previous month. In contrast to the United States, where rates of use are relatively homogenous, there is great variation from one European country to another. EMCDDA estimates that rates of yearly use vary from 0% to 3.1% of the general population (European Monitoring Centre for Drugs and Drug Addiction, 2009a).

Cocaine use in England and Wales increased significantly during the 1990s, from 0.3% in 1992 to 2.0% of those aged 15–59 years in 2000 (European Monitoring Centre for Drugs and Drug Addiction, 2009b). According to UN figures, the rate of cocaine use in Scotland is almost double that in the United States. In the United Kingdom as a whole, including Scotland and Northern Ireland, one million individuals use cocaine every year, giving the United Kingdom the largest numbers of cocaine users in Europe (UNODC, 2010).

The United Nations Office on Drugs and Crimes (UNODC) estimates are not that different than those of the EMCDDA. UNODC estimates that in 2008, the worldwide prevalence of cocaine use was 0.3%–0.4% of the adult population. That would equate to 15–19 million people having used cocaine at least once in the previous year. Compared to 2007, there was a trend toward a decrease in the global number of cocaine users, with most of that change attributed to decreased American consumption. The little qualitative information that is

Table 1.2 Some of the Comparisons Are Startling, Not Just for the Number of Reported Deaths, but for the Types of Drugs Involved

Rank	Drug Name	Number of Mentions	Percent of Total Episodes	Rank	Drug Name	Number of Mentions	Percent of Total Episodes
1	Cocaine	3465	46.00	39	Dextromethorphan	55	0.73
2	Alcohol-in-combination	2944	39.09	40	Doxylamine succinate	46	0.61
3	Heroin/Morphine[a]	2912	38.66	41	Meperidine HCl	42	0.56
4	Codeine	880	11.68	42	Trazodone	42	0.56
5	Diazepam	640	8.50	43	Oxycodone	41	0.54
6	Methadone	431	5.72	44	Chlorpromazine	38	0.50
7	Amitriptyline	414	5.50	45	Mesoridazine	38	0.50
8	D-Propoxyphene	398	5.28	46	Pentobarbital	37	0.49
9	Marijuana/Hashish	359	4.77	47	Ephedrine	30	0.40
10	Nortriptyline	335	4.45	48	Promethazine	29	0.39
11	Diphenhydramine	318	4.22	49	Pseudoephedrine	27	0.36
12	Acetaminophen	298	3.96	50	Oxazepam	26	0.35
13	Methamphetamine/ Speed	234	3.11	51	Brampheniramine maleate	26	0.35
14	Quinine	203	2.70	52	Hydromorphone	25	0.33
15	Doxepin	195	2.59	53	Theophylline	25	0.33
16	Phenobarbital	182	2.42	54	Phenylpropanolamine	24	0.32
17	Amphetamine	155	2.06	55	Quinidine sulfate	24	0.32
18	Lidocaine	155	2.06	56	Ibuprofen	24	0.32
19	Desipramine	151	2.00	57	Lithium carbonate	23	0.31
20	PCP/PCP Combinations	146	1.94	58	Caffeine	22	0.29
21	Unspec. Benzodiazepine	130	1.73	59	Glutethimide	21	0.28
22	Fluoxetine	122	1.62	60	Clonazepam	21	0.28
23	Hydantoin	115	1.53	61	Propanolol HCl	20	0.27
24	Aspirin	113	1.50	62	Amoxapine	19	0.25
25	Alprazolam	109	1.45	63	Hydroxyzine	18	0.24
26	Chlordiazepoxide	99	1.31	64	Benztropine	18	0.24
27	Fentanyl	96	1.27	65	Haloperidol	17	0.23
28	Hydrocodone	92	1.22	66	Lorazepam	17	0.23
29	Imipramine	87	1.16	67	Ethchlorvynol	16	0.21
30	Butalbital	85	1.13	68	Trifluoperazine	14	0.19
31	Chlorpheniramine	70	0.93	69	Triazolam	14	0.19
32	Thioridazine	69	0.92	70	Household/ Commercial subs	13	0.17
33	Meprobamate	68	0.90	71	Amobarbital	13	0.17
34	Temazepam	67	0.89	72	Loxapine	12	0.16
35	Secobarbital	62	0.82	73	Metoprolol	12	0.16
36	Carisoprodol	61	0.81	74	Phentermine	11	0.15
37	Carbamazepine	60	0.80	75	Cyclobenzaprine	11	0.15
38	Flurazepam	56	0.74	76	Metoclopramide	11	0.15

Source: Reproduced from the 1992 DAWN Report; Substance Abuse and Mental Health Services Administration.
Note: Percentages are based on a total raw medical examiner drug abuse case count of 7532.
[a] Includes opiates not specified as to type.

available suggests that the prevalence of cocaine use is low in most parts of Asia. Chinese experts are only now reporting an increase of cocaine use within their country (UNODC, 2013); however, there is very good evidence that consumption in Southeast Asian countries is also rising.

Most of the figures cited earlier derive from survey data that rely on self-reporting, a method that uniformly underestimates the extent of drug use. Within the last few years, a viable, and almost surely more accurate, way to monitor drug use has emerged: measurement of sewage effluent drug content.

In 2005, scientists of the Mario Negri Institute for Pharmacological Research in Milan tested waters from the River Po that flows across northern Italy to the Adriatic Sea. They estimated that the Po carried the equivalent of about 4 kg of cocaine every day, translating to 40,000 doses a day. That result was more than twice the amount that the government had predicted (Hawkes, 2005). Very similar findings have been reported from elsewhere in Italy and London (Orr and Goswami, 2005). French scientists analyzed water from the River Seine in Paris and found cocaine and benzoylecgonine (BZE) at levels ranging from 5 to 282 ng/L and 15 to 849 ng/L, respectively. These measurements also confirmed what clinicians have known, or at least suspected for many years: more cocaine is used on weekends than during the week and thus effluent drug concentrations rise on weekends (Berset et al., 2010; Karolak et al., 2010; van Nuijs et al., 2011). So many papers have been published about this methodology that the process now has a formal name: "sewage epidemiology." While this new technique provides a good indicator of total cocaine consumption, the numerator is never really known; only the amount of cocaine used, not the number of actual users, can be estimated with any certainty.

1.2 Epidemiology

Arguably, the single best source of epidemiologic data in the United States is the DAWN report. This report has two components: one tracks drug-related deaths and the other drug-related emergency room visits (Figure 1.3). Of the two, the former provides the most useful information about national patterns of drug abuse.

According to the most recent DAWN Emergency Department (ED) component available, which was for 2009 and only published in December of 2011, there were nearly 4.6 million emergency room visits that were, in some way, related to drug abuse, misuse, or adverse reactions to drugs (Substance Abuse and Mental Health Services Administration, 2011).

It must be observed that these figures represent only a very small proportion of the population of the United States (Substance Abuse and Mental Health Services Administration, 2012). They are significant as indicators only and do not provide absolute values.

Emergency room visits were divided almost equally between those occasioned by misuse of any drug and adverse drug reactions to legitimate medications (2.3 vs. 2.1 million). Of the 2.1 million emergency room visits involving drug misuse or abuse, 1.2 million visits were the result of prescription drug misuse, while almost 1.0 million visits were related to the use of illicit drugs.

The subgroup of individuals aged 21 and over accounted for 80.9% of emergency room visits (3,717,030). Of these, slightly less than one-half (1,654,784 visits) involved drug

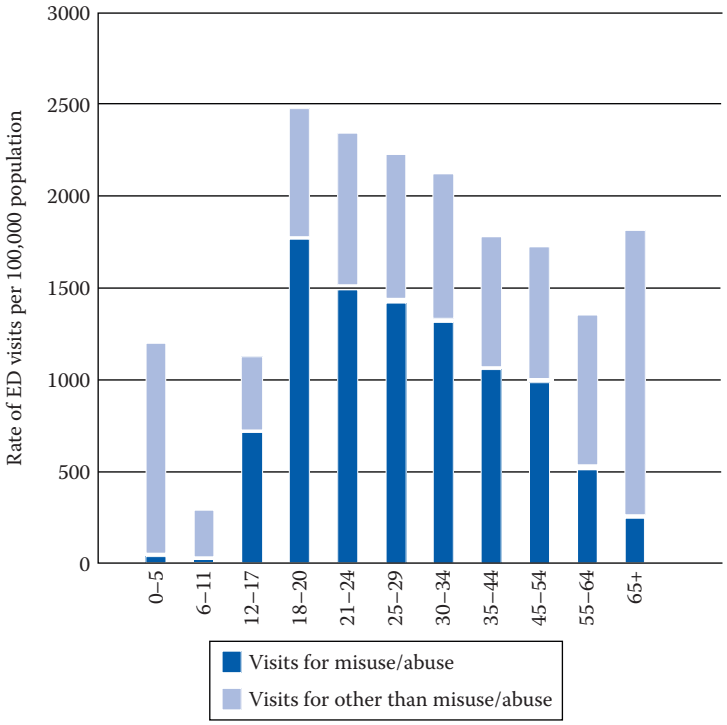


Figure 1.3 Emergency room visits involving the use of illegal drugs in 2011. In 2011, over 125 million visits were made to EDs in general-purpose, nonfederal hospitals operating 24 h EDs in the United States. DAWN estimates that over 5 million of these visits, or about 1626 ED visits per 100,000 population, were related to drugs, a 100% increase since 2004. In 2011, drug-related visits ranged from a low of 288 visits per 100,000 population aged 6–11 to a high of 2477 visits per 100,000 population aged 18–20. The graph shows rates of drug-related ED visits per 100,000 population, by age group, 2011. (From United States Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, Results from the 2011 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-44, HHS Publication No. (SMA) 12-473, Office of Applied Studies, Rockville, MD, 2012.)

misuse or abuse, with a rate of 754.8 visits per 100,000 persons aged 21 or older. Cocaine-related visits are uncommon in younger age groups (less than 30 visits per 100,000 population). Drug use trends change radically over the age of 21. Cocaine then becomes the most common cause of emergency room visits (398,229, amounting to 181.6 visits per 100,000 population, not very different than it had been in 2004). Cocaine was followed fairly closely by marijuana (121.5 visits per 100,000 population) and heroin (89.4 visits per 100,000). Visits related to methamphetamine were still much less common than those caused by cocaine, amounting to only 37.2 visits per 100,000 persons in 2009. Whether that trend continues is not known. Overall, the total number of drug-related emergency room visits for illegal drugs, for all age groups within the United States, increased 81% from 2004 (2.5 million visits) to 2009 (4.6 million visits) (Substance Abuse and Mental Health Services Administration, 2010).

Almost nothing has been published about cocaine demographics since the last edition of this textbook. The most recent Drug and Alcohol Service Information System (DASIS) report was published in 2005 and provided information on the years 2003 and

2004. The National Survey on Drugs and Health (NSDUH) report is more current and contains data through 2009. In that year, 617,000 persons aged 12 or older had used cocaine for the first time within the past 12 months; this averages to approximately 1700 initiates per day. This estimate was similar to the number in 2008 (722,000). The annual number of cocaine initiates, at least in the United States, has declined substantially over the last decade, from 1.0 million in 2002 to 617,000 in 2009. The number of initiates of crack cocaine also declined during this period from 337,000 to 94,000. Most (71.5%) of the 0.6 million recent cocaine initiates were 18 or older. The average age at time of first use among recent initiates who were aged 12–49 was 20 years, similar to the average age in 2008 (19.8 years). At the same time, federal researchers estimate that 1.1 million U.S. citizens are addicted. The data suggest that the probability of becoming cocaine addicted after a single exposure is exceedingly small (Substance Abuse and Mental Health Services Administration, 2011).

As was true for the previous decade, the rate of substance dependence or abuse in 2009, for males aged 12 or older, was about twice as high as the rate for females (11.9% vs. 6.1%). However, in young people aged 12–17, the rates of substance dependence or abuse are similar in both sexes (6.7% vs. 7.4%) (Substance Abuse and Mental Health Services Administration, 2011).

Cocaine abusers tend to cluster within communities and neighborhoods, and clustering suggests a contagious spread of drug use. The probability that a person will develop a particular disease, and the outcome, depends upon the degree to which others in the community already have the disease; the more individuals who are addicted in a particular neighborhood, the more likely it is that nonusers will become users. The late Professor David Musto (January 8, 1936 to October 8, 2010) was the first to use this model to account for the pattern of recurrent cocaine pandemics, and his assumptions appear to have been proven correct, at least in the sense that current drug users must be present in sufficient numbers to encourage use among nonusers (Behrens and Caulkins, 1999). Epidemiologic evidence suggests that the habit of cocaine addiction (i.e., beliefs and behaviors suggesting that cocaine is harmless) is transmitted from neighbor to neighbor and neighborhood to neighborhood. The evidence also shows that these ideas do not penetrate as readily into wealthy neighborhoods (Petronis and Anthony, 2003).

The 2009 NSDUH report indicates that in the last decade, the percentage of youths aged 12–17 who perceived cocaine use as a great threat actually declined. The number of individuals who thought that using cocaine once or twice a week could be dangerous declined from 79.8% to 78.5% (Substance Abuse and Mental Health Services Administration, 2010). The decrease in perceived risk suggests a reemergence of interest in cocaine.

In the complete absence of current data, any analysis of drug-related mortality is nearly impossible. Partially for that reason, the EMCDDA recognizes two different kinds of cocaine deaths (all other drug-related deaths are divided into two groups as well): deaths directly caused by illegal drugs (drug-induced deaths) and deaths among problem drug users. Problems in the latter group may be lifestyle related or the results of the medical complications that inevitably occur after protracted drug exposure. While it may be possible to state some general principles about cocaine and cocaine users, the reality is that the situation varies country by country and is in a state of flux. Still, it appears that both exposure and mortality rates in the United Kingdom and EU now approach, or even exceed, those in the United States (EMCDDA, 2010).

New estimates of the number of problem drug users in England during 2008 and 2009 were published in October 2010. The total number of individuals addicted to cocaine was estimated at just over 190,000. Data from British law enforcement agencies show that prices for crack cocaine and ecstasy (3,4-methylenedioxymethamphetamine [MDMA]) have fallen and that the purity of cocaine powder was only 20% in 2010, a decrease from previous years. However, after adjusting for purity, the price of cocaine powder has almost doubled since 2003 (Davis et al., 2010).

In Spain, where the prevalence of cocaine use is second only to the United Kingdom, and much higher than in the United States, the data suggest that cocaine-related death rates are different within different areas of that country. In 2006, Spain reported only 518 deaths related to all types of drug use, which many, even the government, consider a gross underestimate. Various Scandinavian reports show stabilizing numbers through 2007, but those numbers are nearly 5 years out of date and certainly need revision (UNODC, 2013).

In Australia, the Bureau of Statistics tracks deaths and arrests related to methamphetamine and cocaine and has been doing so since 1997 and then coding them according to the World Health Organization's (WHO) International Statistical Classification of Diseases and Related Problems. In 2001, the rate was slightly above 5 per 100,000 (for all stimulants). The most recently published data, from 2007 to 2008, show only 669 arrests for cocaine and 16,047 for amphetamines as a group (UNODC, 2010). Findings from the 2012 National Drug Strategy Household Survey suggest that 1.9% of the Australian population had used cocaine, though the number dying from cocaine use is not known (Substance Abuse and Mental Health Services Administration, 2012). These numbers bear a striking resemblance to the numbers recorded at the height of the cocaine pandemic in the United States and the pandemic that is only now fully established in Europe and parts of Asia.

The situation in Australia and New Zealand offers a striking example of drug market fluidity. In 2007, fewer than 1000 arrests for cocaine occurred in Australia. In 2005, a paper on drug-related deaths published in the *New Zealand Medical Journal* mentioned neither cocaine nor methamphetamine (Beasley and Reith, 2005). Yet cocaine seizures in that part of the world are now increasingly common, and the Chief of the New South Wales Bureau of Crime Statistics estimates that use within Australia has increased by 50% in the last 2 years. Other parts of the world, such as Russia and the former Soviet Republics, are said to be experiencing significant problems with multiple drugs, including cocaine, but just as in Africa and South America, there is no coherent reporting system and information is, for the most part, neither collected nor reported.

1.3 History

The word "coca" comes from the Aymara Indian *khoka*, meaning "the tree." Coca has nothing to do with the chocolate-producing nut called cocoa, and its only relation to the kola nut is phonetic. Europeans had not heard of coca until Spain colonized South America. When Spain conquered Peru in the 1500s, Spanish soldiers and explorers encountered Indians who had chewed coca leaf for thousands of years.

The experience of the Indians with coca was recounted in Nicolas B. Monardes' monograph, *Joyfulle News out of the New Founde Worlde, wherein is declared the Virtues of Herbs, Treez, Oyales, Plantes, and Stones*. Monardes book was reprinted many times after first being published in Barcelona sometime in the 1560s. He was fascinated by stories brought back by the explorers who claimed that coca allowed users to go without food but continue to work for hours. Monardes was also aware that coca had undesirable side effects. He wrote, "Surely it is a thyng of greate consideration, to see how the Indians are so desirous to bee deprived of their wittes, and be without understanding" (Frampton, 1577). The first illustration of coca to appear in an English publication (*Companion to the Botanical Magazine*) was published in 1836. It was drawn by Sir William Hooker, then director of the Royal Botanical Gardens at Kew (Figure 1.4).

In spite of the magical properties ascribed to coca leaf, the medical community remained unimpressed. The great Dutch physician, botanist, and chemist Hermann Boerhaave (1668–1738) favorably mentioned coca in his textbook on medicinal plants, *Institutiones Medicae* (Leiden, 1708) (Mortimer, 1901), but in spite of Boerhaave's initial enthusiasm, more than 100 years elapsed before the first illustration of coca appeared

Figure 1.4 The first illustration of cocaine. The first illustration of coca to appear in an English publication (*Companion to the Botanical Magazine*) was published in 1836. It was drawn by Sir William Hooker, then director of the Royal Botanical Gardens at Kew. (Illustration courtesy of the library at the Royal Botanical Gardens, Kew, U.K.)

in an English language magazine. In addition to illustrations of the coca plant, the article also contained Hooker's translation of a book by a German explorer and naturalist named Eduard Poeppig. Poeppig thought that coca chewers were very much like opium addicts and warned against the immoderate use of coca (Poeppig, 1835/36). Other travelers and explorers had more positive impressions, but the potential toxicity of coca was known even before it became widely available in Europe.

Johann von Tschudi (1818–1889) was one of the early explorers of the Amazon. He was a prolific writer and his travel books were popular in Europe and the United States. He, too, was impressed with the apparent ability of coca to increase endurance, but he was concerned that Europeans might develop a "habit." His book *Travels in Peru*, published in 1846 and translated into English in 1847, contains the first accurate description of cocaine "binging" (Von Tschudi, 1847). The term describes the tendency of cocaine users to consume, in one session, all the drug in their possession. According to von Tschudi, "They give themselves up for days together to the passionate enjoyment of the leaves. Then their excited imaginations conjure up the most wonderful visions…I have never yet been able to ascertain correctly the conditions the Coquero passes through on returning to his ordinary state; it however appears, that it is not so much a want of sleep, or the absence of food, as the want of Coca that puts an end to the lengthened debauch."

In 1857, Enrique Pizzi (dates unknown), an Italian professor of chemistry and pharmacology, who was teaching at the University of La Paz, isolated the active principle of coca and gave it to von Tschudi. Von Tschudi took a sample to Germany. In Göttingen, von Tschudi gave the sample to his friend, Friedrich Wöhler (1800–1882), the chemist who had first synthesized urea. Wöhler gave the sample to Albert Niemann (1834–1861), his graduate student. Niemann found that the sample contained only gypsum. Wöhler remained curious and, when he heard that Emperor Franz Joseph I (1830–1916) was sending a frigate on an around-the-world training cruise, approached Carl von Scherzer (1821–1903), chief scientist of the expedition, and asked him if he could bring back enough coca leaves to analyze. Von Scherzer returned 3 years later with 60 pounds of leaves and gave them to Wöhler, who again gave them to Niemann.

Given an adequate supply of coca leaf, purification of cocaine proved relatively simple. Niemann published his PhD thesis, "On a New Organic Base in the Coca Leaves," in 1860 (Niemann, 1860). Even after the purification of cocaine, interest in its therapeutic applications remained slight, and reports in journals remained anecdotal. Other than the fact that he was the first to isolate cocaine, very little is known about Niemann; he died shortly after his thesis was published. A *Lancet* editorial published in 1872, 12 years after cocaine had been purified, stated that "there is considerable difference of opinion as to its effects upon the human subject, and the published accounts are somewhat conflicting; but we think that there is strong evidence in favor of its being a stimulant and narcotic of a peculiar kind, and of some power."

Coca-containing wines became popular in France and Italy during the late 1860s (Figure 1.5). Angelo Mariani (1838–1914) manufactured the most famous of these (Figure 1.6). It contained 6 mg of cocaine per ounce and was advertised as a restorative and tonic, which does indeed seem to be how it was used. Satisfied customers endorsing Vin Mariani included Thomas Edison, Robert Louis Stevenson, Jules Verne, Alexander Dumas, and even Pope Leo III. Within 10 years of their introduction in Paris, Mariani's

Figure 1.5 Coca-containing wines. These wines became popular during the 1860s. Among the many competitors, the most famous was Vin Mariani. The average product contained 5–10 mg of cocaine per ounce. (Karch, S.B., MD—private collection.)

wines were in much demand throughout the United States. Mariani's success spawned a host of imitators, including "Maltine" (Figure 1.7), a coca-based wine much like that produced by Mariani. The amount of cocaine contained in these products was modest. It is now known that when alcohol and cocaine are combined, a new metabolite called cocaethylene is formed (Hearn et al., 1991a). It has the same affinity for the dopamine receptor as cocaine itself, which means that it should be just as psychoactive. Because cocaethylene has a half-life that is many times longer than that of cocaine, combinations of alcohol and cocaine may be quite intoxicating. This may explain why virtually all reported pleasant experiences after drinking the wine.

In the early 1880s, Parke-Davis and Company began to sell a fluid extract containing 0.5 mg/mL of semipurified cocaine. At about the same time, physicians began prescribing elixirs containing cocaine for treatment of a variety of ailments, including alcohol and morphine addiction. In spite of the inappropriate use of these mixtures, reports of toxicity and cocaine-related disease were rare. Concurrent with the increased dispensing by physicians, patent medicine manufacturers began adding cocaine extract to nearly all of their products (Figure 1.8). One such promoter was John Stith Pemberton (1831–1888). He went into competition with Mariani and began selling "French Wine Cola." His initial marketing efforts were not very successful. In what proved to be a wise marketing decision, Pemberton dropped the wine from his product and added a combination of cocaine and caffeine. The product, reformulated in 1886, was named "Coca-Cola" (Pentegrast, 2000).

Figure 1.6 Newspaper advertisement for Vin Mariani. Angelo Mariani was a master at self-promotion, and it is difficult to decide for which of his two greatest inventions he should be remembered: the popularization of coca wine or the invention of the modern publicity campaign. Vin Mariani was immensely popular. Mariani sent cases of free wine to celebrities, who would then write thank-you notes or even endorsements that Mariani collected and published. Thomas Edison and Sarah Bernhardt wrote endorsements, as did Pope Leo III. President William McKinley's secretary, John Addison Porter, wrote to Mariani to thank him and assured Mariani that the wine would be used whenever the occasion required. This advertisement, featuring a picture of the Pope, appeared in a London newspaper in 1899.

Figure 1.7 Advertisement for Maltine, a coca-based wine that competed with Vin Mariani. (Courtesy of Bozarth, MA, PhD, University of Buffalo, Department of Psychology, Buffalo, NY.)

Figure 1.8 Advertisement for Burnett's Dandruff Shampoo containing cocaine. The manufacturers never explained why cocaine should be good for the scalp. (Karch, S.B., MD—private collection.)

Two events occurred in 1884 that significantly changed the pattern of cocaine use in the United States and Europe. The first was the publication of Freud's paper, *Über Coca* (Freud, 1884). The second was the discovery made by Freud's roommate at the Vienna General Hospital, Carl Köller (1857–1944). Köller discovered that cocaine was a local anesthetic (Figure 1.9) (Noyes, 1884).

By the time Freud sat down to write his now famous/infamous paper, he already lagged behind American physicians in recommending cocaine as a treatment for heroin addiction! By that time, American physicians had already published more than a dozen papers recommending cocaine in the treatment of morphine addiction (Bentley, 1880). Freud enthusiastically accepted this American notion. In fact, he obtained the references he used for his paper from the *Surgeon General's List* (the predecessor of *Index Medicus* and then *PubMed*) and even elaborated upon them. He recommended using cocaine as a remedy for a host of conditions that are not even recognized as diseases today. Köller's discovery was far more important. The availability of an effective local anesthetic radicalized surgery as it was then performed and had tremendous impact on the way medicine was practiced.

Physicians around the world were soon experimenting with the use of cocaine in a wide range of conditions. Some of the applications, such as eye and hemorrhoid surgery, were quite appropriate. Other applications, such as the treatment of hay fever, were more questionable (Mackenzie, 1884). Still other uses were bizarre and potentially dangerous (Reich, 1886). With so many physicians experimenting with the drug, not much time elapsed

Cocaine 5

October 11, 1884.] THE MEDICAL RECORD. 417

THE OPHTHALMOLOGICAL CONGRESS IN HEIDELBERG.

(From our Special Correspondent.)

MURIATE OF COCAINE AS A LOCAL ANÆSTHETIC TO THE CORNEA—NO RADIATING MUSCULAR FIBRES IN THE IRIS—ACTUAL CAUTERY IN SUPERFICIAL CORNEAL ULCERATIONS—OPTICO-CILIARY NEURECTOMY—IS CATARACT THE RESULT OF CHRONIC BRIGHT'S DISEASE?—PROFESSOR ARLT AND HIS RECENT WORK IN GLAUCOMA.

KREUZNACH, GERMANY, September 19, 1884.

SIR: The usual Ophthalmological Congress in Heidelberg has just closed its session, and a few cursory notes at this early date may interest some readers. At this meeting elaborate papers are not read, but condensed statements are presented of the subjects introduced. The notable feature of this Society is that only new things or new phases of old topics are presented. This is not from any expressed rule, but is from the tacit understanding which controls men who are so diligently investigating the unknown in science as are these eager workers. These men have no patience with mere reiterations. Perhaps the most notable thing which was presented was the exhibition to the Congress upon one of the patients of the Heidelberg Eye Clinic, of the extraordinary anæsthetic power which a two per cent. solution of muriate of cocaine has upon the cornea and conjunctiva when it is dropped into the eye. Two drops of the solution were dropped into the eye of the patient at the first experiment, and after an interval of ten minutes it was evident that the sensitiveness of the surface was below the normal, then two drops more were instilled and after waiting ten minutes longer there was entire absence of sensibility, a probe was pressed upon the cornea until its surface was indented, it was rubbed lightly over the surface of the cornea, it was rubbed over the surface of the conjunctiva bulbi, and of the conjunctiva palpebrarum; a speculum was introduced to separate the lids and they were stretched apart to the uttermost; the conjunctiva bulbi was seized by fixation forceps and the globe moved in various directions. In all this handling the patient declared that he felt no unpleasant sensation, except that the speculum stretched the lids so widely asunder as to give a little discomfort at the outer canthus. Before the experiment his eye was shown to possess the normal sensitiveness, and the other eye, which was not experimented on, was in this respect perfectly normal. The solution caused no irritation of any kind, nor did it at all influence the pupil. The anæsthetic influence seemed to be complete on the surface of the eye, and it lasted for about fifteen minutes and the parts then resumed their usual condition. This first experiment was done in the presence of Professor Arlt, of Professor Becker, of the clinical staff, of Dr. Ferrer of San Francisco, of some other physicians, and of the writer. The next day the same experiment was performed on the same patient in the presence of the Congress and with the same results. This application of the muriate of cocaine is a discovery by a very young physician, or he is perhaps not yet a physician, but is pursuing his studies in Vienna, where he also lives. His name is Dr. Koller, and he gave to Dr. Brettauer, of Trieste, a vial of the solution, to be used in the presence of the Congress by Dr. Brettauer. Dr. Koller had but very recently become aware of this notable effect of cocaine, and had made but very few trials with it. These he had been led to make from his knowl edge of the entirely similar effect which it has for some year or more been shown to have over the sensibility of the vocal cords, and because of which laryngologists pencil it upon their surface to facilitate examinations.

The future which this discovery opens up in ophthalmic surgery and in ophthalmic medication is obvious. The momentous value of the discovery seems likely to prove to be in eye practice of more significance than has been the discovery of anæsthesia by chloroform and ether in general surgery and medicine, because it will have therapeutic uses as well as surgical uses. It remains, however, to investigate all the characteristics of this substance, and we may yet find that there is a shadow side as well as a brilliant side in the discovery. Professor Kühne, who in the Heidelberg Physiological Laboratory worked out the details of Boll's discovery of the visual purple of the retina, received the news of this new discovery with the liveliest interest. We may, perhaps, get from him a further investigation into its properties. The substance makes a clear solution, and is found in Merck's catalogue.

Another notable statement came from Dr. Eversburch, of Munich, as the result of very exact and elaborate studies, to the effect that there are no radiating muscular fibres in the iris; in other words, that the dilator iridis has no existence in man. It is found, he says, in some animals, and especially in those which have oblong pupils, whether vertical or horizontal, and in the form of fasciculi at the extremities of the slit. He absolutely denies the existence of such fibres in the human eye, and asserts that the fibres hitherto described under this name are nerve-fibres. These revolutionary assertions were received with respect and attention, because the investigator was known to be a careful and competent anatomist. If his declarations should be confirmed, and they will not be lightly accepted, we must find out a new theory for the active dilatation of the pupil. A good deal of physiology will have to be cast into a new form. It is true that the anatomical discussion has not been closed on this point, but in favor of the existence of the dilator stand the names of Merkel, Henle, and Iwanoff among recent investigators. Eversburch has in his possession the preparations of Iwanoff, who died a few years ago, and he knows the nature of the contest into which he enters.

The uses of the actual cautery in superficial forms of corneal ulceration and in some other superficial processes, especially in those of micrococcic origin, were discussed both here and in Copenhagen. There seems to be a general consensus as to the usefulness of this treatment in selected cases of superficial corneal disease, viz., in ulcus rodens, in superficial suppurative processes, in atonic ulcers, and by Nieden in xerophthalmus. Nieden will shortly announce his views in full in an article in the *Archives for Ophthalmology*. He presented a most delicate and elegant form of galvano-cautery which he had devised, and to which he had applied a very delicate and promptly acting key invented by Professor Sattler. Another form of cautery is in use in the Heidelberg Eye Clinic, which has been devised by Professor Becker, and is a very small and utilizable Paquelin cautery. Both these instruments can be handled with nicety and delicacy, and without frightening the patient, and also in most cases without giving him any pain. This treatment, as well as the scraping of such ulcers by a sharp spoon, as does Meyer, of Paris, is founded on the micrococcic theory of the pathology of these processes, and marks another forward step in ophthalmic therapeutics.

Optico-ciliary neurectomy as a preventive of sympathetic ophthalmia has not passed out of practice, as to a considerable degree has become the case among us. So able an observer and logical a reasoner as Professor Schweigger, of Berlin, recommends its performance and holds it in higher esteem than enucleation. He divides the internal rectus muscle to gain easy approach to the nerve, and he lifts it from its bed by a sharp double hook and excises 10 mm. of it. He is said to be extremely skilful in this proceeding, and the very small disturbance which he causes in the structures of the orbit may perhaps explain the success which he has had and the confidence which he expresses in its prophylactic virtue. Among over a hundred cases which furnished the material for his conclusions, in two cases he saw occur in the opposite eye an acute neuro-retinitis, with opalescent infiltration, etc. There was no reduction of vision either central or peripheral. In two weeks the appearance

Figure 1.9 Cocaine as a local anesthetic. The discovery that cocaine was a potent local anesthetic revolutionized surgery. It was first reported at an ophthalmology congress in Heidelberg. Shortly thereafter, an account appeared in the Medical Record of October 11, 1884. (From the Medical Library at the University of California, San Francisco, CA.)

before the first reports of cocaine toxicity began to appear. Less than 1 year after Köller's and Freud's papers were published, an article in the *British Medical Journal* described the toxic reactions associated with cocaine use in ophthalmologic surgery (Ziem, 1885). At about the same time, the popular press began carrying accounts of cocaine-related deaths (Thompson, 1886). The first cocaine-related cardiac arrest was reported in 1886 (Thompson, 1886), as was the first cocaine-related stroke (Catlett, 1886). In 1887, a physician named Mattison reviewed 50 cases of cocaine toxicity, 4 of which were fatal. Each of the fatalities had the characteristics associated with cardiac arrhythmias (Mattison, 1887). The following year, Mattison published data on an additional 40 cases, including 2 more fatalities.

None of these negative reports appeared to have had any impact. Patent medicine manufacturers continued to cash in on the popularity of coca by replacing low-concentration cocaine extracts with high concentrations of refined cocaine hydrochloride. Thousands of cocaine-containing patent medicines flooded the market, some with truly enormous amounts of cocaine. Dr. Tucker's Asthma Specific, for instance, contained 420 mg of cocaine per ounce and was applied directly to the nasal mucosa. Absorption was almost 100%. As the cocaine content of the products increased, so did the number of reported medical complications. The situation rapidly deteriorated when users learned they could "snort" cocaine. Until the early 1900s, cocaine had been taken mainly by mouth or by injection. The fact that the first cases of septal perforation and collapse (saddle nose deformity) were not reported until 1904 suggests that "snorting" had only become popular a year or so earlier (Maier, 1926).

As demand for cocaine grew, alternate sources came online, and a Southeast Asian cocaine industry came into existence. Coca was also grown in Nigeria, Sri Lanka, Malaysia, Indonesia, Taiwan, and Iwo Jima (Figure 1.10) (Reens, 1919; Karch, 2003). The Javanese

Figure 1.10 Indonesian coca production. During the 1920s, Indonesian plantations exported more coca leaf than producers in South America. This photograph, taken in 1927, shows workers sorting coca leaf. (Photo courtesy of the Tropen Museum Photo Bureau, Amsterdam, the Netherlands.)

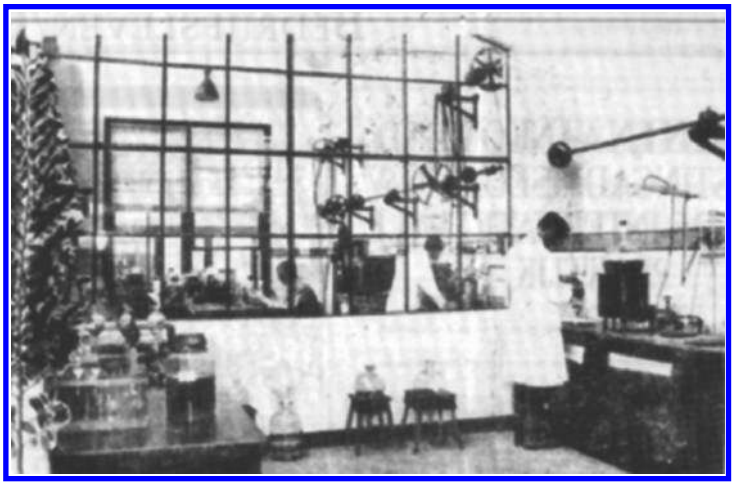


Figure 1.11 The NCF: the Dutch Colonial Development Board and coca growers in Java formed a joint venture and built a refinery to better compete with Merck and the other German cocaine manufacturers. The NCF opened in Amsterdam on March 12, 1900. The Dutch plant was so successful that a second floor was added to the factory in 1902. By 1910, NCF claimed to be the largest cocaine manufacturer in the world, producing more than 1500 kg of refined cocaine per year. This photograph is from a trade publication, *Het Pharmaceutisch Weekblad*, published in 1925. (Courtesy of de Kort, M., the Netherlands Ministry of Health.)

plantations were the main suppliers and Japanese pharmaceutical companies were the main buyers. European drug manufacturers, such as Merck, even bought their own Javanese plantations, shipping dried coca leaves back to Germany for refining. In order to remain competitive, Dutch coca growers, along with the Dutch Colonial Development Board, formed a joint venture and built their own refinery in Amsterdam. The Nederlandsche Cocaine Fabriek (NCF) opened on March 12, 1900, in Amsterdam (Figure 1.11). The Amsterdam plant was so successful that a second floor was added to the factory in 1902. By 1910, coca exports from Java exceeded those from South America (Karch, 2003).

The first histologic studies of cocaine toxicity were published in 1888. Vasily Zanchevski of St. Petersburg, Russia, studied the acute and chronic effects of cocaine in dogs. After a single lethal dose (24 mg/kg), the animals had changes said to be typical of acute asphyxia, although given the design of the experiment, it is unlikely that such changes could have been exhibited. Smaller daily doses given for several weeks caused a marked hyperemic condition of the central nervous system, in contrast to the rest of the organs, which were anemic. Focal degenerative changes were observed in the spinal ganglia, heart, and liver. In some cases, the myocytes had "lost their striae and [were] intensely granular" (Zanchevski, 1888). Although illustrations are lacking, Zanchevski's descriptions suggest that he was the first to observe a lesion called contraction band necrosis (CBN) occurring as a result of catecholamine toxicity, in this case a consequence of cocaine ingestion.

French researchers were the first to study systematically cocaine's psychological effects, largely because cocaine and morphine addiction were such a major problem in Paris. In 1889, at a meeting of the Biological Society of Paris, French psychiatrist Valentin Magnan (1835–1916) presented three cases illustrating that cocaine users were subject to tactile hallucinations. The symptom complex became known as "Magnan's symptom" and is still frequently diagnosed today, though the name of the discoverer has long since been forgotten. In 1914, Georges Guillain contrasted the differences between

cocaine- and alcohol-induced hallucinations, commenting on how variable the effects of a given dose of cocaine could be (Maier, 1926).

Chronic cocaine use causes a very specific disorder that has become increasingly common and is generally referred to as "cocaine-associated excited delirium" or "agitated delirium." The terms are used interchangeably, and to make things more complicated, the American Academy of Emergency Physicians has recently coined still another term for the same disorder, calling it "excited delirium syndrome" (ExDS) (Force, 2009). All of the available evidence suggests that the occurrence of this disorder is an indirect marker for the availability of cocaine (and other stimulants).

Excited delirium first came to widespread public attention in 1914 when the *New York Times* carried a front-page article written by an American physician, Edward Williams. The article described "Cocaine crazed Negros" who were threatening the women of the South (Williams, 1914). Because Williams' writings were patently racist, and because he only described the syndrome as occurring in blacks (at the turn of the last century, black farm laborers were often paid in cocaine) (Karch, 1998), later historians were prone to write off his observations as mere racist hysteria (Kennedy, 1985).

New reports of the syndrome (hyperthermia, followed by agitated psychosis, respiratory arrest, and death) reappeared at the start of the current pandemic. Although the disorder has nothing to do with race, it is quite real (Wetli, 2006) and the underlying neurochemical changes have now been extensively characterized in a large multicenter study (Mash et al., 2009). Excited, or agitated, delirium was a known clinical entity long before the *New York Times* ever thought it was "news fit to print." In 1849, 25 years prior to the commercial availability of cocaine, a physician named Luther Bell published a paper describing exactly the same sort of symptoms in hospitalized psychiatric patients. Bell titled his article: "On a form of disease resembling some advanced stages of mania and fever, but so contra distinguished from any ordinarily observed or described combination of symptoms as to render it probable that it may be an overlooked and hitherto unrecorded malady" (Bell, 1849). In spite of its storied history, many refuse to accept it as a real entity, arguing instead that it is a "phony" defense used to avoid blame when a prisoner dies in custody. Critics also point to the fact that excited delirium is not listed in the American Psychiatric Association's official *Diagnostic and Statistical Manual* (*DSM*-5; American Psychiatric Association, 2013). However, now that the National Institutes of Mental Health no longer rely upon *DSM* for disease classification, that old objection is no longer valid (Insel, 2013).

The first human autopsy study of a cocaine-related death was published in 1922. Bravetta and Invernizzi described a 28-year-old man who had been sniffing cocaine regularly for some months prior to his death. He neither drank nor used other drugs (Bravetta and Invernizzi, 1922)—copies of the original paper are reproduced in the monograph by Hans Maier quoted earlier (Maier, 1926). Hyperemia of the brain, lungs, and adrenals was noted, and the heart was described as "flaccid," a finding that could represent the now well-recognized entity of cocaine cardiomyopathy (Karch, 2005). The poorly reproduced micrographs from the autopsy showed cardiac lesions similar to those described by Zanchevski (and this author). They also demonstrated fairly obvious glomerulosclerosis. Animal studies by the same authors confirmed their autopsy findings and also demonstrated widespread endothelial injuries. These illustrations were also reprinted in Maier's classic text.

The tissue disposition of cocaine was studied at an even earlier date. In 1887, a German chemist, Dr. L. Helmsing, published his technique for the detection of cocaine in urine

and tissues. The technique was fairly sensitive, able to detect cocaine in urine from a cat that had been given 8 mg of cocaine (Helmsing 1887). In 1951, Woods et al. developed a calorimetric technique capable of detecting levels of cocaine as low as 500 ng/mL (Woods et al., 1951). A quarter century later, in 1975, Jatlow and Bailey used gas chromatography to lower the limits of detection to 5 ng/mL (Jatlow and Bailey, 1975).

Shortly after Maier's text was first published, case reports stopped appearing, at least in the U.S. literature. Between 1924 and 1973, only one cocaine-related fatality was reported in the medical literature, and it involved a surgical misadventure. However, trade remained brisk in Europe. As a consequence of international agreements that were incorporated into the peace treaty that ended World War I, laws were enacted worldwide, all more or less similar to those already in effect in the United States. Thus, between 1915 and 1924, cocaine users became marginalized and use was confined mainly to the criminal classes. Usage plummeted, even though the main cocaine producers were still based in Germany. During the Weimar Republic, cocaine was cheap and readily available. The availability of methamphetamine, together with the start of World War II, more or less ended European use of cocaine until the 1970s when, as in the United States, large-scale use began to reemerge (Korff and Verbraeck, 1993; Zaitch, 2002).

In 1977, Suarez first described the "body packer" syndrome, where death results from the rupture of cocaine-filled condoms in the smuggler's intestines (Suarez et al., 1977). The absence of prior case reports no doubt reflected a decline in cocaine use, but the decline itself is difficult to explain. Certainly the passage of laws restricting the sale of cocaine (the Pure Food and Drug Act of 1906 and the Harrison Narcotic Act of 1914) had something to do with it, but other factors were clearly involved (McLaughlin, 1973).

Professor David Musto suggested that cycles of drug abuse begin when a generation of young people no longer remembers the adverse consequences experienced by the preceding generation. Cycles end when drug users realize that other drug users they know are becoming ill. As awareness increases, experimentation falls off, and the cycle eventually ends (Musto, 1987; Behrens et al., 1999). Current drug users tend to recruit new ones, so in this respect, Musto's theory treated cocaine addiction in the same way as any contagious disease: victims can be affected mildly or severely, or even die. Those experimenting with drugs, the "light users," will suffer few adverse consequences from their "disease" but are the ones most likely to recruit new users and spread the disease to others. "Heavy users" will suffer extreme ill effects, even death, and so are unlikely to recruit any new users. Someone who dies of an acute overdose the first time they use a drug is unlikely to infect many others or create new recruits. If this model is correct, then the memories of those who have had bad experiences with a drug will be balanced against the memories of those who have not. When there are more light users who have not had bad experiences, then the use of drugs will increase, always supposing, of course, that the drug supply is elastic—as demand increases, so does supply, although this is not always the case.

Musto's model seems to fit the situation today rather well, in both the United States and Europe. By the 1920s, initial enthusiasm for cocaine had waned, partly because of efforts by the United States to prohibit drug use, but it is impossible to say what really caused the decline in use. It appears that even though there was a great deal of cocaine available, and at very reasonable prices, no one wanted to use it because the downside appeared too great. Cocaine had gone from a wonder drug to a scourge. Apart from a few show business celebrities, those who took cocaine were thought of as deviant losers. During the interwar years, another antidrug element was added—availability decreased and prices increased.

Not only had many people become ill taking the drug, it was now also expensive. That situation is no longer true, which is unfortunate because, together, the high prices and known deleterious effects were a powerful disincentive to users. Even after prices went up and availability went down, cocaine remained available, but only as an expensive indulgence, not a drug for the masses. In 1934, Cole Porter wrote about sniffing cocaine in his play *Anything Goes*; an aristocratic appearing man wearing a top hat and tails sang the song. Working people and college students were not experimenting with cocaine.

Another factor that contributed to the end of the first pandemic may have been the introduction of amphetamines. The cycle seems to be repeating itself now. All indicators show major increases in methamphetamine abuse within the last year and new amphetamine-like designer drugs are introduced almost every day. Significant toxicity from the use of coca leaf and coca extract was never a problem in Europe or the United States. Toxicity only emerged when purified cocaine became readily available and individuals could increase their dosage by orders of magnitude. The small amounts of cocaine in Vin Mariani were apparently harmless, but the huge amounts in Dr. Tucker's formula were occasionally lethal. With the appearance of "crack" cocaine in 1986, another order of magnitude in dosage was achieved (Jekel et al., 1986). That cocaine-related illness is now a significant cause of morbidity and mortality should not be surprising. More people are using the drug, they are using more of it, and they are using it more effectively.

1.4 Current Affairs

Global cocaine seizure rates are hard to assess. Some government surveys indicate that supply has stabilized over the last few years, but press accounts tend to tell an opposite story, with multiton seizures becoming the norm. In its annual drug report for 2012, the UN section dealing with the drug trade states that, since 2007, the total area of land under cultivation for coca bushes has declined by 18%, especially in Colombia, while cocaine use has declined in North America but remained stable in Europe. The UN report also indicated a future trend for an increased use of synthetic drugs and the nonmedical use of prescription drugs "diverted from legal supplies." This increase seems to be substantiated by the almost daily introduction of new designer drugs, produced with the intention of skirting national and international law.

In the United States, deaths from misuse of prescription painkillers such as morphine have increased fourfold since 1999. They now outnumber deaths involving heroin and cocaine. The U.S. government has officially declared prescription drug abuse to be an epidemic—America's fastest-growing drug problem.

The UN forecasts that the number of illicit drug users will increase by a quarter by 2050, roughly in proportion to the growth of the world population. According to the report, the majority of the increases will probably take place in developing countries, most especially involving young people. The UN report suggests that cannabis will remain the most widely used illegal substance.

Efforts to reduce and measure drug supply and demand may have focused on established patterns of production with consumption in the United States. More recently, there are indications of emerging cocaine consumption in countries previously not associated with this phenomenon, and it cannot be excluded that the available indicators do not yet fully reflect the extent of global cocaine demand and supply.

For a time, there were declining numbers of reports of cocaine seizures from both North America and Europe. Whether this was due to increased enforcement efforts or increased seizures in South and Central America is difficult to say. Trafficking through West Africa, which had been steadily increasing between 2004 and 2007, seemed to be declining until 2008, but that was only because drug cartels developed new means of transportation (submarines built by Russian technicians among others) and new routes of transport through Spain and Africa. One large Mexican cartel now supplies the East Coast of Australia where police routinely confiscate shipments of 100 kg or more. Any decreases in consumption within the United States are now more than offset by increasing use in the rest of the world.

The situation in the United Kingdom, a country with a large cocaine market, is unclear. British authorities have been reporting declines in cocaine purity for some time, suggesting decreased cocaine availability but, at the same time, seizures of the drug are still increasing, prices are falling, and there are indications of a switch to new trafficking routes (European Monitoring Centre for Drugs and Drug Addiction, 2009b). Even if these declines are real, any shortfall appears to have been made good by the rising tide of synthetic amphetamines (especially the cathinone derivatives or "bk-amphetamines").

The U.S. Department of Justice reports that cocaine availability has decreased sharply in the United States and that the decreases first became apparent in 2006. Within the United States, every indicator of cocaine availability (seizures, price, purity, workplace drug test results, and emergency room visit data) points to significantly less availability in 2009 than in 2006. For example, federal cocaine seizures decreased 25% from 2006 (53,755 kg) to 2008 (40,449 kg) and remained low in 2009. The price per pure gram of cocaine increased from $94.73 in the third quarter of 2006 to $174.03 in the third quarter of 2009, while cocaine purity decreased from 68.1% to 46.2% (U.S. Department of Justice, 2010).

In 2007, the mean purity of cocaine in Europe ranged between 22% and 57% (European Monitoring Centre for Drugs and Drug Addiction, 2009a; Evrard et al., 2010); at the same time, purity in the United States dropped from the high 60s in 2006 to the high 40s today. Most of the countries with adequate drug surveillance systems report a decline in the purity of cocaine over the period 2002–2007, the only exceptions being Spain and Portugal, both important transit points for Europe-bound cocaine. In 2007, among the 19 EU members reporting data, the mean retail price of cocaine ranged from EUR 44 to EUR 88 per gram, with about half of the EU nations reporting mean prices of between EUR 58 and EUR 67 per gram. In short, European cocaine is becoming cheaper but less pure (European Monitoring Centre for Drugs and Drug Addiction, 2009a; UNODC, 2010; U.S. Department of Justice 2010). If there is a cocaine shortage, and it is beginning to look as if there may be, it would hardly be surprising if a new group of adulterants, such as levamisole or other designer drugs, were to emerge.

1.4.1 Cultivation and Crop Yields

Coca leaf has been grown in the Andean subregion for thousands of years. Coca grows best on the moist, warm slopes of mountains ranging in elevations from 1500 to 5000 ft. If the plants have not been hybridized, or even genetically manipulated, coca shrubs grow to heights of 6–8 ft. The trunk of the plant is covered by rough, somewhat glossy bark that has a reddish tint. Its flowers are small and usually white or greenish yellow. Leaves are elliptical, pointed at the apex, and dark green in color.

Until recently, it had been assumed that all cultivated coca was derived from two closely related species that grow naturally only in South America, *Erythroxylum coca* Lam and *Erythroxylum novogranatense* Hieron. Each species has one distinct variety designated as *E. coca* var. *ipadu* (Plowman, 1985) and *E. coca novogranatense* var. *truxillense* (Rusby) Plowman (Plowman, 1985). All four types are cultivated, although the alkaloid content of the different plants varies considerably (Plowman and Rivier, 1983). *E. coca ipadu* is cultivated only in the Amazon valley of Brazil, Colombia, and Peru. Of all the cultivated varieties, *E. coca ipadu* contains the least alkaloid, less than 0.5%, and very little of that is cocaine. *E. novogranatense* is cultivated more widely and is better adapted to growth in hotter, drier climates.

However, recent studies using DNA resequencing have shown that at least 14 different varieties exist, though they have not yet been sufficiently well analyzed to determine how much, if at all, each contributes to total cocaine production (Plowman, 1985). Although there is some controversy, it seems likely that *E. novogranatense* was the variety cultivated in Java, Ceylon, India, and Taiwan. This variety may contain anywhere from 1% to 3% total alkaloid, with cocaine constituting as much as one-half of the total alkaloid present (Lee, 1981; Bohm et al., 1982; Plowman and Rivier, 1983; Plowman, 1985; Schlisinger, 1985).

A strain of *E. novogranatense* cultivated in the desert coast region of Peru, near Trujillo, is the plant used to flavor Coca-Cola and other cola beverages. Colombia remains the world's leading producer of cocaine, accounting for at least 75% of all cocaine production. There are a number of factors that could change the situation quite quickly. According to reports from the Drug Enforcement Agency (DEA), production in Bolivia and Peru is increasing, perhaps because of increased enforcement efforts in Colombia. And, of course, the possibility of resumed production in Southeast Asia always remains real.

The real concern is genetic manipulation. Irrefutable evidence exists that Bolivian coca producers have been hybridizing *E. coca* var. *ipadu* in order to increase its cocaine yield. Several years ago, U.S. government scientists collected and analyzed a total of 132 coca samples from the highest producing areas of Bolivia. These samples were then compared for cocaine content with other specimens in their library. The age of the plants, and the original planting date, were estimated when the samples were collected and confirmed with DNA fingerprinting.

When overlapping DNA patterns were correlated with the samples, several clustered linkages were apparent. For example, one cluster of five samples, all with very closely related DNA, was found to have come from an area in the northern state of Guaviare and the adjacent state of Meta. The findings support the notion that regionalized populations of *E. coca* var. *ipadu* are being developed rapidly in Colombia in a number of areas. The main center for these new populations is the Caquetá-Putumayo region of Colombia; at least 5–8 new populations of *E. coca* var. *ipadu* have been or are being developed and several of those strains have gained regional widespread use. It is also clear that in several regions of Colombia, new strains are being field tested and developed. The process is probably relatively informal and occurs independently of any cartel interference (Johnson et al., 2003).

Whether as a result of traditional techniques of plant hybridization or modern genetic manipulation, the coca now being grown in the Andes has been modified in such a way that it contains more cocaine than in the past. The most obvious consequence is that more cocaine comes to market. Just how much is anyone's guess; without knowing the yield of the plants being grown, it is impossible to estimate output. Table 1.3 shows the U.S. and European seizures from 1986 to 2012.

Table 1.3 Cocaine Seizures in the United States from 1986 through 2012

DEA Domestic Drug Seizures					
Calendar Year	Cocaine (kgs)	Heroin (kgs)	Marijuana (kgs)	Methamphetamine (kgs)	Hallucinogens (dosage units)
2013	22,512	965	267,957	3,990	116,215
2012	36,694	999	388,059	4,622	870,203
2011	32,374	1,075	575,960	2,485	3,954,732
2010	30,053	713	725,858	2,188	2,604,797
2009	50,704	618	671,650	2,007	3,422,593
2008	50,461	605	662,137	1,518	9,311,715
2007	98,065	623	360,708	1,112	5,677,739
2006	71,604	816	328,275	1,804	3,745,560
2005	118,128	622	283,382	2,161	8,868,465
2004	117,844	669	266,088	1,656	2,196,988
2003	73,720	788	254,242	1,680	3,038,916
2002	63,513	709	238,646	1,347	11,824,798
2001	59,415	747	272,120	1,634	13,863,756
2000[a]	58,674	546	331,964	1,771	29,293,957
1999	36,163	351	338,247	1,489	1,717,305
1998	34,447	370	262,180	1,203	1,139,524
1997	28,674	399	215,348	1,147	1,099,825
1996	44,735	320	192,059	751	1,719,239
1995	45,309	876	219,830	876	2,768,046
1994	75,031	490	157,181	769	1,368,437
1993	55,528	616	143,055	560	2,714,575
1992	69,322	722	201,483	352	1,308,018
1991	67,016	1,174	98,593	289	1,294,273
1990	57,021	535	127,792	272	2,832,084
1989	94,939	758	286,371	896	13,125,011
1988	60,951	728	347,305	694	10,467,864
1987	49,666	512	629,839	198	6,556,891
1986	29,369	278	490,607	234	4,146,711

Source: http://www.dea.gov/resource-center/statistics.shtml. Accessed April 23, 2015.
Note: CY 2013 statistics are preliminary and subject to updating.
[a] CY 2000 had several large LSD seizures.

Major coca-growing areas in the Andes share many characteristics. Yungas, which is close to La Paz, has an average annual rainfall of 45.7 in. Chaparé, which is close to Cochabamba, has an annual rainfall of 102 in. The plantations in Yungas can be harvested three times per year. Each harvest yields from 2 to 2.7 tons/ha/year. Chaparé leaf contains, on average, 0.72% cocaine. It is estimated that present refining techniques are only 45% effective (less than half the cocaine is actually recovered from the leaf). As a result, at least 390 kg of Chaparé leaf are required to produce 1 kg of cocaine base. The requirements would be higher in Yungas, where coca is said to have an alkaloid content of 0.85% (Abruzzese, 1989). Plant hybridization with resultant increases in crop yield probably also helps to account for the fact that cocaine supplies at the retail level have only decreased slightly in the last few years, in spite of major decreases in the amount of coca leaf produced.

Recent surveys of the Andean region conducted by the UN indicate a major decline in cultivation and in the amount of cocaine actually refined in Colombia (see above). In 2008, the areas under coca cultivation dropped by 18% to 81,000 ha, while production was down 28%, from 600 metric tons in 2007 to 430 metric tons in 2008 (UNODC, 2013). This decline would have been more impressive if it were not known that the cartels are shifting production out of Colombia to Central America. Similarly, declines in leaf production seem more impressive than they really are. At the same time that cultivation was decreasing in Colombia, it was increasing in Bolivia (6%) and in Peru (4.5%). In Bolivia, potential production of cocaine rose 9% to 113 metric tons, while in Peru, it was up 4.1% to 302 metric tons (UNODC, 2010).

1.4.2 Cocaine Production

Until very recently, the Amazon was the main route for smuggling the chemicals necessary for drug processing from Brazil to cocaine laboratories in Bolivia, Colombia, Peru, and even laboratories operating in the western part of Brazil near the Paraguayan and Bolivian borders. That process seems to be in a state of flux. No matter in which country the cocaine is processed, the same general methodology is followed. This is illustrated in Figure 1.12. It is difficult to be more specific about the process because so many different solvents can be substituted in the process.

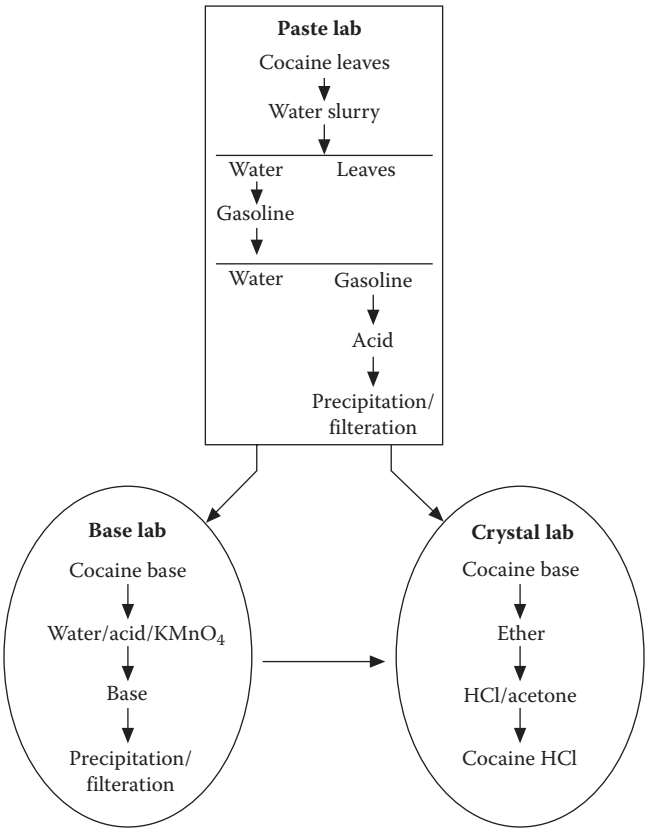


Figure 1.12 Flow chart of illicit cocaine processing. The preparation of purified cocaine from leaf. (Reproduced from the Drug Enforcement Agency's website.)

The U.S. government was largely successful in its attempt to keep the cartels out of Caribbean shipping lanes. As a result, more and more of the finished product is routed through Mexico. As a consequence, cocaine paste (semirefined cocaine) is now shipped to Central America, a zone notorious for both lack of leaders and lack of resources. Large sections of some Caribbean states are no longer under effective government control (Johnson, 2011).

Brazil has a huge chemical industry, much of it located in uncharted territory. The availability of these chemicals provides the cartels with ample opportunity for criminal labs to convert coca leaf into semirefined cocaine (called paste). Brazil's role as South America's leading manufacturer of acetone and ether, the top two solvents used in the processing of cocaine, has become a key factor in the complex cocaine economy of the region. An estimated 25,000 registered chemical factories, trade, and transport companies and an unknown number of unregistered plants and companies handle large quantities of processing chemicals as well as the precursors necessary for making ecstasy, methamphetamine, and cocaine. Extraction is a two- or three-step process carried out in a series of laboratories.

The first steps occur on site. Immediately after harvesting, coca leaves are placed in a shallow pit lined with heavy plastic. The leaves are then soaked in a dilute solution of water and strong alkali, such as lime, for 3 or 4 days. An organic solvent is added. Methyl isobutyl ketone is the solvent of choice for this purpose, but nearly a dozen other solvents have been identified in samples that have been confiscated by the DEA and other agencies (INCB, 1999). In recent years, the use of ethyl acetate and *n*-propyl acetate has become increasingly popular, but kerosene, gasoline, or even acetone can be used if no other solvents are available.

Extracted coca leaf is discarded and sulfuric acid is then added to the extract, dissolving a complex mixture of alkaloids in the aqueous layer. If the alkaloid content of the leaves is very high (as in Bolivia), hydrochloric acid may be used instead of sulfuric. The organic solvent is then removed, and the remaining aqueous solution is made alkaline by the addition of lime, ammonia, or the equivalent, causing the more basic alkaloids to precipitate out. This crude form of cocaine, called coca paste, is allowed to dry in the sun. The site where the initial steps occur is referred to as a "paste lab." Laborers, called pisacocas, keep the alkali–coca leaf mulched by stirring it with their hands and walking through it with their bare feet. The fluid is quite corrosive, and the workers quickly develop large extremity ulcers. The pisacocas tolerate the ulcers only because they are given a constant supply of coca paste to smoke (Weatherford, 1988).

The dried product is a mixture of cocaine, *cis*- and *trans*-cinnamoylcocaine, tropine, tropacocaine, hygrine, cuscohygrine, ecgonine, BZE, methylecgonine, and isomers of truxillines (ElSohly et al., 1991). The mixture also contains a host of soluble organic plant waxes and benzoic acid. Depending on the alkaloid content of the coca leaves and on how the leaves were processed, it takes between 100 and 150 kg of dry leaf to produce 1 kg of coca paste (Montesinos, 1965; Brewer and Allen, 1991). In fact, the actual cocaine content of coca base is extremely variable, depending mostly on what precursors are available and also the skill of the chemist. Cocaine concentrations ranging from 20% to 90% have been measured in confiscated samples (Lopez-Hill et al., 2011). While the cocaine content may be variable, paste is almost always found to have been adulterated with 5%–20% caffeine (Lopez-Hill et al., 2011), though why this adulterant is almost universally present is not known.

At base labs, such as those now proliferating in Central America, paste is dissolved in dilute sulfuric acid. Potassium permanganate (potassium dichromate or sodium hypochlorite can be used just as effectively) (Figure 1.13; INCB, 1999) is added until the solution

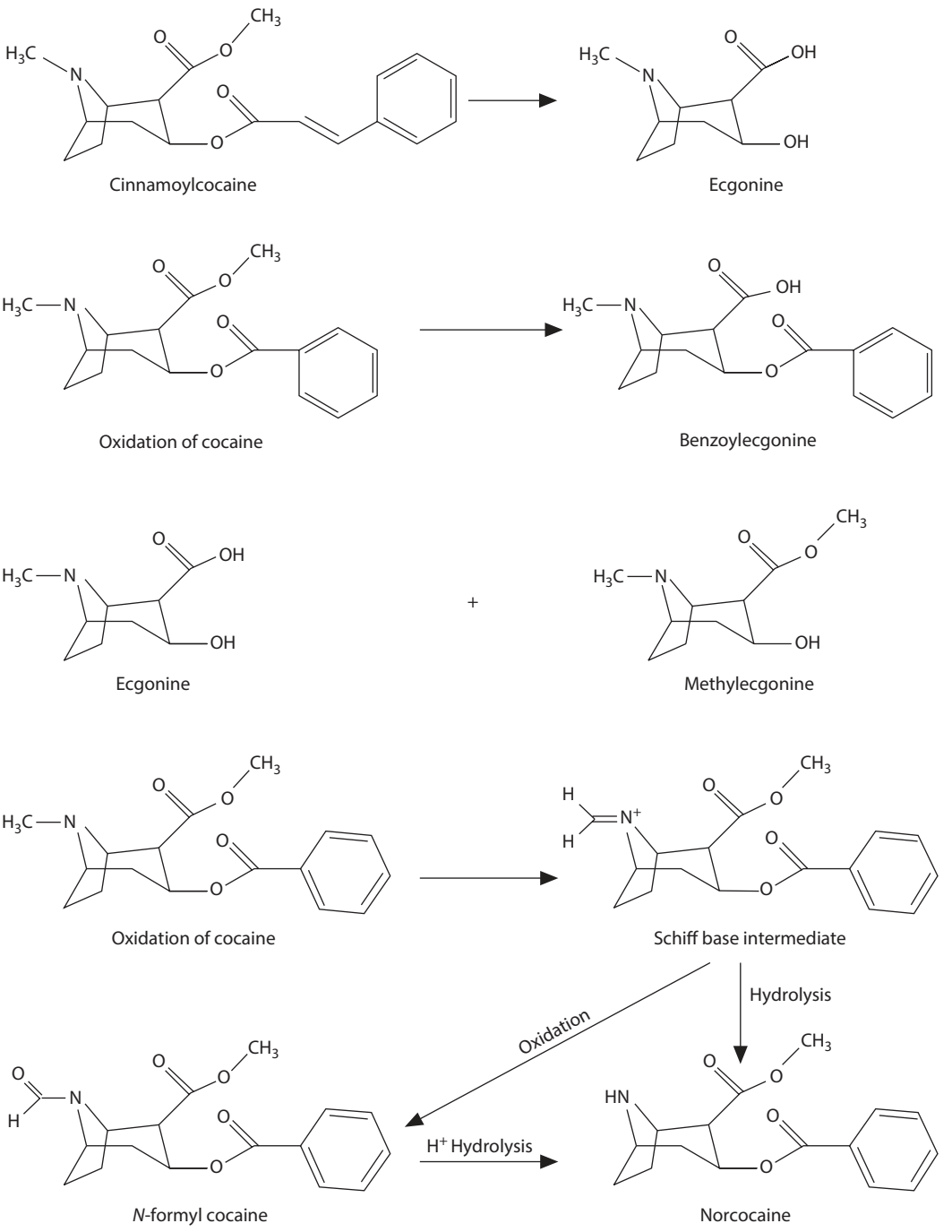


Figure 1.13 The chemistry of cocaine refining. Cocaine refiners often add potassium permanganate to remove impurities. Cinnamoylcocaine is converted to ecgonine, which is water soluble and easy to separate from cocaine. If the process is allowed to continue for too long, the cocaine itself is degraded and the yield drops. Norcocaine, which may be hepatotoxic, is formed at the same time.

turns pink, thereby destroying the cinnamoylcocaine isomers present as impurities in the paste. The isomers of cinnamoylcocaine are converted to ecgonine and, because ecgonine is very water soluble, it is easy to separate it from the cocaine. The job of the clandestine chemist is to stop the oxidation process (usually by adding ammonia or some other alkali) before the cocaine starts to oxidize and the yield drops. Analysis of impounded samples suggests that permanganate oxidation is used only about 60% of the time.

The reddish-pink solution is allowed to stand, then it is filtered and the filtrate is made basic with ammonia. The cocaine base precipitates out. The precipitate is filtered, washed with water, and then dried. Finally, it is dissolved in diethyl ether or acetone. After filtering, concentrated hydrochloric acid and acetone are added, causing purified cocaine hydrochloride to precipitate out. This final step may be done on site or the semipurified cocaine may be transported to a "crystal lab" usually located in one of the larger Colombian cities, although some drug producers have begun to set up labs in the United States. As much as 50 kg may be processed at one time (Lee, 1981).

Semipurified cocaine is then dissolved in a solvent, often ether. Hydrochloric acid is then added, along with a bridging solvent such as acetone, and white crystals precipitate out. The crystals are collected by filtration. Traces of the solvent remain, and their presence can sometimes be used to identify the origin of cocaine samples.

In coca-producing countries, there is a significant market for the semipurified paste itself. Paste is smoked, rolled up in pieces of newspaper, or packed into cigarettes. Many of the ingredients introduced during the manufacturing process are still present in the coca paste and are inhaled as pyrolysis products. Coca paste smoking is a major cause of morbidity in coca-producing countries, but there is a paucity of scientific data about it (Paly et al., 1982).

When permanganate is added during the refining process, the *N*-methyl group of cocaine is oxidized, leading to the formation of *N*-formyl cocaine. Hydrolysis of *N*-formyl cocaine leads to the formation of norcocaine. The presence of these last two compounds can have forensic and clinical significance. Because *N*-formyl cocaine is a product of permanganate oxidation, it is not present in coca paste. Accordingly, the presence of this compound may yield valuable information about how, and possibly where, the cocaine sample was produced (Brewer and Allen, 1991).

Norcocaine is potentially hepatotoxic but, as a rule, only small amounts are formed in humans, and then usually only when ethanol is also present. The norcocaine content of raw cocaine is low as well. Analysis has shown norcocaine concentrations in illicit samples ranging from 0.01% to 3.70% (Lurie et al., 1987; Moore et al., 1993; Stein and Kraatz, 1996).

As expected, detailed chemical analysis of coca paste will disclose the presence of all the elements used during its manufacture, including benzoic acid, methanol, kerosene, sulfuric acid, cocaine sulfate, and other coca alkaloids (Jeri, 1984; Moore and Casale, 1994). Paste can be broken down into neutral, acidic, and basic fractions. Gasoline residues are particularly common in the neutral fraction (ElSohly et al., 1991) and their presence is generally an indicator that the preferred solvents are not readily available.

Amphetamines may occasionally be contaminated with substantial amounts of lead but coca paste samples rarely contain more than trace levels. However, paste can contain large amounts of manganese, and the amount of manganese present is a marker for where the paste was produced. Colombian paste is manganese-rich while Peruvian is not (ElSohly et al., 1991).

The total synthesis of cocaine is possible and clandestine cocaine laboratories have been confiscated. The process is, however, a great deal more demanding than the synthesis of amphetamine and has never been attempted on a large scale, though new methods for total

synthesis of cocaine's tropane ring have been discovered (Cheng et al., 2011), increasing the probability that totally synthetic cocaine could, in the near future, come to market. The synthetic origin of the cocaine will be evident from the contaminants that are also found in the sample. Diastomers of cocaine, such as pseudococaine, allococaine, and the *d*, *l* form (which does not occur in nature), are not found in cocaine refined from coca leaf (Soine, 1989).

1.4.3 Price and Quality

Beginning in 2006, cocaine availability began to decrease significantly. Every national-level indicator of cocaine availability (seizures, price, purity, number of positive workplace drug tests, and number of emergency room visits) indicates that cocaine was significantly less available in 2009 than in 2006. Federal cocaine seizures decreased 25% from 2006 (from 53,755 to 40,449 kg) in 2008, and they remained at roughly the same level in 2009. The price per pure gram of cocaine increased from $94.73 in the third quarter of 2006 to $174.03 in the third quarter of 2009, while cocaine purity decreased from 68.1% to 46.2% (Figures 1.14 and 1.15) (U.S. Department of Justice, 2010).

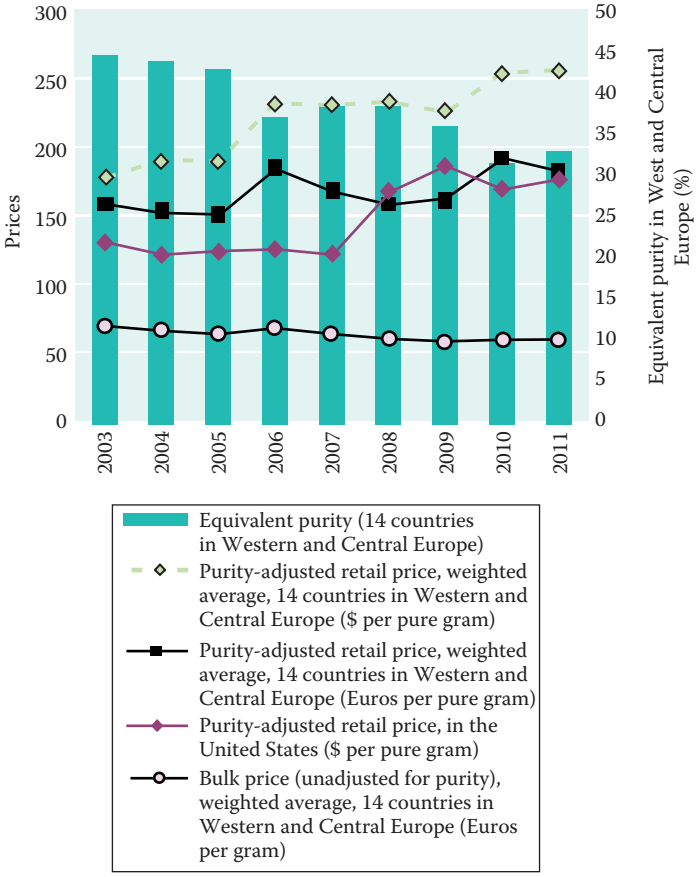


Figure 1.14 Cocaine retail prices in the United States and Western and Central Europe, 2003–2011. (Estimates based on annual report questionnaire and data from Europol and the United States Office of National Drug Control Policy; Reproduced from United Nations Office on Drugs and Crime, World Drug Report, 2013, United Nations, New York, 2013.)

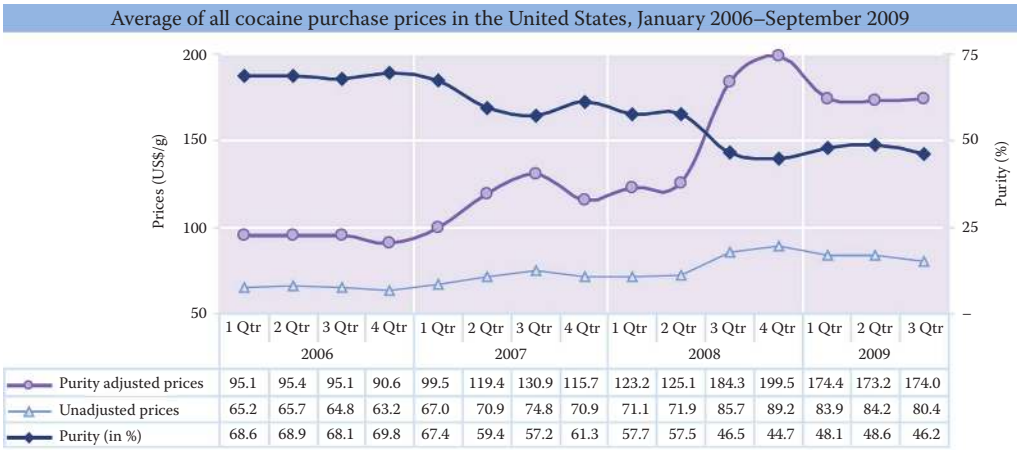


Figure 1.15 Price versus purity of cocaine sold in the United States, 2006–2009. (Reproduced from the United States World Drug Report, 2011; US Drug Enforcement Agency.)

1.5 Metabolism of Cocaine and Its Metabolites

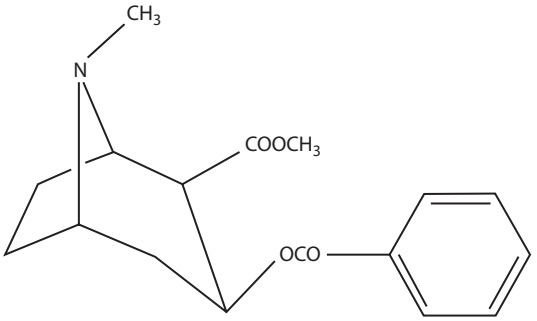
1.5.1 Cocaine (Table 1.4)

Cocaine is rapidly cleared from the bloodstream at an estimated rate of 2.0 L/min (Figure 1.17) (Inaba et al., 1978). In healthy human adults, cocaine has a half-life that has variously been reported as between 0.5 and 4.0 h, depending mainly on whether the individual is a chronic or naïve user (Chow et al., 1985; Moolchan et al., 2000). However, many other factors must be considered when attempting to evaluate postmortem measurements of any drug, including cocaine (Skopp, 2004). For example, the half-life of cocaine is 10–12 times longer in the newborn than in adults, on the order of 11–12 h (Dempsey et al., 1998).

The results of animal, as well as human clinical, and autopsy studies demonstrate that cocaine is stored in deep body compartments and that its rate of excretion changes as the drug accumulates (Weiss and Gawin, 1988; Cone and Weddington, 1989; Burke et al., 1990; Jufer et al., 1998; Levisky et al., 2000; Preston et al., 2002). Excretion rates are also altered by the amount of cocaine consumed: "crack" smokers consume multiple gram quantities of the drug, not just milligram doses (Gossop et al., 1994), and even though clearance rates are substantial, traces of cocaine remain in the body for weeks and in the hair for months (years in the dead). Ethical considerations prevent pharmacokinetic studies performed with realistic doses of cocaine.

In an analysis of 104 postmortem urine specimens that had already been tested and were known to be positive for cocaine metabolite (either BZE or EME, or both), cocaine was found to be present in 66% of the specimens, sometimes in very high concentrations (0.07–78 mg/L). In this study, when the BZE concentration of the urine sample was greater than 2.0 mg/L, cocaine was detected in 83% of the specimens, but the detection rate dropped to only 30% when BZE levels were under 2.0 mg/L (Ramcharitar et al., 1995). In a study of 99 cocaine-related deaths, the mean urine cocaine concentration in 48 individuals actually dying of cocaine toxicity was 1.1 ± 2.7 mg/L, but only 0.49 ± 0.75 mg/L in those where cocaine was an incidental finding (Karch et al., 1998). The difference was not statistically significant.

Table 1.4 Physiochemical Properties and Pharmacokinetics of Cocaine

Chemical Name	Methyl-3-benzoyloxy-8-methyl-8 azabicyclo[3.2.1]octane-4-carboxylate	
Physiochemical properties, structure, and form	Usually as hydrochloride salt or free base, soluble in water CAS 50–36–2 (base) CAS 53–21–4 (HCl) MW 303.353 (base) pKa 8.6	
Synonyms	The name "cocaine" is now universal. There are simply too many languages and local names to list but common names include crack, snow, and coke. Cocaine HCl is used as a local anesthetic and in ENT surgery as a 2%–10% solution. "Crack cocaine," as illustrated in Figure 1.16, is absorbed more rapidly through the lungs, but the pharmacology remains the same.	
Pharmacokinetics (cocaine)	Bioavailability	30% (oral, nasal)
	C_{max} (nasal)	220 ng/mL (106 mg) (Jeffcoat et al., 1989)
	C_{max} (smoked)	203 ng/mL (50 mg) (Jeffcoat et al., 1989)
	C_{max} (s.c.)	300 ng/mL after 75 mg (Kolbrich et al., 2006)
	T_{max}	Within minutes for nasal and smoking routes
	V_d	1–3 L/kg
Common blood concentrations in drug users	Quite variable depending on dose, frequency of use, and time since last administration but can easily rise to 1 mg/L for short periods. Unstable in blood and is rapidly hydrolyzed to benzoylecgonine post collection.	
Blood terminal elimination half-life	About 30 min to 2 h but is dose dependent (Jufer et al., 1998).	
Metabolism	Unstable in blood and solution, hydrolyzed to benzoylecgonine; also metabolized to ecgonine methyl ester and ecgonine; active cocaethylene is formed by transesterification when alcohol is also consumed; anhydroecgonine methyl ester is produced when smoked.	
Urinary excretion	Intravenous: cocaine 1%–9%, benzoylecgonine 35%–55% Intranasal: cocaine 4%, benzoylecgonine 16%–36% Smoked: benzoylecgonine ~16% Excretion pH dependent	
Postmortem artifacts	Blood concentrations can approximately double after death, even in peripheral blood. However, cocaine metabolites are unstable and converted after death, largely to ecgonine methyl ester (EME).	
Interactions	When alcohol is co-consumed with cocaine, it is converted into cocaethylene, which is also active and possibly more toxic. Ethanol decreases clearance of cocaine and increases bioavailability of oral cocaine (Parker and Laizure, 2010). Cocaine also increases clearance of buprenorphine and methadone (McCance-Katz et al., 2010).	
Published major papers and reviews	Wilkinson et al. (1980), Barnett et al. (1981), Cone (1995), Karch (2005), and Barroso et al. (2009)	

More than a dozen different cocaine breakdown products have been identified, but they are rarely measured, mainly because their presence seems to be of little clinical or forensic significance. In Blaho's series of 46 symptomatic "crack" smokers, the mean concentrations of cocaine and its principal metabolites, listed in decreasing order, were as follows: BZE (1.4 ± 0.19 mg/L) > ecgonine methyl ester (EME) (0.53 ± 0.05 mg/L) > ecgonine

Figure 1.16 "Crack" cocaine. Unlike cocaine hydrochloride, "crack" sublimates at a lower temperature, enabling it to be smoked. (From the website of the Drug Enforcement Agency.)

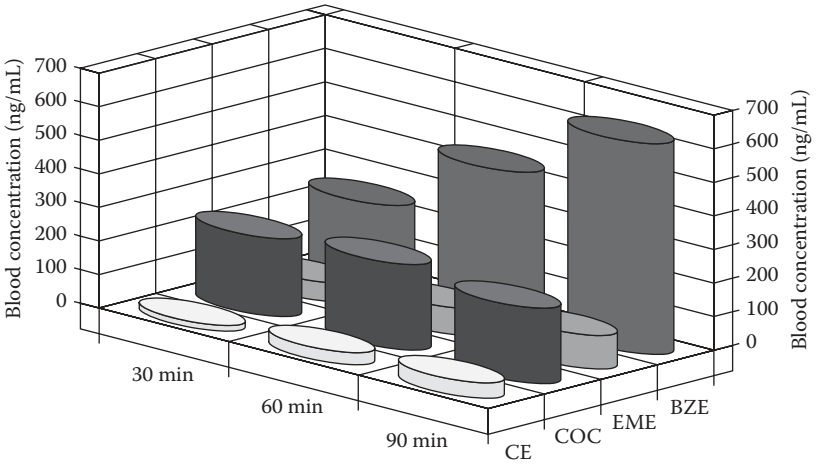


Figure 1.17 Blood concentrations of cocaine and its metabolites in humans. Average blood concentrations of cocaethylene (CE), cocaine (COC), ecgonine methyl ester (EME), and benzoylecgonine (BZE) in eight subjects given 2 mg/kg of intranasal cocaine and 5 mL/kg of 10% ethanol. (Data adapted from Pirwitz, M.J. et al., *Arch. Intern. Med.*, 155, 1186, 1995.)

(0.53 ± 0.07 mg/L) > cocaine (0.18 ± 0.06 mg/L) > norcocaine (0.04 ± 0.03 mg/L) > cocaethylene (0.02 ± 0.01 mg/L) (Blaho et al., 2000).

Most hospital laboratories use commercial immunoassay kits for urine screening. There are many products to choose from, but almost all of these screening kits are designed to detect only BZE. Other metabolites, even if present, go undetected or produce only minimally positive cross-reactions. Over the last decade, researchers have begun to introduce other diagnostic techniques (gas chromatography–mass spectrometry [GC/MS] or some mode of tandem mass spectrometry such as liquid chromatography–tandem mass spectrometry [LC–MS/MS]) that allow for the simultaneous measurement of cocaine and all of its metabolites, including even pyrolysis products (Klingmann et al., 2001; Lewis et al., 2004). More importantly, the price of these measurements continues to decline (Cognard et al., 2006). The same situation applies to heroin and its metabolites (Rook et al., 2005).

In some laboratories, LC–MS/MS has already replaced use of immunoassay screening. One implication of this development is that physicians trying to interpret laboratory results will require some knowledge of the role, if any, played by many different drug metabolites that can and will be identified. This will require a steep learning curve for the pathologists and considerable effort on the part of the toxicologists; it will also require a great deal of money. Funding these new technologies will prove to be a considerable challenge for even the most affluent of medical examiner's offices.

In the absence of alcohol, the principal breakdown products of cocaine are BZE (also abbreviated as BEG) and EME (Figure 1.18). Three distinct esterases (hCE-1, hCE-2, and hCE-3) are involved in the process. Cocaine is converted to BZE by hC-1 and EME by hC-2. BZE is the primary metabolite that appears in the urine. However, depending on storage conditions, cocaine will transform into BZE spontaneously. If ethanol and cocaine are both present, hC-1 will transesterify cocaine to form cocaethylene.

In cases of cholinesterase deficiency, more cocaine is shunted via the BZE route. There is very little evidence, however, that cholinesterase levels are associated with toxicity; neither does it appear that either BZE or EME exerts any measurable activity at all in humans. A negligible amount of cocaine undergoes oxidative metabolism to form norcocaine. There are two alternate pathways by which this may occur. One route involves only cytochrome CYP3A4 (LeDuc et al., 1993). The alternative pathway also requires the participation of a flavin-containing monooxygenase in a two-step process; cocaine *N*-oxide is formed as an intermediate and then demethylated to form norcocaine (Kloss et al., 1983). Unless the collection tube contains NaF as a preservative, any cocaine present in postmortem blood will be converted to BZE by esterases that continue to operate for some time after death. Similarly, unless it is collected with NaF preservative, anhydroecgonidine (a cocaine metabolite produced by "crack" smokers) also undergoes breakdown to cocaine (Fandino et al., 2002).

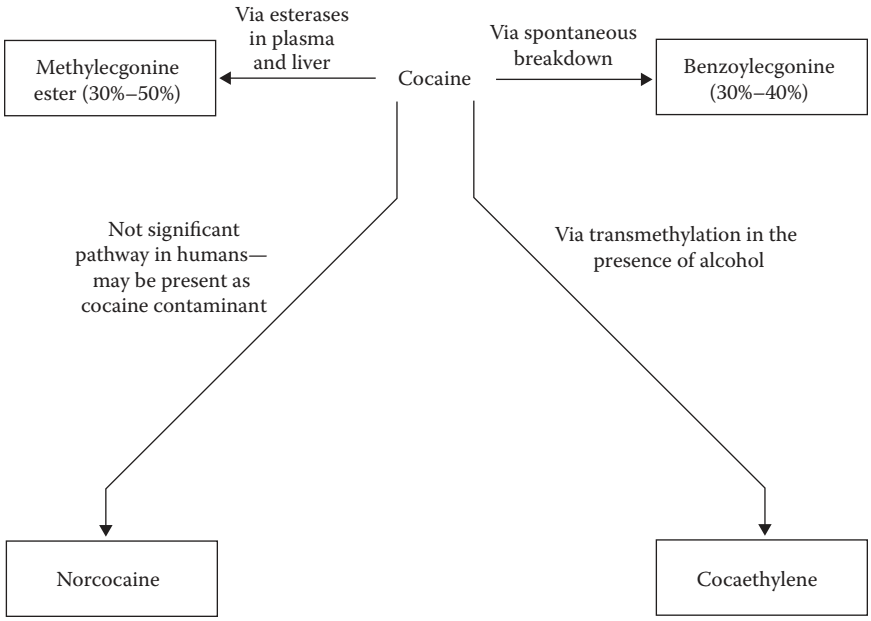


Figure 1.18 Metabolic fate of cocaine.

1.5.2 Benzoylecgonine and Ecgonine Methyl Ester

In the absence of alcohol, the principal metabolites of cocaine are BZE and EME (Figure 1.13). In the living, the relative proportion of BZE and EME detected appears to have very little to do with the route of administration. In a study of 111 emergency room patients, BZE concentrations were consistently the highest (generally by a factor of 10 of 11) of any metabolite detected (1.4 mg/L in "crack" smokers, 1.9 mg/L in insufflators, 1.9 mg/L in intravenous users, and 1.6 mg/L in subcutaneous injectors) (Blaho et al., 2000).

Each of the different cocaine metabolites has a slightly different excretion rate and a markedly different steady-state volume of distribution. In fact, formal pharmacokinetic measurements have been performed for only one cocaine metabolite—BZE. The V_{ss} for BZE is only one-third as high as that for cocaine (Ambre et al., 1991). Urinary detection times have been determined for all of the principal metabolites: BZE, EME, norcocaine (NCOC), benzoylnorecgonine (BNE), *m*-hydroxy-BZE (*m*-HO-BZE), *p*-hydroxy-BZE (*p*-HO-BZE), *m*-hydroxy-COC (*m*-HO-COC), and *p*-hydroxy-COC (*p*-HO-COC). Results of studies performed in closed metabolic wards indicate that the excretion pattern is, at least in part, related to the pattern of ingestion. No matter which route was used, C_{max} values were BZE > EME > COC > BNE and *p*-HO-BZE > *m*-HO-BZE > *m*-HO-COC > NCOC > *p*-HO-COC.

Elimination half-lives for cocaine and its metabolites are generally shorter following smoking, slightly longer after intravenous injection, and longest following "snorting." The metabolite with the longest half-life was *m*-HO-BZE (mean range 7–8.9 h). Cocaine itself displayed the shortest half-life (2.4–4 h). However, some of the difference may be more apparent than real; the result depends upon the cutoff level chosen or the sensitivity of the assay (Cone et al., 2003).

The half-lives of BZE and EME are both much longer than the half-life of cocaine (Brzezinski et al., 1994). EME has a half-life on the order of 4 h, while BZE may be detected in the blood for 4–6 h and in the urine for 2–3 days after snorting 100 mg, or 1.5 days after injecting a single 20 mg dose. In neonates, the half-life of BZE appears to be 1.5–2.1 times longer than in adults (Dempsey et al., 1998). As a consequence, BZE is likely to be detectable in plasma for at least 24 h after ingestion (Javaid et al., 1983). In chronic users (>8 g/day), a 20 mg dose of BZE given intravenously is likely to be detected for an even longer period. BZE can be detected in the blood of chronic users for at least 5 days (Verstraete, 2004). Measurement of the ratio of cocaine, the parent compound, to metabolite is not meaningful, because the volume of distribution of the parent compound is so much greater than that of the metabolites. Neither is it legitimate to combine the concentrations of the different metabolites to estimate the amount of cocaine ingested nor to calculate a "body burden," and for the very same reason, the V_d of each metabolite is very different. This means that a different percentage of each compound will reside in the blood/plasma at any given time.

Another possible source for detecting BZE in a forensic urine sample is "spiking" (the purposeful contamination of a specimen in order to cause a false-positive test). Claims of spiking are sometimes made by athletes who test positive after a competition. Analyzing the specimen for other cocaine metabolites, particularly those that only occur in vivo, and detecting *m*-hydroxybenzoylecgonine, *p*-hydroxybenzoylecgonine (*p*-OH-BZE), and *N*-desmethylbenzoylecgonine prove that the individual had, in fact,

been using cocaine. It also proves that BZE had not been maliciously added to the sample (Klette et al., 2000). Another variation on this theme, also used by some athletes, is to claim that cocaine was surreptitiously added to their food. In such an event, the presence of normal in vivo metabolites would be expected, but if they are present in high concentrations, it stretches credulity to suppose that the victim would not have noticed something wrong at the time the food was eaten. And, in any case, could by ruled out by hair testing.

Some authors have suggested that low plasma cholinesterase (PCE) levels explain cocaine toxicity (Jatlow et al., 1979), but there is very little evidence to support this idea. Normal PCE levels vary tremendously from individual to individual and these levels change depending on the physiologic state. In one study purporting to show that "complications" were more common in individuals with low PCE levels, some of the individuals with "complications" actually had higher PCE levels than controls (Om et al., 1993). Clearly, individuals with genetic defects and atypical forms of cholinesterase, as indicated by low dibucaine numbers (a measure of PCE activity), will metabolize cocaine more slowly than individuals without that defect, but that difference does not prove toxicity is any more likely.

There is no evidence that a reduction in cholinesterase activity increases the chances for toxicity, although the converse is not true. A substantial body of experimental animal evidence suggests that treatment with an exogenous esterase can at least ameliorate, if not eliminate symptoms of cocaine intoxication (Collins et al., 2012).

One reason that exogenous esterase in humans may prove to be ineffective is that almost all the complications of chronic cocaine abuse occur after continuous, long-term use (Smart and Anglin, 1987; Karch et al., 1998). When an individual who has been using cocaine dies, postmortem blood testing will disclose total overlap between blood cocaine concentrations in fatal cases of cocaine toxicity, and concentrations of cocaine and its metabolites are identical (Karch et al., 1998). Cholinesterase activity is not even necessary for EME production. Small amounts of EME continue to form in hepatectomized animals, and BZE concentrations continue to rise after hepatectomy, even when PCE inhibitors are given (Kambam et al., 1993).

Finally, it should be observed that, while the blood ratio of cocaine to BZE or any of its metabolites is meaningless, the same measurements made on brain homogenates might, in fact, be diagnostic. Almost 20 years ago, Spiehler showed that if brain, not blood, is the testing matrix, the ratios of cocaine/BZE in the toxic cases (brain mean 14.7 and blood mean 0.64) were clearly different from those found in cases where cocaine was obviously an incidental finding (brain mean 0.87 and blood mean 0.27). The brain–blood ratios of cocaine and BZE concentrations generally are characteristic of the time elapsed since cocaine dosing. In instances of cocaine overdose, the mean brain–blood ratio was 9.6 for cocaine and 0.36 for BZE (Spiehler and Reed, 1985).

The difference is explained by BZE's inability to cross the blood–brain barrier (BBB). Any BZE found in the brain would have to be derived from cocaine that had previously crossed the BBB. It follows that brain ratios of cocaine to BZE are meaningful and do reveal important information about toxicity and time of ingestion. Spiehler's work was repeated in a much larger study with the same results (Bertol et al., 2008), except that the blood–brain ratios in cases where cocaine was actually the cause of death were substantially greater than those reported by Spiehler. A very strong case could be made for the use of brain as a primary analyte.

1.5.3 Cocaethylene

The DAWN report for 2003 states that, of all drug-related emergency room visits, nearly one quarter were related to the consumption of alcohol, or alcohol in combination with other drugs. The most frequently listed "other" drug was cocaine (25,049 visits), which was detected nearly five times more often than heroin (5,160 visits) (Substance Abuse and Mental Health Services Administration, 2004). By 2010, the total number of alcohol mentions had risen to 685,574, all but 13,000 of which occurred in conjunction with the use of other drugs (Substance Abuse and Mental Health Services Administration, 2010). The observation is important because when ethanol is consumed with cocaine, it leads to the creation of a unique metabolite called cocaethylene (Hearn et al., 1991a).

Cocaethylene is synthesized in the liver by a transesterification reaction that replaces the methyl group of cocaine with an ethyl group (Figure 1.19). The reaction occurs in the microsomal fraction and is catalyzed by a nonspecific carboxylesterase that not only catalyzes the transesterification of cocaine to cocaethylene but also converts cocaine to BZE (Brzezinski et al., 1994). The enzyme that performs the conversion is a broad-spectrum bioscavenger. It also catalyzes the deacetylation of heroin to 6-acetylmorphine and even the detoxification of organophosphate chemical weapons, such as sarin, soman, and tabun (Bencharit et al., 2003). Cocaethylene is detectable for much longer periods than cocaine, in both urine and blood, because it does not bind as strongly to the carboxylesterase (Brzezinski et al., 1997).

When cocaethylene was first discovered, many thought it might be the key to explaining why cocaine toxicity occurs in some, but not all abusers. That is not the case. There is generally no evidence that the combination of alcohol and cocaine does more than enhance additively the already strong tendency of each drug to induce a variety of physical

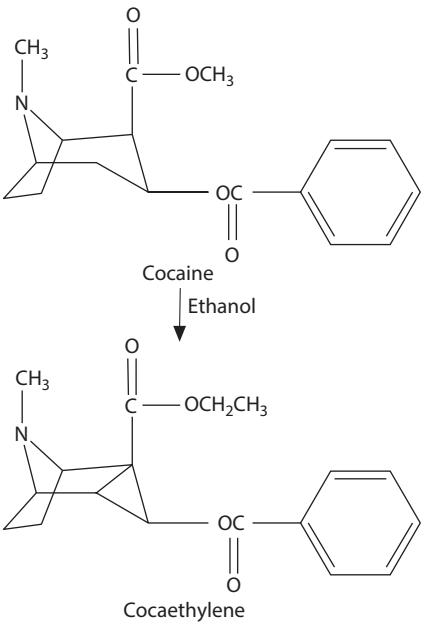


Figure 1.19 Cocaethylene formation. Cocaethylene is formed in the liver by a transesterification reaction, which adds an extra methyl group to cocaine. Cocaethylene has a much longer half-life than cocaine, but cocaethylene binds to the dopamine receptor with the same affinity as cocaine.

and psychological disorders, though there is some evidence that the combination of alcohol and cocaine tends to have greater-than-additive effects on heart rate (Pennings et al., 2002).

In controlled studies, cocaethylene is less potent in elevating heart rate than equivalent doses of cocaine. In the same fashion, cocaethylene was found to produce less notable psychological changes than cocaine. No matter the drug dose, there were significant increases in systolic blood pressure relative to placebo, but no significant effect on diastolic blood pressure. Cocaethylene has a slower rate of clearance and a larger volume of distribution than cocaine and, therefore, has a correspondingly longer elimination half-life.

Drug concentrations and autopsy findings were compared in a sample of 72 accidental deaths where only cocaine, cocaine metabolites, and no ethanol was detected in two-thirds of the cocaine-related deaths. When ethanol was present, no other differences between the two groups could be identified. This suggests that acute cocaine toxicity is not enhanced by ethanol–cocaine interactions. However, ethanol concentrations were generally low in this study, and it is possible that increased toxicity only becomes apparent when much larger quantities of alcohol have been consumed (Hearn et al., 1991a).

In most postmortem studies, concentrations of cocaethylene are very modest, probably because the amount of alcohol present is the rate-limiting step in cocaethylene production. Most cocaine users simply do not ingest enough alcohol to produce significant amounts of cocaethylene. In the case of the developing fetus, it appears that, even when cocaethylene is produced in significant quantities, the placenta prevents its passage from mother to child (Morishima et al., 1999).

In Hearn's original autopsy study, cocaethylene was found in only 62% (77/124) of decedents testing positive for both cocaine and ethanol. Further analysis showed that, in the 47 cases where cocaethylene could not be detected, ethanol concentrations were usually less than 0.1 mg/L (Hearn et al., 1991a). Using a somewhat different but very sensitive technique, Jenkins and Goldberger (1997) found cocaethylene in the blood of only one of 13 decedents who tested positive for cocaine (range 23–2088 ng/mL) and that the individual with the highest cocaine level had died of a gunshot wound, not cocaine toxicity. In another study of 41 patients with detectable cocaethylene concentrations, there was no significant correlation between plasma ethanol and plasma cocaethylene concentrations. The lack of correlation was thought to be a consequence of the very high ethanol concentrations in most of the patients, which, contrary to the usual situation, made the cocaine concentration the rate-limiting step in cocaethylene formation (Bailey, 1996).

The relationship between cocaine and alcohol has been evaluated in healthy volunteers (Farre et al., 1993; McCance-Katz et al., 1993). In the first study, individuals with a history of recreational drug use were given cocaine alone or a drink containing 1 g/kg of vodka followed by a 100 mg dose of cocaine hydrochloride (snorted). Cocaethylene was detected only in the samples from the group that had been pretreated with alcohol. The peak cocaethylene concentration was 55 ± 8 ng/mL, and serial blood measurements were consistent with a half-life of 109 min. Norcocaine levels were also much higher in the group that had been pretreated with alcohol.

The pharmacologic properties of cocaethylene and cocaine, though largely similar, do differ in some important aspects. In vitro studies suggest that cocaethylene is a more potent blocker of the inward sodium channel than the parent compound (Xu et al., 1994), but a less potent blocker of the hERG potassium channel. If that is the case in humans, then high levels of cocaethylene would be more likely to produce cardiac conduction abnormalities than would high levels of cocaine. In fact, Brugada syndrome, a conduction abnormality

due to the presence of an abnormal sodium conductance channel in cardiomyocytes, has been reported after cocaine use, but never in a situation where cocaethylene was also present (Bebarta and Summers, 2007). This result is hardly surprising given that the cocaine concentrations normally encountered in regular cocaine users would be expected to interact with hERG and the sodium conductance channel and would very likely be proarrhythmic anyway, particularly if the abuser were heterozygous for hERG (Ferreira et al., 2001; Guo et al., 2006).

1.5.4 Anhydroecgonine Methyl Ester (Methylecgonidine)

Anhydroecgonine methyl ester (AEME; Figure 1.20) is the major pyrolysis product of cocaine. It is excreted only in the urine of "crack" smokers (Jacob et al., 1990), and it can also be detected in their blood, although plasma concentrations were generally quite low (3–34 ng/mL when measured in 13 admitted "crack" smokers) (Toennes et al., 1999). The pharmacology and pharmacokinetics of this compound have still not received very much attention.

Structurally, AEME shares features with other chemicals such as anatoxin and arecoline that have cholinergic properties, raising the possibility that anhydroecgonine may be toxic in its own right. Studies in animals confirm that AEME inhalation decreases airway conductance (Chen et al., 1995). AEME may, or may not, have something to do with the reported increases in incidence and severity of asthma within the inner city (Tartasky, 1999; Rome et al., 2000; Tashkin, 2001).

The detection of AEME is of questionable forensic value, at least for proving that "crack" was smoked, because the testing procedure cannot be relied upon, even in cases where AEME is known to be present. Pyrolytic production with pentaflouro-AECG (the derivatizing agent often used for BZE, ecgonine, and *m*-hydroxybenzoylecgonine) can completely mask any AEME originally present in a biological sample (Toennes et al., 2003; Cardona et al., 2006). AEME can also be formed as an analytic product if injection port temperatures are too high, but there are effective means of measurement that will survive forensic challenge (Cognard et al., 2006; Yang et al., 2006).

1.5.5 Norcocaine (Figure 1.21)

Oxidation of cocaine plays a minor role in human metabolism of cocaine, but there is only a modicum of evidence that oxidized cocaine, in the form of norcocaine and norcocaine nitroxide, may play an important role in human cocaine toxicity. Norcocaine is

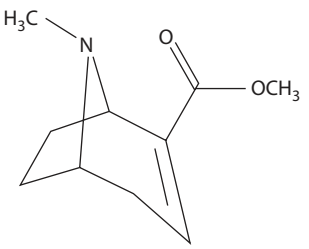


Figure 1.20 Anhydroecgonine.

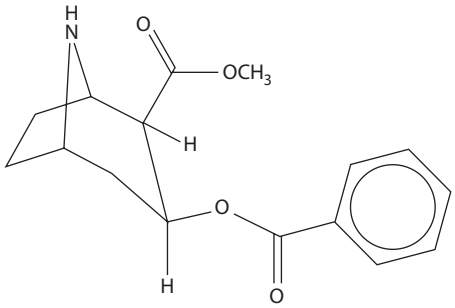


Figure 1.21 Norcocaine.

metabolized by *N*-hydroxylation, a conversion catalyzed by several different members of the CYP family (1A, 2A, 3A, and possibly 3B) (Pellinen et al., 2000). Other minor pathways are known to be involved in the hydrolysis of hydroxybenzoylecgonine, but they have not been identified and their significance remains obscure. Animal brains and liver microsomes can reduce norcocaine nitroxide to form superoxide radicals, in turn leading to lipid peroxidation and lipid peroxyl radical formation. The result compromises the antioxidant systems that are designed to protect the heart (Kovacic, 2005).

Whether this mechanism helps to explain the fibrosis normally observed in the hearts of chronic cocaine users, or even some of the hepatic damage that is also seen, remains unknown. Myocardial fibrosis is a multifactorial process and the most likely explanation is that the presence of norcocaine is just one more contributory factor to cardiac myotoxicity and repair. It also appears that cocaine itself, even in the absence of high levels of norepinephrine (NE), can induce myocardial apoptosis (Zhang et al., 1999), and apoptosis appears to play a key role in cocaine-induced myocardial remodeling. It had been thought that humans produced only minute amounts of norcocaine, but Blaho et al. (2000) detected significant amounts in the plasma of symptomatic cocaine abusers, thereby raising the possibility that this route might actually be responsible for human toxicity. In their study of 111 cocaine users presenting for emergency room treatment, the mean norcocaine concentration was 30 ± 17 ng/mL.

1.6 Fetal Metabolism

Like any other abused drug, cocaine may interfere with the placental transfer of endogenous compounds. In vitro studies have shown that cocaine, nicotine, and cannabinoids all can inhibit amino acid transport in the placenta. Specifically, cocaine decreases the activity of the placental amino acid transport systems A, N, and possibly others as well (Myllynen et al., 2005). Any interference with placental transporters may potentially have adverse effects on fetal development and placental handling of endogenous compounds by interfering with the placental blood flow through vasoconstriction (Woods, 1998; Lipton et al., 2002). Interestingly, this ability is not confined to cocaine. Nicotine and alcohol also can induce placental vasoconstriction. In fact, nicotine and alcohol exert very similar effects on the placenta (Ganapathy, 2011).

1.7 Routes of Ingestion

1.7.1 Overview

Ethical considerations prevent the administration of cocaine in quantities approaching those consumed by real addicts during a binge. As a consequence, cocaine pharmacokinetics in real cocaine abusers remains largely unstudied and poorly understood, although an abundance of clinical trials using limited amounts of the drug exist.

In 2005 and 2006, large, population-based studies of heroin addicts taking realistic doses were finally performed, mainly because it is possible to give a narcotic antagonist and still safely measure opiate pharmacokinetics. Studying stimulants of any sort in abusers is much more difficult because their cardiovascular effects are pronounced and not readily reversed.

However, a handful of important pharmacokinetic studies have been published. In one of these, an uncontrolled clinical study, plasma cocaine concentrations in 111 symptomatic cocaine users were studied and an attempt made at correlation of plasma concentrations with clinical symptoms (Blaho et al., 2000). The study was unsuccessful to the extent that no correlation between dose and effect in real cocaine abusers was demonstrated. Failing to find a difference is, presumably, a result of cocaine tolerance, a process that begins to emerge after the first dose (Howell and Ezell, 1990; Mendelson et al., 1998). Using human volunteers, Van Dyke et al. (1982) showed many years ago that tachyphylaxis occurs after even single dose of cocaine.

In a different controlled study of established cocaine addicts, also treated on a locked metabolic ward, Moolchan found very much the same thing as Van Dyke et al., where doses were estimated in a group of addicts admitted to a closed ward (Moolchan et al., 2000). Studies using realistic doses of the principal metabolite, BZE (Ambre et al., 1991), have also been performed. The results of these studies suggest that (1) chronic users may consume multigram quantities of cocaine with relative impunity; (2) a variety of cocaine metabolites previously thought to be insignificant (EME, norcocaine) are, in fact, formed in fairly substantial quantities; and finally, (3) the half-life of cocaine may be much variable than had previously been thought. All of these variables are partly a function of the route of administration (Figure 1.22).

1.7.2 Coca Leaf Chewing

Coca has been chewed for over 3000 years. Carbon-14 (^{14}C) dating from mummies of the Alto Ramirez culture confirms coca leaf chewing by the people who lived in the valleys and coastal areas of Northern Chile. Of 11 bodies recovered from the burial site of Pisagua, 7 were analyzed and 2 of the samples tested positive for cocaine. One mummy, estimated to be slightly more than 3000 years old, was found to have a cocaine value of 13.3 ng/10 mg of hair. The concentration in a second mummy was 5.6 ng/10 mg (Rivera et al., 2005). The pharmacokinetics of leaf chewing has only recently been characterized. Habitual users chew an average of 12–15 g of leaf three or four times a day. Depending on the quality of the leaf, the alkaloid content is usually less than 0.5%. Thus, the total amount of coca consumed at any one time is unlikely to amount to more than 75 mg. In one experiment, novice chewers who spit out their saliva had average peak plasma concentrations of 38 ng/mL at 1 h. Experienced users, who swallow their saliva, had mean values

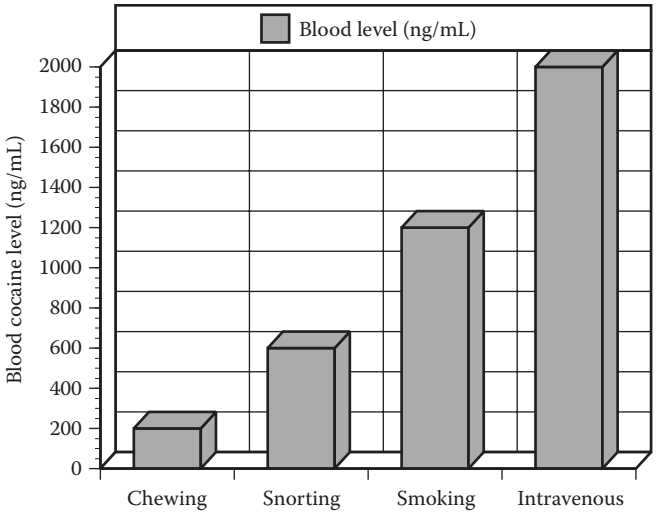


Figure 1.22 Blood levels and routes of administration. The route of ingestion determines cocaine blood levels. "Crack" smokers may have blood levels three or four times as high as leaf-chewing Indians. Levels after snorting are intermediate.

of 249 ng/mL; however, the range was extremely variable—from 130 to 859 ng/mL (Paly, 1979). These concentrations are toward the lower end of the spectrum of levels attained when the drug is snorted.

1.7.3 Snorting (Insufflation)

When cocaine is snorted, peak plasma concentrations are proportional to the amount of cocaine ingested (Wilkinson et al., 1980). Absorption is rapid and bioavailability is good; the half of the absorbed cocaine is less than 2 min (Jeffcoat et al., 1989). However, because cocaine is also a vasoconstrictor, it inhibits its own absorption, and the time required to reach peak concentration becomes longer as the dose increases. One hundred milligrams, approximately the equivalent of two to three "lines," will produce a plasma concentration of 50–100 ng/mL, sufficient to cause transient increases in heart rate and blood pressure (Javaid et al., 1983; Foltin et al., 1988).

In a 1976 study, intranasal application of 1.5 mg/kg (equivalent to 90 mg in a 60 kg man) produced peak plasma concentrations of 120–474 ng/mL within 30–60 min of administration (Van Dyke et al., 1976). When somewhat larger doses were given (2 mg/kg), peak plasma concentrations ranged from 131 up to 1012 ng/mL, with an average of 370 ng/mL observed at 30 min, falling to 295 ng/mL at 60 min, and 223 ng/mL at 90 min (Brogan et al., 1992).

Laboratory studies have shown that nasal insufflation is at least as efficient a method for administering cocaine as intravenous injection or smoking. There is evidence that behavioral and vascular tolerance begins to emerge almost as soon as the second dose is taken. In a 1994 study (Foltin and Fischman, 1994), gave two doses of intranasal cocaine, separated by 40 min, to four volunteers (0.06, 0.34, 0.69, and 1.37 mg/kg) and dose–response curves were measured. Intranasal cocaine produced dose-related increases in ratings of "positive" drug effects, heart rate, and blood pressure. Plasma cocaine levels peaked following the second cocaine insufflation of each session, while metabolite levels continued to increase.

Although the plasma cocaine level approximately doubled following the second cocaine administration, the ratings of positive drug effects, heart rate, and blood pressure did not increase after the second cocaine administration. Clearly, acute within-session tolerance develops during repeated administration of intranasal cocaine (Foltin and Haney, 2004).

In practice, the amount of cocaine taken by users is considerably greater than the test doses given in the laboratory. Blaho et al. (2000) reported that plasma concentrations in four symptomatic cocaine "insufflators" were 0.21 ± 0.20 mg/L. That value is not significantly different than a mean cocaine concentration of 0.18 ± 0.06 mg/L measured in 46 symptomatic "crack" smokers who presented at hospital emergency departments for treatment (Blaho et al., 2000). The plasma concentrations observed in the symptomatic patients are surprisingly close to results observed in volunteers taking cocaine in a controlled setting. Perhaps the most striking aspect of the Blaho study was the enormous amount of drug being ingested. Given that cocaine has a half-life of roughly 1 h and the fact that many of those included in the study had had symptoms for several hours, it is obvious that at one point, some of the patients would have had plasma concentrations of more than 5 mg/L!

Concerns have been raised that "snorting" may help facilitate transmission of hepatitis C (HCV), particularly among gay men who are already HIV infected. This makes sense because anal sexual practices lead to rectal bleeding; snorting drugs in the setting of increased HCV prevalence clearly is a risk factor for acute hepatitis C (Schmidt et al., 2011). Indeed, some evidence suggests that even the straws used to "snort" cocaine, together with risk-taking behaviors, may be responsible (Caiaffa et al., 2011).

1.7.4 Surgical Application

Cocaine is still used by otorhinolaryngologists as well as cosmetic and plastic surgeons, but this practice continues to decline. The explanation has partly to do with physicians having found alternative anesthetics that are equally effective and partly because of the decreasing number of physicians with experience administering cocaine. Nonetheless, the combination of cocaine and epinephrine is still used, especially in the management of children with lacerations (Kennedy et al., 2004), and complications with this approach seem quite rare.

If cocaine-soaked pledgets are used to control epistaxis, considerable cocaine is absorbed and plasma concentrations may exceed 600 ng/mL within the first half hour (Liao et al., 1999). High plasma levels may explain why myocardial infarction has occasionally been reported in conjunction with the practice (Laffey et al., 1999; Makaryus et al., 2006).

At the turn of the twentieth century, a mixture of epinephrine and cocaine was routinely used for anesthesia (Mayer, 1924). The practice has now largely been abandoned, but cocaine is still occasionally mixed with an epinephrine solution to produce something known as cocaine paste (although today the term "cocaine paste" more commonly refers to an intermediate product of the cocaine alkaloid extraction process that results in a high content of chemical impurities). Occasionally, bicarbonate is added to the mixture in the hopes of retarding absorption, but even then the resultant plasma levels can be very high. Concentrations of over 2000 ng/mL have been observed (Lips et al., 1987; Bromley and Hayward, 1988).

Potential for the occurrence of an untoward event after the use of "mud/paste" is even higher than after the insertion of cocaine-saturated pledgets, and the threat of myocardial

infarction is very real (Meyers, 1980; Chiu et al., 1986; Littlewood and Tabb, 1987; Ashchi et al., 1995; Noorily and Noorily, 1996; Laffey et al., 1999; Bell et al., 2001; Osula et al., 2003). It is not clear whether the addition of epinephrine to cocaine effectively improves hemostasis. In controlled studies where epinephrine was added to lidocaine used during septoplasty, blood loss was no different than in the group in which lidocaine alone was given (Thevasagayam et al., 2007).

The surgical application of even small amounts of cocaine will cause patients to test positive for cocaine for up to 3 days. When patients undergoing lacrimal duct surgery were anesthetized with less than 3 mL of topical 4% cocaine hydrochloride, urine specimens obtained 24 h later were almost all found to have cocaine concentrations that exceeded 300 ng/mL, used by the National Institute on Drug Abuse (NIDA) as a cutoff point. Urine from some of patients still exceeded the NIDA cutoff at 48 h and, in a few instances, cocaine is still detectable 72 h later, though at levels less than 300 ng/mL (Cruz et al., 1991). In a second study of the same problem, patients undergoing septoplasty received either 160 or 400 mg of cocaine applied topically. Ninety percent of the drug was absorbed within the first 15 min. Plasma from the group receiving 400 mg, drawn 20 min after application, had a mean concentration of 0.608 ± 0.09 mg/L (Liao et al., 1999). Given the high concentrations, it is possible that surgeons may accidentally contaminate themselves and test positive for cocaine.

At least one study has addressed the possibility of accidental exposure, an issue that could be of great concern to medical staff. The study considered several possible exposure scenarios (Bruns et al., 1994). The study involved 22 patients about to undergo routine nasal surgery as well as the surgeons who were to operate on them. The surgeons participating in the study used their fingers to mix 4 mL of 4% cocaine into cotton pledgets that were then inserted into the patients' nostrils. The surgeons wore masks at all times.

In six cases, the surgeons wore gloves, and in six cases, they did not. In order to test for cumulative effects, a separate experiment was performed: a single physician handled cocaine on two consecutive days. On the first day, pledgets were prepared once every 2 h for 6 h, and on the second day, once every hour for 6 h. The cotton pledget was handled for 2 min, and the surgeon then washed his hands 15 min later.

When the surgeons wore gloves, no cocaine metabolite was detected in their urine. When the surgeons did not wear gloves, BZE appeared in their urine, although at levels well below NIDA or Department of Defense cutoffs, either for screen assays or GC/MS confirmation. The mean BZE concentration was 30.1 ng/mL at 8 h and 18.8 ng/mL at 24 h. The highest BZE level recorded was 53 ng/mL. However, much higher levels were observed when one surgeon handled the cocaine-soaked cotton for the 2-day study, and a cumulative effect was definitely observed. Twelve hours after the first exposure (once every 2 h for 6 h), the urinary BZE concentration was approximately 90 ng/mL. Eighteen hours after the second exposure, urine concentrations measured by GC/MS had risen to 245 ng/mL, just below the initial urine-screening cutoff but well above the GC/MS confirmation cutoff of 150 ng/mL.

1.7.5 Intravenous Use

Intravenous cocaine produces much higher plasma cocaine concentrations than does coca leaf chewing. This difference explains why intravenous users are more likely to experience complications from cocaine abuse. A 40 mg intravenous bolus of cocaine, when given

to a human volunteer, produced plasma concentrations of between 204 and 523 ng/mL at 10 min (Kumor et al., 1988). A 32 mg dose given to volunteers produced peak levels of approximately 250 ng/mL, with a maximum increase in heart rate at 7.3 min (Chow et al., 1985). Barnett et al. (1981) observed concentrations of 700–1000 ng/mL only 5 min after the injection of 100 mg. The levels exceeded 2500 ng/mL after an injection of 200 mg (Barnett et al., 1981). Blaho et al. (2000) reported that typical symptomatic emergency room patients, who had came to the emergency room after injecting unknown amounts of cocaine, had relatively low plasma cocaine concentrations of 170 ± 0.24 ng/mL, but much higher concentrations of BZE, with a median plasma concentration very near 2000 ng/mL (Blaho et al., 2000).

Intravenous cocaine produces a more intense and immediate response than ingestion by any other route except for "crack" smoking, but it does require multiple frequent injections to maintain a "high." Narcotic users, by comparison, inject infrequently. The increased number of injections places the cocaine user at greater risk for infection with human immunodeficiency virus, as well as all of the other infectious complications of intravenous drug use. In the past, investigation of fatal cases of "speedballing" (combining cocaine and heroin) often disclosed that the heroin had been injected and the cocaine smoked, though the current trend seems to favor the injection of both drugs at the same time (Lankenau and Clatts, 2005; Buchanan et al., 2006).

1.7.6 Genital Application

Cocaine is promptly and completely absorbed through all mucous membranes and high blood levels are quickly achieved. In addition, cocaine acts as a local anesthetic, which may make certain sexual practices more pleasurable. However, the main mechanism of genital application today is smuggling. Drug couriers conceal packets of drugs in their vagina or rectum (though swallowing still remains the preferred route) (Mebane and De Vito, 1975; Gallup et al., 1991; Benjamin et al., 1994; Klein et al., 2000).

Smuggled drugs are often wrapped in semipermeable materials so that often, but not always, drug and/or drug metabolite may appear in the smuggler's urine. A 2008 study analyzed urine samples from 64 suspected body packers for cocaine and opiates, positive point of care results were confirmed with GC/MS testing, and each suspect was also x-rayed. In 48 of 64 cases (24 positives and 24 negatives), screening results were confirmed both by GC/MS assay and abdominal x-ray films. Even though measured urinary drug concentrations were always below 50 ng/mL, it does appear that point of care testing can be used to identify this type of drug smuggler (Marchei et al., 2008).

A 2011 paper described a body packer who developed symptoms of cocaine toxicity requiring laparotomy, even though none of the packets had ruptured. The plasma cocaine concentration after one hour of symptoms was 594 ng/mL, while a spot urine test performed at the same time showed urine concentrations of cocaine, BZE, and EME of 12,858, 80,142, and 137,433 ng/mL, respectively (Hantson et al., 2011). Concentrations of such magnitude would, of course, be readily detectable by virtually all point of care devices. However, concentrations this great cannot be explained by diffusion through permeable packing membrane. It seems far more likely that one partially torn packet passed unnoticed.

Fatalities have been reported after direct vaginal or rectal application during sexual activities (Doss and Gowitt, 1988; Greenland et al., 1989). Except for a pregnant

woman who died of air embolism after her partner blew "crack" smoke into her vagina (Rosse et al., 1994), the clinical histories in these patients suggest that death was due to arrhythmia but, interestingly, reported plasma concentrations in these deaths have not been high.

Toxicity from genital application is not limited to females. Priapism has been reported in men after application of cocaine to the glans (Mahler et al., 1988; Fiorelli et al., 1990; Rodriguez-Blaquez et al., 1990; Munarriz et al., 2003) and occasionally leads to serious surgical complications (Altman et al., 1999). Biopsy of the corpora in these individuals demonstrates fibrosis (Minardi et al., 2004). Drugs and alcohol, not just cocaine, are commonly introduced into the rectum to promote sphincter relaxation and to ease the discomfort of anal dilatation. Plasma cocaine concentrations after rectal application have never been measured. Nor should it be automatically assumed that priapism is drug related. Some medical disorders, such as sickle cell disease, and some drugs, such as risperidone and other antipsychotics, are well known for their ability to induce priapism (Sharma and Fleisher, 2009; Penaskovic et al., 2010).

1.7.7 Dermal Absorption

Cocaine adheres to the skin and can be absorbed through it. Controlled experiments have shown that cocaine can remain on the skin for at least 3 days after external exposure (Kidwell et al., 1997) and even that it is not easily removed. The most reasonable explanation is that positively charged drugs will bond ionically to skin proteins just as they bond to protein in hair (Levisky et al., 2000; Kidwell and Smith, 2001). That would explain why cocaine can be recovered from the skin of individuals who have handled "crack" cocaine, even after thorough hand washing or swabbing the skin with 70% isopropyl alcohol. It is quite unlikely that anyone could absorb enough cocaine via this route to cause a NIDA positive urine test (although that can occur when an individual has handled bulk cocaine) (Maloney et al., 1994).

In one very small study, where 5 mg of cocaine hydrochloride and 5 mg of cocaine free base, dissolved in alcohol, were painted on the forearm skin of a volunteer, the maximal urinary BZE concentration from the free base was 55 ng/mL at 48 h. Much less of the cocaine hydrochloride was absorbed, with a peak urine level of only 15 ng/mL at 48 h (Baselt et al., 1990). A similar study, done with cocaine paste, yielded much the same result (ElSohly, 1991). These levels are so low that medical personnel need not be overly concerned about false-positive test results. Even if drugs are detectable, plasma concentration secondary to environmental exposure will be significantly lower than those observed in abusers, and the two conditions should not be confused.

Environmental skin contamination is not the only explanation for the presence of cocaine in or on the skin. Cocaine has a relatively large volume of distribution (V_{ss}). Various reports suggest values of 2.5–3.0 L/kg, which means that cocaine will distribute throughout the tissues in the body, including the skin. Studies of tissue obtained at autopsy have shown high concentrations of abused drugs in abdominal skin and subcutaneous fat, even of drugs that are generally not thought of as being highly lipophilic. The rate at which such drugs move from subcutaneous tissue to the surface, if they do at all, is simply not known (Levisky et al., 2000).

Skin patch testing is still used in some settings to monitor individual drug use, and absorption through the skin is also of theoretical concern for convicted drug takers being

monitored with sweat-collection patches. It has been suggested that exposure to low levels of drug in the environment could lead to skin absorption, entry of very small amounts of drug into the bloodstream, subsequent secretion of the drug into the sweat, and eventual absorption of drug-containing sweat by the patch. The results of several studies suggest that this scenario, while logically possible, simply does not occur. However, the possibility of absorption by the patch has never been entirely ruled out (Kidwell et al., 2003). The body has a great deal of skin, and there is no reason why it should not act as a drug repository. Even though the amounts of cocaine released into the bloodstream at any one time may remain below limits of detection, cocaine could, nonetheless, still be absorbed by a sweat detection patch (Yang et al., 2006).

Cocaine solutions are occasionally used to anesthetize lacerations either with, or without, further injection of a different local anesthetic. Several different mixtures containing cocaine have been designed specifically for this purpose, the best known being TAC (tetracaine, adrenaline, and cocaine). These solutions have been used quite safely with no adverse events reported. Resultant plasma cocaine concentrations are rarely monitored, but the few times they have been measured, plasma concentrations have either been negative or in the 1–2 ng/mL range (Vinci et al., 1999; Tadicherla and Berman, 2006).

1.7.8 Inhalation

The first medical reports of "crack" smoking came from the Bahamas in 1983 (Jekel et al., 1986), followed by the first mentions of "crack" smoking in New York City in 1985 (Gross, 1985). The origins of "crack" smoking are not entirely clear. It has been alleged by conspiracy theorists that introduction of the drug was either (1) a genocidal plot to destroy the black community or (2) a cynical approach toward fundraising, adopted by an administration badly in need of cash to support covert military operations in South America. Neither theory is supported by a substantial body of evidence, though it must be admitted that the financing of wars by selling drugs is a very old tradition, first introduced into North America by the Spanish more than 500 years ago (Karch, 1998). Economists (Caulkins, 1997) have disproved another popular theory, namely, that "crack" is cheaper than cocaine hydrochloride. Ongoing monitoring studies continue to show that is simply not the case.

"Crack" prepared on the streets contains variable amounts of bicarbonate and other contaminants. In order to produce "crack" for clinical experiments, cocaine hydrochloride is mixed with an equal weight of sodium bicarbonate in sterile water and the mixture placed in a boiling water bath. Cocaine base precipitates out and forms small pellets or rocks when the water is cooled. "Crack" prepared in this fashion is quite pure; smoking 50 mg of base will deliver between 16 and 32 mg of cocaine to the subject (Foltin and Fischman, 1991).

The composition of "crack" has a great deal of clinical significance, especially since virtually all street cocaine currently sold in the United States and Europe is contaminated with levamisole, an old anthelmintic drug that has now become the cocaine adulterant of choice. Levamisole has a much lower melting point than cocaine (60°C vs. 90°C) (Moffat et al., 2004), so it is not clear whether levamisole would even survive the conversion from cocaine hydrochloride to crack, but law enforcement data suggest that more than 70% of

cocaine sold on the street is contaminated with levamisole, and a very high percentage of abusers who do test positive for levamisole (Buchanan et al., 2011) are not crack smokers at all but rather insufflate or inject their cocaine. The salting out process used to make crack removes many, if not most, of the adulterants and contaminants contained in the cocaine and might even partially remove levamisole. Removal of cocaine adulterants may make the occurrence of medical complications less likely. For example, the entity of eosinophilic myocarditis (an allergic disorder) in cocaine abusers, all but disappeared after "crack" was introduced.

There have been numerous anecdotal reports of cocaine–fentanyl combinations being sold on the street, usually represented as pure cocaine. However, the traces of fentanyl found in some of these exhibits may actually be the result of contamination, either from reuse of the plastic "baggies" or accidentally introduced by the distributor during formulation (i.e., selling a cocaine–fentanyl mixture was never intended). Clearly, this explanation does not apply in every case because the percentage of fentanyl in some of the samples has been so extraordinarily high (23%) that it could only have been purposely added (Anon, 2006).

When faced with arrest, many "crack" smokers swallow the drug packets they are carrying. The act is referred to as "body stuffing" as opposed to "body packing," which is the term for smuggling drug-containing packets in the rectum, vagina, or intestines. Some have accidentally aspirated the small drug packets and choked to death while trying to hide the evidence. The fatality rate for "packing" (concealing large amounts of drug in the intestines) is much higher. A newspaper article published in 2006 described a transatlantic flight that had to be diverted because one of the passengers had fallen ill and was vomiting; he had swallowed more than 1.5 kg of cocaine and began to regurgitate the packets in midflight (Anon, 2002). When cocaine is smuggled in this fashion, it is usually wrapped in latex (see Figure 1.27). If the package leaks, the results can be fatal (Bednarczyk et al., 1980; Wetli and Mittlemann, 1981; de Beer et al., 2008).

If the "crack" has been formulated in such a way that it contains substantial amounts of bicarbonate, the rock is likely to pass through the stomach without dissolving and without producing symptoms. If the "crack" contains more cocaine than bicarbonate, it may dissolve rapidly and produce toxicity. Today, "crack" is most often sold in small plastic vials; there are anecdotal reports of body stuffers who have died not from cocaine poisoning but from aspirating the plastic vials or small rocks covered with plastic wrapping (Pollack et al., 1992; Zaas et al., 2002).

In controlled laboratory studies, plasma cocaine concentrations measured 6–12 min after smoking 50 mg of "crack" ranged from 250 to 350 ng/mL. In a second study from the same laboratory, two 50 mg doses of free base were smoked 14 min apart; the peak plasma concentration was 425 ng/mL 4 min after smoking the last dose (Foltin and Fischman, 1991). In one subject, smoking a 50 mg dose every 14 min, for a total of four doses, produced a plasma concentration of over 1200 ng/mL. In both humans and experimental animals, changes in heart rate and blood pressure are dose dependent and correlate temporally with peak plasma cocaine concentrations (Boni et al., 1991), but there is a great deal of variation between experimental subjects, animal and human.

Environmental exposure to cocaine is a real hazard for inner-city children. Nearly 2.5% of children examined at a metropolitan emergency department tested positive for cocaine or cocaine metabolite. The number would probably have been higher, but children

with signs of cocaine toxicity or a history of cocaine exposure were specifically excluded from the survey (Kharasch et al., 1991). While a number of papers have been published describing the effects of environmental exposure on children (Ackerman et al., 2008; Chae and Covington, 2009), plasma levels in children living with cocaine-using parents have never been reported.

1.7.9 Gastrointestinal Absorption

When cocaine is orally ingested, resultant plasma concentrations seen in drug smugglers are generally far lower than in body packers (see below). Cocaine is well absorbed via the gastrointestinal tract. Coca leaf chewers who swallow their saliva have higher plasma cocaine concentrations than those who do not. Cocaine's hydrochloride salt is absorbed even more completely than cocaine liberated from masticated leaf (Wilkinson et al., 1980). Plasma levels in coca chewers have been measured (Paly, 1979), and the gastrointestinal absorption of cocaine hydrochloride has been studied in at least two controlled clinical trials.

In the first of these trials, now nearly two decades old, human volunteers were given increasing doses of cocaine over the course of several weeks; participants were eventually receiving doses of cocaine hydrochloride of up to 2000 mg/day. Peak plasma concentrations occurred approximately 1 h after administration. The maximum concentrations produced by doses ranging from 1250 mg (five separate doses of 250 mg each) to 2000 mg/day (five separate doses of 400 mg each), ranged from 653 to 1899 ng/mL (Jufer et al., 1998).

In 2009, human volunteers took a series of increasing doses of cocaine in a ranging study. They were given five, 175 mg, oral doses of cocaine (Walsh et al., 2009). After a rest period of 4 days, the same experiments were repeated again. Pharmacokinetic as well as psychological measures disclosed plasma concentrations of cocaine similar to or higher than those produced by intravenous and smoked doses of cocaine in prior human laboratory studies. Peak plasma cocaine concentrations ranged from 500 to 725 ng/L, while concentrations of BZE and EME were 2500–3000 and 1000–1400 ng/mL, respectively. Peak concentrations of cocaine first appeared approximately 5 h after ingestion, followed by EME and BZE at slightly more than 5 h. Plasma concentrations of norcocaine peaked at 50 ng, also at 5–6 h. These concentrations are illustrated in Figure 1.23.

Gastrointestinal absorption assumes particular importance in the "body packer" syndrome (Figures 1.24 through 1.27). Most packets are round to oval in form and are easily seen with plain x-ray, though CT scanning is generally considered a more sensitive diagnostic tool. The pattern of x-ray attenuation observed suggests the content of the packets: hashish is denser than stool, cocaine appears similar to stool, and heroin has a gaseous transparence. Asymptomatic packet ingestion is managed conservatively, but leakage from the packets can constitute a surgical emergency. In a review of 17 cases collected over a decade in Jamaica, there were 11 cases of bowel obstruction, 2 of delayed passage of pellets, 3 of ruptured packets with cocaine toxicity, and 1 patient who panicked and requested surgery. The distal ileum was the most common site of obstruction. It appears that actual cocaine poisoning only occurs when the packets rupture in the upper gastrointestinal tract. Obstructing packets must, of course, be removed, but if unruptured, the packets may be allowed to pass spontaneously (East, 2005).

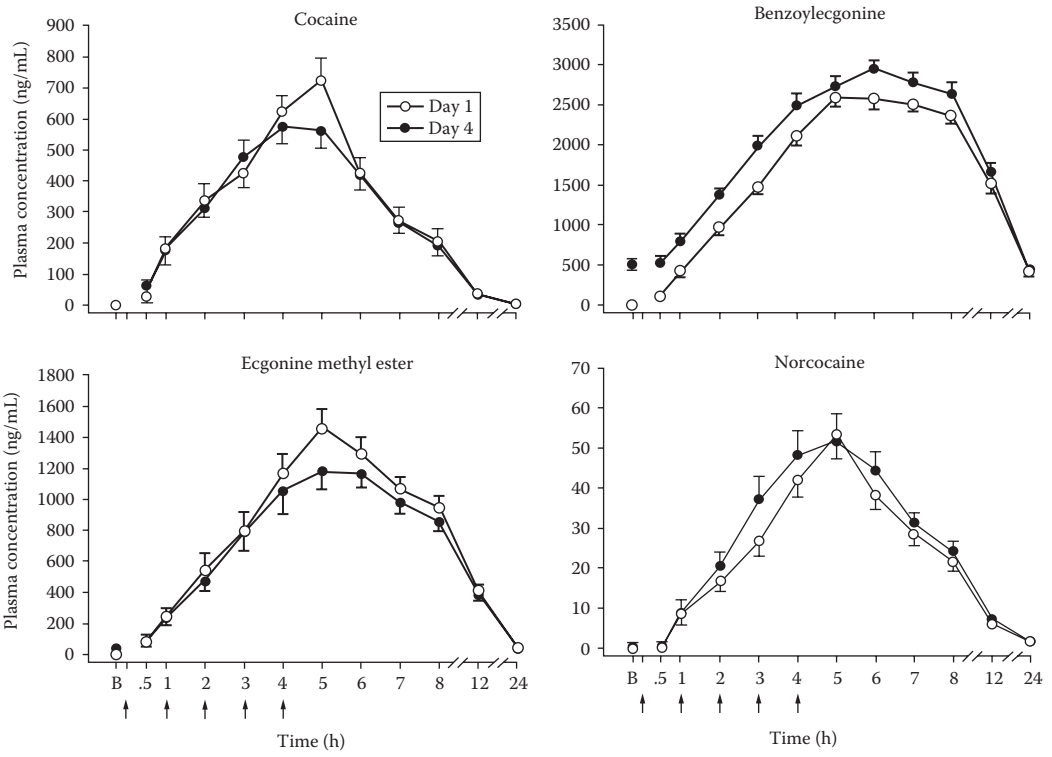


Figure 1.23 Plasma cocaine and its metabolites after oral administration of cocaine to human volunteers. (Reproduced from Walsh, S.L. et al., *Exp. Clin. Psychopharmacol.*, 17(4), 205, 2009, doi:10.1037/a0016469. With permission from PubMed Central [PMC] January 25, 2010.)

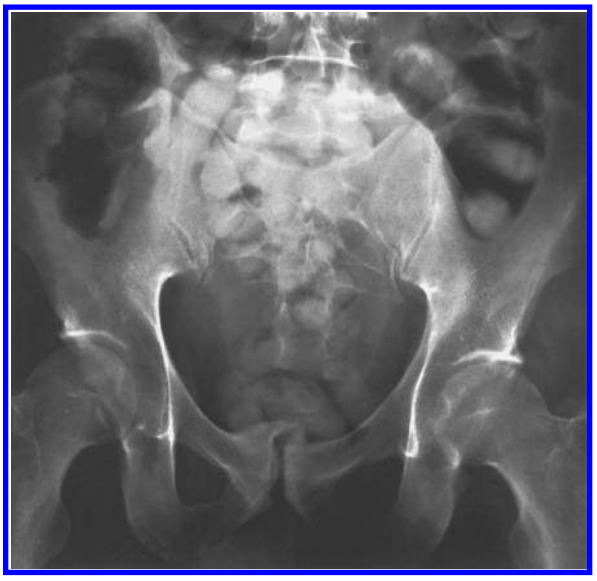


Figure 1.24 "Body packer" syndrome. Drug couriers can be diagnosed with plain abdominal x-rays, although occasionally CT scanning is required. This plain film clearly demonstrates cocaine-containing packets. (Courtesy of Dr. Meyers, M., *Abdom. Imaging*, 20, 339, 1995, State University of New York, Health Sciences Center, New York.)

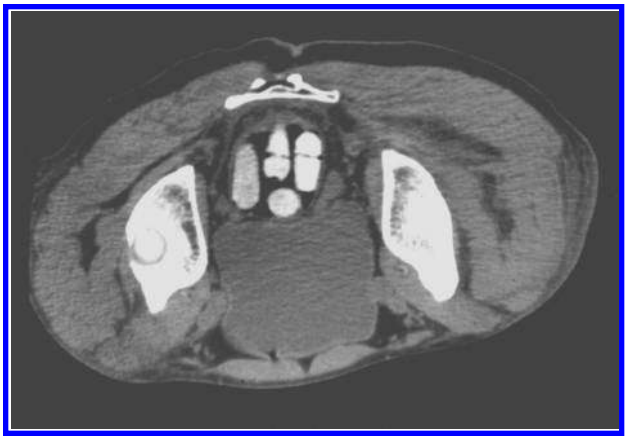


Figure 1.25 "Body packer" syndrome. This syndrome was first described in 1977. Smugglers who swallow multiple, rubber-coated packets of drugs are at grave risk for massive overdose. (Courtesy of Dr. Meyers, M., *Abdom. Imaging*, 20, 339, 1995, State University of New York, Health Sciences Center, New York.)

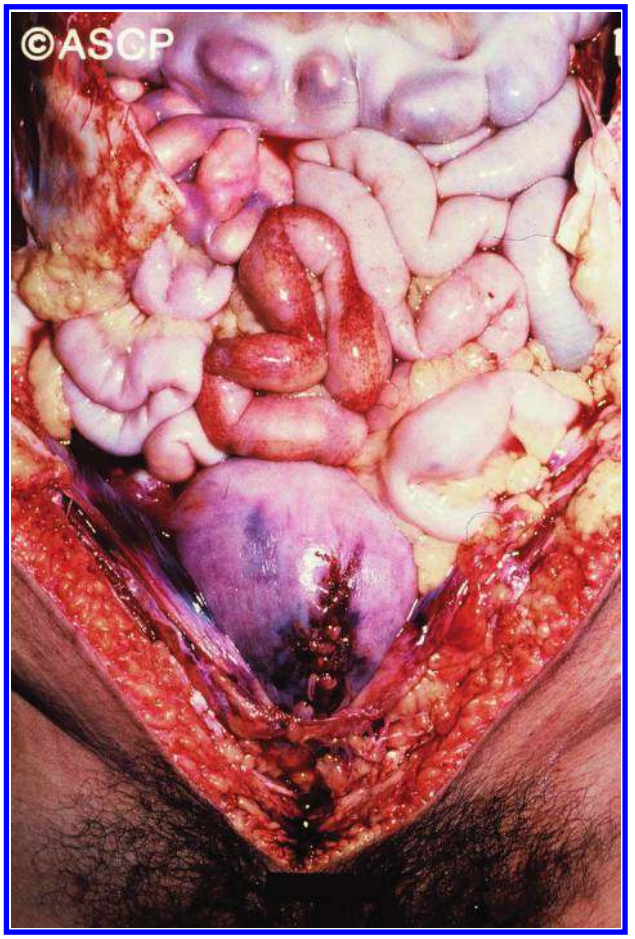


Figure 1.26 Pregnant drug courier. Pregnant uterus is visible in lower section of picture, while cocaine-filled condoms are apparent throughout the intestinal tract; see Figure 1.27. (Photo courtesy of the College of American Pathologists, Northfield, IL.)

Figure 1.27 Drug packets. Cocaine packets removed from decedent shown in Figure 1.26. Reports suggest that some may ingest up to a kilogram or more in this fashion. (Photo courtesy of the College of American Pathologists, Northfield, IL.)

The practice of "body packing" was first described in 1977 (Suarez et al., 1977). Low-level smugglers ("mules") swallow packets containing hundreds of grams of cocaine. The drugs are wrapped in a condom, plastic bag, or aluminum foil, with each packet containing 3–6 g of drug; a rare individual courier may carry nearly 2 kg of drug. If a cocaine-containing packet should rupture, fatal seizures will result, usually with concomitant pulmonary edema and heart failure. If the drug courier happens to be a regular user with some degree of tolerance, even a massive overdose may not produce the classical symptoms, and the outcome is not necessarily fatal (Bettinger, 1980; Howell and Ezell, 1990). Occasionally, body packers may present first with toxic psychosis, rather than fever (Fernandez Moyano et al., 1998).

Plasma concentrations in body packer fatalities have ranged from 3 to 11 mg/L, well in excess of levels normally seen after intravenous abuse. Even if the cocaine-containing packets do not rupture, small amounts of cocaine may still appear in the urine, and urine testing may be diagnostic for the syndrome (Gherardi et al., 1988). Most major international airports are now equipped with facilities for on-site enzyme multiplied immunoassay technique urine testing and special toilet facilities with which to collect the contraband.

Gastrointestinal absorption can also occur via mother's milk (Dickson et al., 1994; Golding 1997; Winecker et al., 2001). In the most recent study, breast milk was collected from 400 women and analyzed using liquid chromatography with mass spectrometry. Few of the women had any cocaine detectable in their milk. Even in the one, self-identified,

cocaine-addicted mother, the concentration of cocaine found in the breast milk was only 5 ng/mL (Marcheia et al., 2011). Given that cocaine is readily soluble in nonpolar solvents, the appearance of small amounts of cocaine in mother's milk is hardly surprising.

Still another form of gastrointestinal absorption involves drinking coca-based teas and, more recently, coca-containing soft drinks. These are popular in many parts of South America where they are sold commercially. At the end of 2005, an Indian-owned company based in Colombia began to sell its own brand of coca soda as an alternative to Coca-Cola®. It is called Coca Sek, which, in the local dialect, means "coca of the sun." It is a carbonated drink made from coca leaf extract and is said to taste like lemonade and ginger ale.

Coca is also consumed in South America as a tea. The average coca leaf tea bag contains approximately 1 g of dried coca leaves. The content of the commercial tea bags is quite variable, depending on where they are manufactured. A reasonable approximation is that 1 g of dried, shredded coca leaf will contain 4–5 mg of cocaine plus variable amounts of BZE and EME, along with trace amounts of *trans*-cinnamoylcocaine. Depending on the origins of the tea, urinary BZE concentrations in those who consume it range from 4000 ng/mL to almost 5000 ng/mL at 3.5–10 h after ingestion (Jenkins et al., 1996).

Coca-containing tea has become an increasing problem for workplace testing programs. In one instance, an enlisted man who was not a cocaine user had to face courts-martial because of a positive urine cocaine test. He claimed not to be a user but did admit to drinking a tea called mate de coca. When a single tea bag was placed into 100 mL of water and boiled for 10 min, 5.35 mg of cocaine, 0.64 mg of EME, and 0.26 mg of BZE were extracted. This would have been more than enough to result in a positive urine test (Anon, 2005). Similarly, the *British Journal of Sports Medicine* reported positive tests for BZE in jockeys who had been drinking mate de coca tea (Turner et al., 2005). Of course, even possessing coca tea in the United States is a crime.

In a recent study of five healthy adult volunteers who consumed coca tea and then underwent serial quantitative urine testing for cocaine metabolites, urine cocaine concentrations exceeded 300 ng/mL by 2 h after ingestion. Three out of five participants' samples remained positive at 36 h. Mean urine BZE concentration in all postconsumption samples was 1777 ng/mL (95% confidence interval [CI], 1060–2495) (Mazor et al., 2006).

1.7.10 Special Maternal/Fetal Considerations

Not a lot is known about maternal/fetal cocaine drug ratios. Indeed, it is not known whether such measurements have any clinical significance. By midpregnancy, the fetus may come into direct contact with high concentrations of cocaine that have been absorbed in the amniotic fluid (Woods, 1998). How much cocaine, if any, the human fetus actually absorbs via this route is not known nor is it known whether the amount absorbed has any medical significance, though there is some epidemiologic evidence that 20% of infants exposed to cocaine in utero will develop hypertension later in life (Salisbury et al., 2007).

In one study, cocaine, nicotine, caffeine, and their metabolites were measured in the cord blood of 36 neonates. Eighteen had detectable concentrations of BZE, and 50% of these were also positive for cocaine. Cocaethylene was not found in any case. The maximum plasma cocaine concentration measured in any newborn was 88 ng/mL (mean, 39 ng/mL). The maximum plasma BZE concentration observed was 3880 ng/mL (mean, 844 ng/mL). Among BZE-positive babies, the mean plasma drug levels were as follows: nicotine, 1.8 ng/mL; cotinine, 94 ng/mL; and caffeine, 1205 ng/mL. Among the

BZE-negative babies, the mean plasma drug levels were as follows: nicotine, 5.2 ng/mL; cotinine, 97 ng/mL; and caffeine, 1440 ng/mL (Dempsey et al., 1999). In the same study, the half-life of cocaine and BZE was found to be 11.6 and 12.8 min, respectively.

In another study, cocaine concentrations were measured in stillborn fetuses (Meeker and Reynolds, 1990). Data collected from 25 cases of fetal or newborn death associated with maternal cocaine use are reported. The average week of gestation at which fetal death occurred was week 30. Abruptio placentae was observed in seven cases and placental infarct was found in four cases. The average fetal blood cocaine and BZE levels were 0.26 and 1.73 μg/mL. The average maternal levels were 0.14 and 1.80 μg/mL, respectively. It would be unwise to consider these levels as reliable as cocaine was not considered the cause of death of any single case. The study does, however, demonstrate that transplacental transmission exists.

Within the U.S. court system, the transfer of cocaine through breast milk has been alleged to be fatal, and nursing mothers have charged with abuse or even manslaughter. However, given the infant's daily consumption of milk and given the cocaine concentrations that have been measured in human breast milk, it seems unlikely that toxic quantities of cocaine could be transferred by breast-feeding. When breast milk was collected from 11 cocaine-using mothers, cocaine was detected in only 6 of the specimens. When cocaine and one or more of its metabolites were detected, the concentration of parent compound was greater than any of the metabolites. The highest cocaine concentration ever found in any report was a surprisingly high 12 mg/L (Winecker et al., 2001), but concentrations of this magnitude have never been confirmed. Spanish researchers tested milk samples from one admitted cocaine user and found a cocaine concentration of 5 ng/mL in one woman but did not specify exactly how the milk sample was collected (Marchei et al., 2011).

One case report described milk kinetics after a 0.5 g dose of cocaine (Chasnoff et al., 1987). A peak concentration of 40 ng/mL was measured in the milk 12 h after the dose; drug and metabolites were cleared after 36 h. In another case report, milk cocaine concentration was 8 ng/mL 6 days after consumption of an unknown amount (Sarkar et al., 2005). The elimination $T_{½}$ of cocaine was 12 h (Chasnoff et al., 1987).

The sampling techniques used by Chasnoff in his 1987 study, where cocaine concentrations as great as 12 mg/L were measured, were employed before it was recognized that cocaine concentrations in the first milk expressed are very different from concentrations in the last milk expressed at the end of feeding (Stowe et al., 2003). The only way to be sure of the amount of drug delivered is to collect all the milk produced over a 24 h period and measure the average concentration; having a woman express some milk and measuring the cocaine concentration is simply unacceptable—the concentration of any drug in breast milk tends to increase as more and more of the milk is expressed. Neither can breast milk concentrations of a drug be calculated by analogy to the behavior of other similarly structured molecules. Milk does concentrate drugs to higher concentrations than plasma, but to what extent depends on the actual molecule; even though molecules may be related (e.g., methamphetamine and amphetamine), that does not guarantee that the same concentration will occur in the milk.

Screening of amniotic fluid samples from 450 women at various stages of pregnancy yielded only five samples of cocaine by GC/MS screening, and only one sample was positive for cocaethylene (Ripple et al., 1992). In a second study, cocaine or BZE was detected in 74% of amniotic fluid samples taken from 23 known cocaine abusers. In the 23 positive

cases, amniotic BZE concentrations ranged from 400 to greater than 5000 ng/mL, while concentrations of cocaine ranged from trace to 250 ng/mL for cocaine (Jain et al., 1993). Interestingly, BZE concentrations were significantly greater in the amniotic fluid than in the urine of the newborns (1800 and 280 ng/mL, respectively; $p = 0.0001$), suggesting that not a great deal of drug had been absorbed, either via the skin or through the gastrointestinal tract.

The results of additional studies suggest that, in spite of maternal use, fetal cocaine absorption does not necessarily occur in every case. A case report published in 1994 described a 26-year-old woman who was an admitted intravenous cocaine user. She had injected herself daily throughout the course of her pregnancy. She also used hashish on a weekly basis during the first trimester of the pregnancy, drank alcohol on occasion, and smoked one to two packs of cigarettes a day throughout the pregnancy. Her history was confirmed when samples of her hair were obtained 2 months after delivery: they contained nicotine, cotinine, and BZE (concentrations in various segments of hair ranged from 0.8 to 2.3 ng/mg). Urine from the child was negative for BZE and cannabinoids, and only cannabinoids were detected in the meconium. Hair samples obtained from the child at birth were negative for cocaine and BZE but did contain nicotine and cotinine (Potter et al., 1994).

Similar anomalies have been reported in other cases. When cocaine and its metabolites were measured in urine, meconium, and amniotic fluid specimens collected from 30 mother–infant pairs, each with histories of maternal cocaine use and presumed prenatal cocaine exposure, there was qualitative, but not quantitative, agreement between results in mother and child. This applied to maternal urine, amniotic fluid, infant urine, and meconium. Even though all of the mothers in this study admitted to using cocaine during their pregnancy, cocaine or its metabolites were detected only in 20 cases where cocaine was used within 3 weeks before delivery (Casanova et al., 1994). Eyler et al. (2005) conducted private structured interviews with women who had a prior history of perinatal cocaine use. In most instances, the history provided by the mothers corresponded with the toxicologic findings. However, in five cases of infants born to women who admitted using cocaine during their pregnancies, tests of hair, urine, and meconium were all negative (Eyler et al., 2005).

Given the number of published case reports, it appears that maternal cocaine ingestion does not guarantee fetal cocaine ingestion in every case. This is an important observation, particularly if a pregnant woman is charged with transmitting drug to her unborn fetus. Clearly, the potential exists, but it cannot be assumed to have occurred.

1.8 Problems of Cocaine Test Interpretation

1.8.1 Introduction

Drugs may be measured in parts per billion and recovered from almost any tissue. Yet highly accurate measurements are seldom of any help in differentiating deaths due to drugs from those deaths where the presence of drugs is simply an incidental finding or, as is increasingly recognized, an interaction between two drugs to produce a third and even more toxic drug. Why are such precise measurements of so little value? Because, except for episodes of massive overdose (as might occur in a drug mule) where the mechanism of death is perfectly clear, most cocaine-related deaths occur in chronic drug users in whom death is a consequence of neurochemical and anatomic changes induced over a period of months or even years.

Long-term cocaine users have changes in their hearts (Karch et al., 1998) and in their brains (Volkow et al., 1993) that favor the occurrence of sudden death. If the abuser is truly unlucky, he or she may be heterogeneous for one of several abnormal hERG ion channels (Guo et al., 2006) or polymorphic catecholamine receptors (Ghimire et al., 2012). These changes explain why, in both the living and the dead, it is absolutely impossible to correlate a specific blood or plasma concentration with a specific type of toxicity or even speculate whether cocaine-related toxicity occurred at all (Jenkins and Goldberger, 1997; Karch et al., 1998; Blaho et al., 2000).

In addition to problems caused by direct cocaine toxicity, less privileged drug users such as sex workers or those without access to sterile syringes are at increased risk for a host of lifestyle diseases such as hepatitis, tuberculosis, and HIV (Inciardi, 1995; Figueroa et al., 2005). Given these realities, the accurate certification of a drug-related death requires knowledge of (1) the decedent's past medical history, (2) an account of what happened at the scene, (3) a thorough postmortem examination, and (4) the results of DNA testing to rule out hereditary forms of heart disease or even myocarditis; the cost of such testing is rapidly decreasing to affordable levels. Though it will still be some time until it becomes widely available.

The definition of just what constitutes a "complete" autopsy is not as simple or clear as it once was. Does the examination of one section of myocardium constitute a "complete" examination of the heart? When a young person dies suddenly, is it proper to refer to their heart as normal when cardiac ion channels have not been measured? In unexplained cases of drug death, should the examination be considered complete if the P450 metabolizer status is not determined? The answer to all of these questions is "no." As a consequence, cause of death determinations are often based solely on toxicologic measurements, significantly raising the chances for a missed or incorrect diagnosis. Because cocaine is so widely used, cocaine-related deaths present a special set of problems.

There is seldom any reason for clinicians to measure plasma concentrations of cocaine or its metabolites. Unlike alcohol intoxication, where specific blood concentrations can generally be related to specific physiologic and psychological states, cocaine blood concentrations do not relate to symptoms (Karch et al., 1998; Blaho et al., 2000), not even in the laboratory setting. Accordingly, treatment must be based on the patient's symptoms, not on the plasma level of cocaine.

When cocaine is given to volunteers, correlations can be drawn between the degree of mood elevation and peak blood levels, but only when cocaine concentrations are rising. If blood concentrations are falling, the exact same concentration that resulted in a "high" when concentrations were rising can be associated with a dysphoric reaction when concentrations are falling. Cardiovascular effects and feelings of euphoria decline more rapidly than do cocaine blood concentrations (Javaid et al., 1978), but the "rush" experienced by cocaine users follows a different time course than the cardiovascular changes.

1.8.2 Tolerance

Postmortem blood concentrations are much more difficult to interpret than drug concentrations in the living. At one time, cocaine blood concentrations of more than 5 mg/L were thought to be uniformly fatal (Wetli and Mittlemann, 1981). With more experience, it has become apparent that isolated postmortem blood concentrations cannot

be used to determine the cause of death at all. Tolerance on a massive scale occurs, and cocaine concentrations well in excess of 5 mg/L can be encountered in trauma-related deaths where the presence of cocaine is clearly an unrelated finding (Pagel et al., 1994; Shannon et al., 1996). For example, one case report described a man who was shot while drinking in a bar. Prior to being shot, the man's behavior was said to have been normal. When he was autopsied several hours later, after having undergone extensive attempts at resuscitation, including aggressive fluid replacement, multiple blood specimens showed a blood cocaine concentration of 30 mg/L (Howell and Ezell, 1990). In a similar case, a young woman with a history of chronic cocaine abuse was found dead at home. The woman was not a body packer attempting to smuggle drugs, and there was no evidence that she had purposefully overdosed; the blood concentration was over 300 mg/L (Peretti et al., 1990).

On the other hand, individuals who are chronic abusers will already have established changes in their hearts and brains (and perhaps elsewhere). In these individuals, death and toxicity may occur after the use of trivial amounts of drug (Smart and Anglin, 1987; Jenkins and Goldberger, 1997; Karch et al., 1998) or even when no cocaine is detected. For example, a cocaine addict just released from a rehabilitation program could still die of an arrhythmia secondary to cocaine-induced myocardial fibrosis (Stephens et al., 2004a,b) even though neither cocaine nor any of its metabolites were present. These considerations would not apply in naïve users who do not suffer from underlying or undiagnosed heart or brain disease. Tolerance is certainly one explanation for the overlap; the other is the occurrence of postmortem redistribution.

1.8.3 Postmortem Redistribution

The concentrations of basic drugs, such as tricyclic antidepressants, some narcotic analgesics, local anesthetics, and stimulants such as methamphetamine and cocaine, are all likely to increase after death (Prouty and Anderson, 1990; Pounder, 1993). Provided that the blood remains liquid, drugs sequestered in the lungs rapidly redistribute into the pulmonary venous blood and then into the left chambers of the heart. Because of the lung's very great blood supply, the absolute amount of drug in the pulmonary circulation will be much greater than in other organs. Postmortem, drugs temporarily sequestered in the pulmonary circulation may diffuse through the thin-walled pulmonary veins, falsely elevating the blood concentration within the left ventricle (Moriya and Hashimoto, 1999).

Accordingly, left ventricular blood should never be used for toxicologic evaluation because the concentrations reported are likely to be considerably higher than they were at the time of death. Blood taken from the right ventricular cavity, however, is unlikely to be subject to the same sort of postmortem concentration increase seen in the left heart and femoral artery blood even less so (Drummer, 2004). Regrettably, many autopsy reports still do not specify from which part of the body the blood sample was obtained, let alone from which side of the heart. This is an omission that pathologists completing a death certificate need to accept and consider before attributing death to a drug simply because the concentration of that drug is high. If the concentration of cocaine is found to fall within the "toxic range" (a bizarre concept given that the "toxic range" is derived in humans, not cadavers!), knowledge of that number will contribute little or nothing to the interpretative process

(Karch et al., 1998). In chronic abusers, changes in the heart and brain favoring sudden death would still be present, even if cocaine had not been used that day. Even if evidence of myocardial remodeling is wanting, the decedent may have been suffering from an undiagnosed channelopathy, making them more vulnerable to sudden death in the presence of cocaine (Karle and Kiehn, 2002).

1.8.4 Cocaine-Related Deaths

Cocaine is directly toxic to the myocardium (Peng et al., 1989) and the process appears to be multifactorial. Cocaine induces apoptosis (Kajstura et al., 2006), and at the same time, it increases production of calmodulin kinase II (CMKII), in both the heart (Sun and Quamina, 2004) and brain (Zhang et al., 2005). The end result is that the hearts of chronic cocaine users undergo a process known as myocardial remodeling; myocardial collagen content increases and ventricular fibrosis becomes apparent. It has been known for nearly two decades that the hearts of cocaine users are enlarged and fibrotic (Tazelaar et al., 1987; Brickner et al., 1991) (see Section 1.10).

Fibrosis is not the only change that occurs within the myocardium. Growth initiators such as angiotensin II, and even the mechanical strain produced by cocaine-related increases in blood pressure, lead to the generation of even more reactive oxygen species (ROS) causing damage to the myocardium, the death of additional myocytes, and the production of even more fibrosis. The role of activated metalloprotease enzymes in this process is not entirely clear, but it appears that their production leads to downregulation of cardiac β receptors. If that is the case, it would ultimately prevent the release of calcium from the myocardium, resulting in decreased cardiac output (Opie et al., 2006).

Regardless of the underlying etiology, the presence of myocardial fibrosis and enlargement leads to electrical instability that favors the occurrence of arrhythmic sudden death. When cocaine is the cause of the remodeling, sudden cardiac death is even more likely because cocaine binds to the hERG (rapid delayed potassium rectifier) channel (O'Leary, 2001). Under most circumstances, cocaine–hERG binding is probably of little consequence. However, cocaine interacts strongly with certain mutated hERG channels (Guo et al., 2006) that favor the occurrence of arrhythmias.

If the appropriate morphologic changes can be identified in the heart of a known cocaine abuser, it would not be unreasonable to designate cocaine as the cause of death, even when little or no cocaine is detected in postmortem blood specimens (Stephens et al., 2004a,b). Conversely, the same conclusions cannot be drawn about naïve cocaine users or those with anatomically normal hearts. The finding of very low blood and tissue cocaine concentrations may just be a consequence of environmental contamination and is very likely to be totally unrelated to the cause of death. Cocaine is universally present in our environment; even the currency of most countries is contaminated (Mirchandani et al., 1991; Oyler et al., 1996; Smith and Kidwell, 1996; De Giorgio et al., 2004). In the absence of any anatomic alteration, low levels of cocaine or its metabolites should only be considered evidence of cocaine exposure. In situations where the postmortem examination is totally unrevealing and modest amounts of cocaine are detected in the blood or hair (and suitable measures have been taken to protect the hair from body fluids liberated by the autopsy), it is likely that a heritable channelopathy or other cardiac mutation is the cause of death, and genetic testing for these entities should be carefully considered.

1.8.5 Estimating Time of Ingestion

Though certainly an attractive idea, the measurement of cocaine to BZE ratio in postmortem blood cannot be used as an indicator of when the cocaine was taken, nor can the concentrations of cocaine and BZE be combined and then used to extrapolate dosage. These two compounds have very different volumes of distribution, invalidating such calculations. The V_{ss} constant for cocaine is thought to be between 2 and 3 L/kg (Chow et al., 1985; Cone, 1995), while that of BZE is only 1.0 (Ambre et al., 1991). What this means is that in a living person, at steady state, most cocaine resides in the tissues and most of the metabolite in the blood. Diffusion of cocaine back into the bloodstream after death makes accurate extrapolation to the immediate antemortem period impossible. Much the same situation pertains to morphine and its metabolites, where the steady-state volume of distribution of the metabolites is less than one-tenth as high as morphine itself (see Section 5.8.3 for a fuller discussion of morphine).

Cocaine rapidly crosses the BBB but BZE does not; any BZE found in the brain is the breakdown product of cocaine that has crossed the BBB (Spiehler and Reed, 1985; Bertol et al., 2008). It follows that the ratio of cocaine to BZE in the brain can be used to estimate time of ingestion: high brain cocaine concentrations with low BZE concentrations mean that ingestion was very recent, while the opposite finding indicates that use was remote. Postmortem redistribution is not an issue in the brain, because, except for the blood already present in the cerebral circulation, no additional drug can be delivered to the brain after death.

1.8.6 Low Cocaine Concentrations

Chronic cocaine users sequester cocaine in deep body stores. Small amounts of this sequestered cocaine can leach back into the bloodstream and saliva for days after the drug was last used (Cone and Weddington, 1989; Burke et al., 1990). There is also evidence that in chronic users of large amounts of cocaine, the plasma half-lives of cocaine and BZE are longer than those observed in occasional users (Preston et al., 2002). In studies performed in a closed metabolic ward, 63% of the volunteers studied tested positive for longer than the expected 48 h window of detection. By self-report, the mean time to last positive urine after last use of cocaine was approximately 81 ± 34 (34–162) h and small amounts of BZE were still present at 72 h. For those reasons alone, attributing significance to very low cocaine levels is not a good idea unless, of course, appropriate anatomic changes are present as well.

As discussed earlier, cocaine can be recovered from most of the currency in circulation in most industrialized countries (Oyler et al., 1996; Dressler and Muller, 1997; Jenkins, 2001; Furton et al., 2002), and it can also be detected in the sweat and hair of the children of drug abusers, even those in rehabilitation! In one study, 85% of the children living with cocaine-using parents had detectable levels of cocaine in their hair (Smith and Kidwell, 1996). Whether the positive tests were a result of inhaled cocaine being deposited within the hair from the circulation or were a result of environmental cocaine deposited on the hair has not been determined.

Volatilized cocaine, just like cigarette smoke, can be passively absorbed. The process has been described in children and can even produce transient neurologic syndromes (Bateman and Heagarty, 1989). Whether such exposure can lead to serious consequences

is not clear, but it certainly can lead to positive urine, blood, and hair tests. If parents use cocaine within their home environment, no matter what their wishes, it will end up in their children.

In general, the courts have tended to rule that the presence of detectable amounts of cocaine, or cocaine metabolite in a child, is proof of willful child endangerment. One of the most frequently cited studies in this regard described 16 dead children who had cocaine or cocaine metabolite in their blood. Scene investigations documented that shortly before death these infants had been exposed to "crack" smoke. Most of the infants were under 3 months of age, none had revealing autopsy findings, and their mean cocaine blood level was 76 ng/mL, just barely over the level required to produce any measurable physiologic effects (Mirchandani et al., 1991). On the basis of this evidence alone, and in the absence of any plausible alternative mechanism, sudden infant death syndrome (SIDS) would appear to be the more likely diagnosis, especially given the absence of pathologic findings. Of course there are two problems with this interpretation. The first is that if drugs are detected in an infant who dies suddenly, the diagnosis is, by definition, not SIDS. The second is that these infants are rarely, if ever, tested for heritable channelopathy, which is not a rare disease.

In fact, low levels of cocaine detected in a fetus do not even constitute evidence of maternal cocaine use. Side-stream intake of volatilized cocaine can occur in non-drug-using mothers (Cone, 1995), and if the mother is exposed to this vapor, then cocaine and its metabolite could appear in the infant, although the mother herself was not a voluntary cocaine user. Thus, the identification of cocaine in an infant is not necessarily proof of abuse by the mother, though it might very well be considered endangerment. Similar considerations apply to breast milk. There is evidence that cocaine becomes concentrated in breast milk (Bailey, 1998), though it has never been demonstrated that any child ever died after exposure to milk from a cocaine-abusing mother.

Because there is so much cocaine in the environment of industrialized countries and because the possibilities of accidental contamination are so real, NIDA has promulgated regulations for drug testing that include "cutoffs." For BZE, the cutoff is 150 ng/mL; levels below that value are reported as negative. Of course, these "cutoffs" were formulated with living patients in mind, but the reasoning is still valid. In the absence of any other information, cocaine or cocaine metabolite levels of less than 50 ng/mL are chiefly of historic interest and do not provide evidence of very recent ingestion. Levels on the order of 50 ng/mL, or even less, are frequently seen in postmortem blood samples and must be interpreted with great care. Cocaine has a relatively large volume of distribution. After death, as the body decomposes, cocaine will be slowly released from deep tissue stores. As a consequence, concentrations found in postmortem blood can increase significantly from levels measured in the immediate antemortem period. Cocaine blood concentrations only rise after death (Hearn et al., 1991b) and, depending on how and where the specimen is collected, the rise may be very considerable, but it may also be transient. High cocaine concentrations will remain high, provided that sodium fluoride preservative is added to the sample. It has been shown that the use of 2% NaF is more effective at cocaine preservation than the traditional 1% "gray top" tube used in clinical medicine (Rees et al., 2012). If NaF is not added to the specimen, conversion of cocaine to BZE will continue and cocaine concentration will decrease. A substantial drop may occur in as little as a few hours.

1.8.7 Cocaine and Prescription Drug Interactions

Although the use of illicit drugs is widespread, there are few experimental or clinical data regarding the effects of these agents on common prescription therapies (Lindsey et al., 2012). Formal studies of cocaine–prescription drug interactions have rarely been undertaken, though some interactions of importance have been identified. Cocaine users who are treated with disulfiram have significantly higher plasma cocaine concentrations when they are taking disulfiram than when they are not. They also develop higher systolic and diastolic blood pressure and higher mean heart rates than when they are just taking cocaine (McCance-Katz et al., 1998). It is not known if this interaction has any clinical significance. Similar considerations apply to documented interactions with amitriptyline, procainamide, and quinidine, all of which inhibit human plasma butyrylcholinesterase, slowing the breakdown of cocaine. Clinical measurements are lacking, but in vitro studies have shown that the breakdown of cocaine is slower in the presence of drugs that inhibit butyrylcholinesterase (Bailey, 1999).

The *N*-demethylation product of cocaine is norcocaine, produced by the enzymatic action of hepatic CYP3A4. This is the same enzyme involved in the metabolism of various antiretrovirals, so cocaine use by individuals taking these drugs may lead to elevated cocaine concentrations; protease inhibitors and many other antiretrovirals are also CYP3A4 inhibitors (Ladona et al., 2000). The most potent inhibitors are ritonavir, indinavir, and efavirenz, but ketoconazole (Nizoral), nefazodone, erythromycin, and clarithromycin (Biaxin) are also CYP3A4 inhibitors (Wynn et al., 2005). On the other hand, antiretrovirals that induce CYP3A4 activity, such as nevirapine, may shift cocaine metabolism so that more of it undergoes *N*-demethylation and less undergoes hydroxylation, which means that production of norcocaine would increase, as would the chance of liver toxicity (Roberts et al., 1991).

Cocaine interacts with the hERG K^+ channel (rapidly delayed rectifier potassium channel) and can increase or decrease the channel's normal function (Karle and Kiehn, 2002). Much of the time this effect is more theoretical than real, but the hERG channel is polymorphic and, in the presence of the combination of the appropriate drug and existing structural changes (such as fibrosis and cardiomegaly), there is enormous potential to disrupt the heart's electrical cycle. Diverse drugs from many therapeutic classes, such as quinine, amiodarone, and methadone, exert cardiotoxic side effects by inducing torsades de pointes (TdP), a life-threatening cardiac arrhythmia, due to interaction with hERG channels. Men are known to be at a lower risk for drug-induced TdP than women, suggesting a role of androgens and estrogens in modulating the sensitivity of cardiac potassium channels, particularly those encoded by hERG (Zunkler and Wos, 2003). In general, the higher the dose, the more likely it is that TdP will occur (Krantz et al., 2002).The neuroleptic agents haloperidol, pimozide, and fluspirilene are all capable of inducing TdP. Testosterone seems to oppose the effect. The action of these neuroleptics is voltage dependent, an effect most consistent with an open-channel blocking mechanism (Shuba et al., 2001).

1.9 Cocaine Adulterants

1.9.1 Traditional Adulterants

During the 1980s and 1990s, adulterants (substances added to an illicit drug in order to enhance weight and volume) were generally benign (Table 1.5). A 1984 analysis of 12,000 samples of street-grade cocaine and heroin found there was little difference between the two groups;

Table 1.5 Types of Cocaine Adulterants

A.	*Sugars*
	Dextrose
	Lactose
	Mannitol
	Sucrose
B.	*Stimulants*
	Caffeine
	Ephedrine
	Phenylpropanolamine
	Phentermine
C.	*Local anesthetics*
	Lidocaine
	Benzocaine
	Procaine
	Tetracaine
D.	*Inert agents*
	Inositol
	Corn starch
E.	*Others*
	Acetaminophen
	Aminopyrine
	Ascorbic acid
	Aspirin
	Boric acid
	Diphenhydramine
	Fentanyl
	Niacinamide
	Phenacetin
	Quinine

Source: Based on information supplied by the Drug Enforcement Agency and Shannon, M., *Ann. Emerg. Med.*, 17(11), 1243, 1988.

Note: According to the DEA, 69% or more of cocaine and 3% of heroin seized by the U.S. Drug Enforcement Administration (DEA) as of 2009 contained levamisole. The percentage today may be greater. See Section 1.9.2 for a detailed discussion.

numerous agents, most not particularly toxic (except perhaps for quinine, which is still added for historical reasons—in the early 1900s, it was thought it might prevent malaria in heroin injectors) were identified, but only 11 adulterants were found in concentrations of greater than 5%. Quinine, mannitol, lactose, and procaine were the nonnarcotic compounds most commonly found. Other substances found included caffeine, inositol, lidocaine, starches, methapyrilene, sucrose, acetylprocaine, and dextrose (Cunningham et al., 1984; Shannon, 1988).

1.9.2 Levamisole/Aminorex

The profile of cocaine contaminants and adulterants did not change for the next 20 years. In 1998, an Italian survey found that lidocaine was still the most common adulterant in cocaine (14.7%), followed by caffeine (9%) (Fucci and De Giovanni, 1998). However, in

2004, levamisole made its first appearance in heroin being sold in the United States (Anon, 2004). Its presence was perceived not so much as a health menace but as a curiosity in need of explaining. It was not until 4 years later in Europe, when 28 kg of cocaine hydrochloride were confiscated in Rome, that the worldwide nature of the phenomenon was appreciated. The cocaine was found to contain nearly 10% levamisole (Fucci, 2007). A 2009 report from the Netherlands found the standard adulterants (phenacetin, hydroxyzine, and diltiazem) were comparable to those found in earlier years, but the authors of the report also observed that cardiac and hallucinatory effects in the region's cocaine abusers were, for no apparent reason, being reported more frequently. They suggested that the adverse effects might have been due to the adulterants. One factor that might have explained the apparent increase in hallucinogenic effects would have been the presence of levamisole, but this was not apparent at the time.

Late in 2007, levamisole began to appear in Colombian cocaine shipments destined for the U.S. wholesale market. In 1991, the U.S. Food and Drug Administration had initially approved levamisole (under the brand name of Ergamisol) for use as adjuvant therapy, along with fluorouracil, in the treatment of colorectal cancer. In spite of the limited FDA approval, levamisole was used "off label" as an immunomodulator in the treatment of rheumatoid arthritis, as a primary anthelmintic agent, and as an adjunct drug in the treatment of HIV/AIDS, ulcerative colitis, chronic hepatitis B, nephritic syndrome, malignant melanoma, breast cancer, acute myeloid leukemia, and even amyotrophic lateral sclerosis. Except as an anthelmintic, where it is extremely effective (Chang et al., 2010), levamisole never proved to be very useful for any of these indications (John et al., 2008; Chang et al., 2010). It was withdrawn from the U.S. market in 1999 because of a clear association with the occurrence of agranulocytosis (Ruuskanen et al., 1976; Czuchlewski et al., 2010) but remains available as a veterinary medicine in the United States, Canada, and South America.

Levamisole adulteration first came to the official attention of U.S. authorities in 2006, and in 2008, the Drug Enforcement Administration (DEA) published an analytic method for levamisole detection (Casale et al., 2006). Data from the DEA's Signature Program (a federally sponsored program that tracks the composition of cocaine seizures within the United States) showed that less than 10% of the samples tested in 2008 contained levamisole. By 2009, that number had risen to approximately 71%. Because levamisole was found in kilogram quantities of cocaine, not just in highly diluted street drug, it is reasonable to assume that Colombian producers have adopted the practice of adding levamisole at the end of the production process, possibly to enhance the effects of the cocaine (see below) (U.S. Department of Justice, 2010).

In the past, it was widely assumed that the choice of adulterants was determined largely by availability. Mannitol is less expensive than levamisole, but if a relatively innocuous white substance can be purchased more cheaply or, better yet, stolen, it can and will be used as an adulterant. Such was the reasoning when diltiazem was first detected as a cocaine adulterant in 2004 (Peters, 2004), although it was also suggested that diltiazem and levamisole were being added as "markers" (Maietti et al., 2009) so that the cartels could trace the distribution of their product. The ubiquity of levamisole argues against that explanation.

It has been suggested that cocaine and levamisole act synergistically. Levamisole acting together with cocaine could enhance cocaine-induced euphoria (Raymon and Isenschmid, 2009). The authors of this theory propose that adding levamisole causes

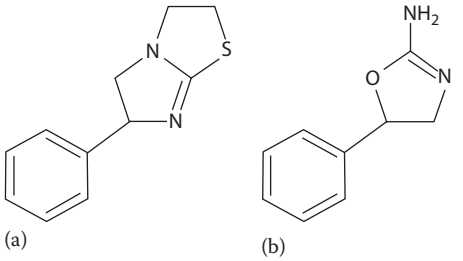


Figure 1.28 Diagram comparing the structure and conversion of (a) levamisole to (b) aminorex. This conversion occurs in man and animals. Aminorex, like many other drugs that disrupt serotonin metabolism, can lead to the occurrence of pulmonary hypertension, but the chances of making the diagnosis in life are small; if the disease is not advanced, it will not be clinically apparent. Nonetheless, histologic changes consistent with idiopathic pulmonary hypertension can be seen in chronic cocaine abusers, even if they are asymptomatic. The lung changes are illustrated in Figures 1.30 and 1.31. See Section 1.16.2 for illustrations and further discussion and also MacLean and Dempsie (2009).

increased peripheral sympathetic activity and increased central neurotransmission, enhancing the intense feelings of pleasure produced by the drug. The theory may be plausible, but a much more logical explanation has recently been proposed. It has been demonstrated, first in the horse and then in man, that levamisole is metabolized to aminorex (Figure 1.28), an appetite suppressant formerly sold in Europe (Ho et al., 2009; Karch, 2012). Experimental studies proved years ago that daily administration of aminorex could cause pulmonary hypertension in experimental animals (Stepanek and Zak, 1975). Aminorex was withdrawn from the European market when it became obvious that users were prone to the development of pulmonary hypertension too (Gaine et al., 2000). For a time aminorex enjoyed some popularity on the illicit market in the United States as a methamphetamine alternative (synthesis is very simple), and aminorex is said to have some MDMA-like properties, but it long ago fell out of favor and is no longer encountered on the U.S. market.

Cocaine adulterated with levamisole is not just found in the United States. There have been isolated reports of cocaine/levamisole-related illness in Canada (Wiens et al., 2010), France (Evrard et al., 2010), Italy (Fucci, 2007), and Spain (Ventura Vilamala et al., 2011). Why little or no levamisole is added to cocaine intended for the United Kingdom and the EU is not clear. The principal cocaine adulterants encountered by European toxicologists remain phenacetin, hydroxyzine, and diltiazem (Brunt et al., 2009). All three of these agents are potentially toxic, but they would be "salted out" in the process of converting cocaine to crack. Equally important, all three of the adulterants commonly seen in Europe are relatively water soluble and, therefore, less likely than the usual cocaine adulterants to produce sclerosis in the veins of intravenous users.

Levamisole now permeates the U.S. cocaine supply (Brunt et al., 2009; Kinzie, 2009; Knowles et al., 2009; Bradford et al., 2010; Buchanan et al., 2010; Chang et al., 2010; Czuchlewski et al., 2010; Duflou et al., 2010; Waller et al., 2010; Walsh et al., 2010; Khan et al., 2011; Ventura Vilamala et al., 2011; Belfonte et al., 2012; Blanc et al., 2012; Buchanan and Lavonas, 2012; Casale et al., 2012; Farmer et al., 2012; Freyer and Peters, 2012; Gulati and Donato, 2012; Larocque and Hoffman, 2012; Lee et al., 2012; Tran et al., 2012; Wolford et al., 2012), suggesting that no matter the route of ingestion, three disorders should become

increasingly common: agranulocytosis, necrotizing vasculitis (Picazo et al., 2003; Buchanan et al., 2010; Bradford et al., 2010), and idiopathic pulmonary hypertension (IPH) (Figures 1.29 through 1.31). None of these conditions is very common and their diagnosis, particularly in a young person, should initiate a search for levamisole-contaminated cocaine.

Clusters of cocaine/levamisole-related agranulocytosis were reported in the United States at the end of 2009 (MMWR, 2009) and in the spring of 2010 (Czuchlewski et al., 2010). Levamisole-contaminated cocaine seems to result in a distinctive clinical syndrome

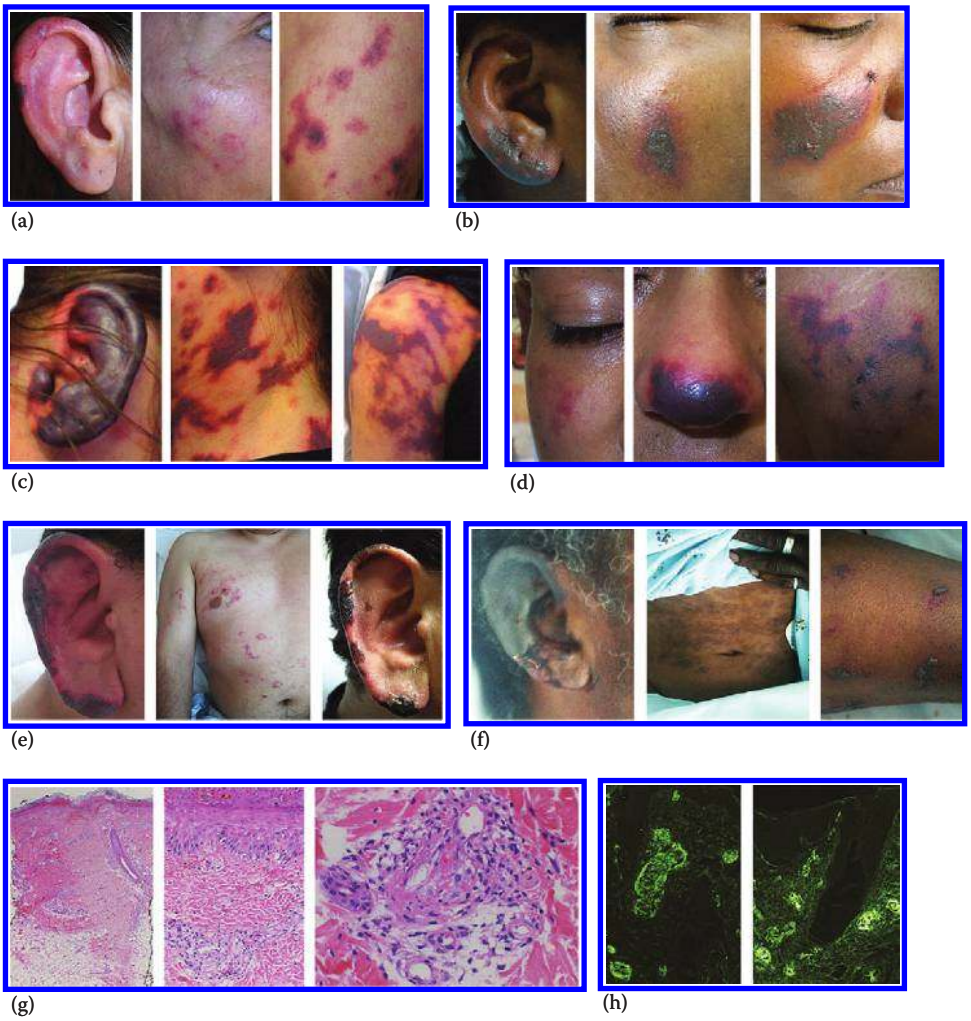


Figure 1.29 Typical presentations of levamisole-induced cutaneous vasculitis. This complication of cocaine use is being reported with increasing frequency. (a) Ear, cheek, and thigh of 46-year-old woman. (b) Ear, left cheek, and right cheek of 57-year-old woman. (c) Ear, neck, and thigh of 46-year-old woman. (d) Cheek, nose, and thigh of 22-year-old woman. (e) Ear (*left*) and trunk (*middle*) of 37-year-old man on admission and same ear resolving 1 week later (*right*). (f) Ear, abdomen, and arm of 50-year-old man. (g) Representative histopathology from ear of patient (e) showing leukocytoclastic vasculitis at ×2.5 (*left*), ×20 (*middle*), and ×40 (*right*) magnification. (h) Representative direct immunofluorescence of ear biopsy specimen demonstrating positive IgM (*left*) and positive fibrin (*right*) in vascular and perivascular spaces. (From Chung, C. et al., *J. Am. Acad. Dermatol.*, 65(4), 722, 2011. With permission.)

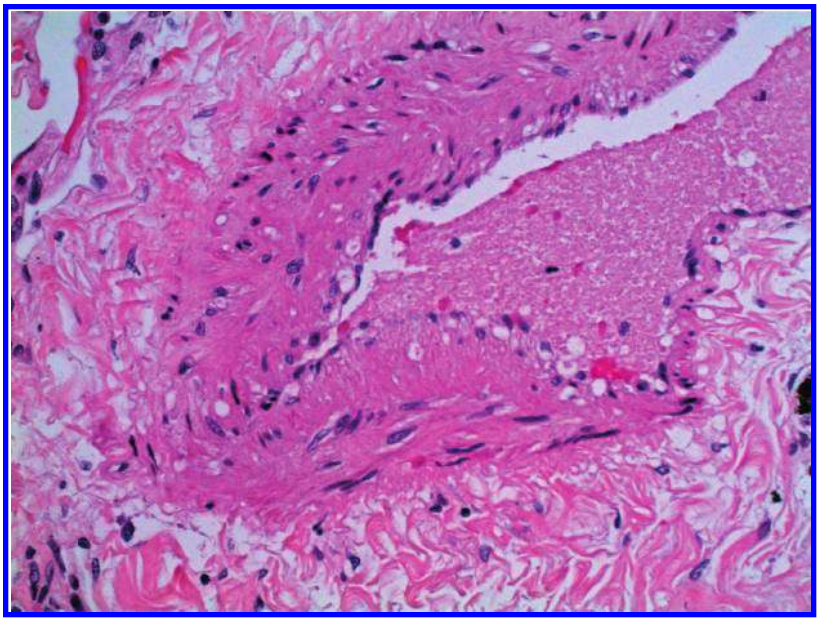


Figure 1.30 Small pulmonary arteriole in a cocaine user. Note the surrounding areas of perivascular fibrosis and the numerous differentiating fibromyocytes.

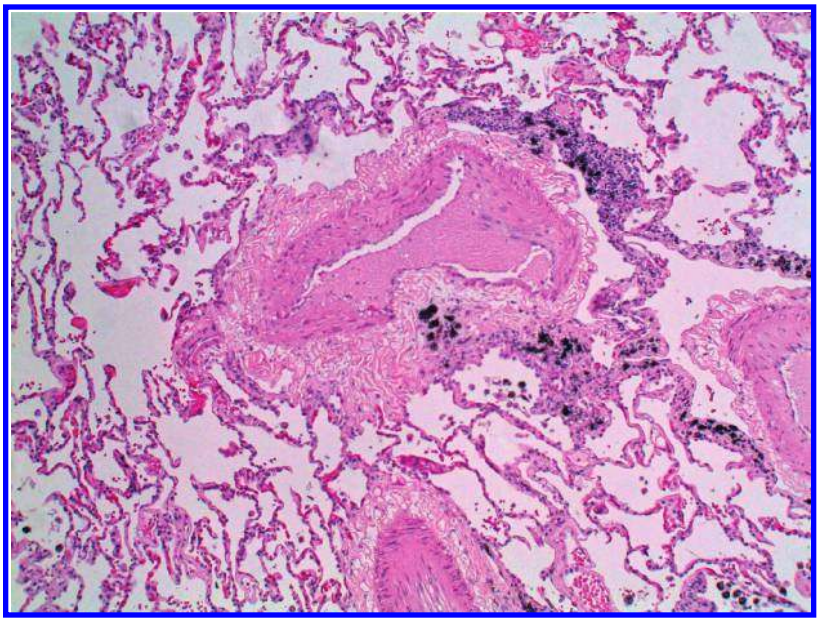


Figure 1.31 Lung from a crack smoker. In addition to the obvious thickening of the vessel walls and modest thickening of the septa, note the deposition of anthracotic pigment, typical for crack smokers. The findings suggest pulmonary hypertension.

that includes the presence of circulating plasmacytoid lymphocytes, increased bone marrow plasma cells, and mild megakaryocytic hyperplasia. More than half of those who become ill will have antineutrophil and HLA-B27+ antibodies (Czuchlewski et al., 2010; Strazulla, 2013). Vasculitis induced by levamisole has been recognized for more than a quarter of a century and also appears to be the result of circulating immune complexes (Macfarlane and Bacon, 1978). Neither disorder has been reported as a consequence of cocaine use in the United Kingdom; perhaps the reports are in press.

1.10 Cocaine Tissue Disposition and Postmortem Redistribution

Low-affinity cocaine receptors are identifiable in animal heart, lungs, gut, kidney, and testes, but the highest-affinity receptors are located in the brain (Calligaro and Eldefrawi, 1987). Distribution of C11-labeled cocaine has been studied in humans using positron emission tomography (PET) scanning. The rate of uptake and clearance of C11-labeled cocaine varies from organ to organ: peak uptake occurred in lung at 45 s, but not until 2–3 min later in the heart and kidneys, and not until 7–9 min later in the adrenal glands. Liver uptake began at 10 min and peaked at 15 min. Half-peak clearances were 90 s in the lungs, 10 min in the heart and kidneys, and 22 min in the adrenals. Lung radioactivity paralleled that of plasma, but active uptake was highest in the heart, kidneys, adrenal, and liver (Volkow et al., 1992). It is now understood that cocaine binds to several types of receptors (including sigma), and the methodology used in many of the tracer studies is not able to distinguish which receptors are involved. Nonetheless, tracer studies do indicate where uptake has occurred, that is, the results indicate where cocaine is most likely to be detected. Unfortunately, this information is valid only in the living and tells us nothing about cocaine's postmortem distribution.

Drug concentrations measured during life may bear little or no relation to drug levels measured after death (Pounder and Jones, 1990; Prouty and Anderson, 1990). The differences are especially great for basic drugs, including drugs of abuse such as cocaine. Postmortem cocaine blood levels vary depending on where the blood sample was drawn (Hearn et al., 1991b) and the concentrations measured may be considerably higher or lower than they were during life (Sylvester et al., 1998; Flanagan et al., 2003; Crandall et al., 2006). The magnitude of the change depends on the postmortem interval, the means of collection, the temperature at which the cadaver was stored, the position in which the cadaver was lying, whether cardiopulmonary resuscitation (CPR) had been performed, the portion of the organ sampled, and the physical properties of the drug itself (Pounder et al., 1996a,b; Moriya and Hashimoto, 1999).

Postmortem redistribution is the process by which drugs move from regions of higher concentration to regions of lesser concentration. Drugs that are hydrophilic will be released from cells as they die. Blood taken from the left side of the heart will yield higher concentrations than that from the right (Pounder et al., 1996a). This is a function of the large amount of blood present in the pulmonary circulation and the thin-walled structure of the pulmonary veins. Drug concentrations are higher in the heart than the femoral vessels, partly because concentrations within the lung may not have equilibrated at the time of death and partly because of migration of drug back into the heart from the lungs.

Blood from the femoral vessels seems less subject to redistribution but, at autopsy, unless the proximal vessels are first ligated, or if more than 25 mL of blood is collected, aspiration

from the femoral vein is likely to yield blood from the liver, where drug concentrations would be expected to be much higher. After death, cocaine quickly disappears from blood and liver but it can be detected in brain and muscle for some time. The same phenomenon has been observed in in vitro studies, suggesting that brain and femoral muscle may be the most suitable tissues for postmortem analysis (Moriya and Hashimoto, 1999) and that the brain–blood ratio may be a reliable indicator of overdose death (Spiehler and Reed, 1985). In fact, the only conclusion that can be reached with certainty about postmortem cocaine blood samples is that cocaine is present and that the amount present may be large or small. Neither conclusion is particularly helpful in determining the cause of death.

1.10.1 Human Postmortem Measurements

Nearly 30 years ago, Poklis et al. analyzed the cocaine content in five individuals who died after taking cocaine (three by intravenous injection and two by smoking) (Poklis et al., 1985). Data from that study are reproduced in Table 1.6. Unfortunately, similar studies have not been repeated in man, although at least one study has been performed in rats. Interestingly, the pattern seen in rats was similar to that observed in man. In addition, these studies have also shown that cocaine and BZE both leach readily into formalin storage solutions. BZE can even be detected in serum samples stored at room temperature (Hilal et al., 2009). In buffered formalin (pH 7.4), cocaine is hydrolyzed to BZE in agreement with pseudo-first-order reaction kinetics (half-life time approximately 7 days), whereas in unbuffered formalin (pH approximately 3.5), it is relatively stable over a period of 30 days (Viel et al., 2009).

1.10.2 Brain

Cocaine blood levels measured at autopsy bear little relationship to levels at the time of death (Spiehler and Reed, 1985), but there is good evidence that concentrations of cocaine in the brain do, making brain the best matrix for postmortem cocaine determinations (Hernandez et al., 1994). The possibility was well illustrated in one case study where brain concentrations of cocaine, BZE, and methylecgonine were 21.2, 3.8, and 3.3 mg/kg, respectively (Giroud et al. 2004).

Cocaine freely crosses the BBB (Misra et al., 1975), as does cocaethylene (Hearn et al., 1991a). Receptors with varying affinities for cocaine are found throughout the brain, particularly in the stratum. However, when cocaine is taken in doses that cause behavioral change, uptake can also be observed in other, lower-affinity sites within the brain such as the frontal and occipital cortices (Calligaro and Eldefrawi, 1987; Volkow et al., 1995). In experimental animals and in autopsied cases of human cocaine-related deaths, the

Table 1.6 Cocaine Content in Five Individuals Who Died after Taking Cocaine

Specimen	Blood	Bile	Brain	Heart	Kidney	Liver	Lung	Spleen	Skeletal Muscle	Adipose Tissue	Urine	Vitreous
Case #1	1.8	10.0	4.0	6.1	26.0	1.6	3.40	22.0	6.1	1.0	39.0	2.4
Case #2	6.9	18.0	24.0	—	26.0	17.0	—	25.0	—	—	41.0	—
Case #3	31.0	—	59.0	—	58.0	6.5	69.0	42.0	48.0	5.9	270.0	—
Case #4	13.0	25.0	83.0	—	53.0	10.0	24.0	—	30.0	—	—	14.0
Case #5	3.9	5.2	6.4	5.3	34.0	15.0	27.0	15.0	—	0.7	27.0	—

Note: The values shown are in mg/L.

concentrations of cocaine found in the brain are almost always many times higher than in plasma, even if the measurements are made only hours after drug administration (Spiehler and Reed, 1985; Reith et al., 1987). Bertol et al. found that the brain ratio of cocaine to BZE in 84 cases of overdose was 10.28:1 in brain, but only 0.69:1 in blood. However, in cases where cocaine was only an incidental finding, the ratios were much closer: 0.21 in blood and 0.71 in brain (Bertol et al., 2008).

BZE, the principal metabolite of cocaine, crosses the BBB with great difficulty (Misra et al., 1975). Only two autopsy series have addressed this issue. Years prior to the study by Bertol et al., Spiehler and Reed's autopsy study of 37 patients who had died of cocaine toxicity revealed a mean blood cocaine concentration of 4.6 mg/L (range 0.004–31.0 mg/L), while the mean BZE concentration was 0.88 mg/L (range 0–7.4 mg/L). The mean concentration of cocaine found in the brain was 13.3 mg/kg (range 0.17–31 mg/kg), while that of BZE was 2.9 mg/kg (range 0.1–22 mg/kg). In most cases, the blood–brain ratio was close to 4. In a second study of 14 deaths, where cocaine was only an incidental finding (instances of murder, accidental death, etc.), the average blood–brain ratio was only 2.5 (Spiehler and Reed, 1985).

Measurement of the brain cocaine and BZE concentration ratios generally provides a good indication of what the levels were at the time of death and allows reasonably accurate estimates relating time of ingestion to death. Because any BZE detected in the brain had to have been formed in the brain, it can reasonably be inferred that an individual with a brain concentration of 8 mg/kg of cocaine and 0.5 mg/kg of BZE must have taken the drug just before death. Had a longer period elapsed, cocaine concentrations would be lower and BZE concentrations higher. Patients with excited delirium are usually found to have only modest postmortem blood cocaine concentrations (Mash et al., 2009) but high concentrations of BZE, a result of "binging," where large amounts of cocaine are ingested over several days.

A handful of human case reports suggest that brain concentrations are lower in the fetus than in the mother. In one case report, the maternal-to-fetal brain cocaine ratio was 6.5:1 (Mittleman et al., 1989). In another study of fetal demise, which included 47 cases where both blood and brain cocaine levels were determined, mean blood concentration was 800 ng/mL, while the mean brain concentration was 1100 ng/mg (Morild and Stajic, 1990). Cocaethylene concentrations, both in the fetus and adult, remain poorly characterized, although there are now good methods for measurement of all the cocaine metabolites in this matrix (Lowe et al., 2006).

1.10.3 Hair

Disagreement continues to exist as to the route (or routes) by which cocaine is incorporated into hair, but melanin binding appears to be the most likely (Cone, 1996). There is good evidence for passive diffusion from blood into the hair follicle, which could lead to cocaine incorporation, but hair is coated with sebum, which may also contain just about any drug, including cocaine. This means that the possibility of external contamination, from sebum or some external source of the drug, is ever present (Blank and Kidwell, 1993; Kidwell et al., 2000). This is an important consideration, especially when the hair samples are collected during an autopsy. There is a real chance that fluids and aerosols liberated during the procedure might accumulate on the hair. Whether or not the color of hair or the race of the individual has any effect on the avidity with which hair binds cocaine is also disputed, with some evidence suggesting that it does, although the results of other experiments suggest it does not (Hoffman 1999; Kelly et al., 2000; Lester et al., 2002).

When deuterium-labeled cocaine is given to human volunteers, the parent drug, cocaine, is the predominant analyte detected in hair, in juxtaposition to the situation that occurs in blood, where BZE is the major analyte. The estimated minimum ingested dose required for detection of cocaine in hair is 25–35 mg of drug administered intravenously; a single dose of cocaine is detectable for 2–6 months in hair, although very large intraindividual variation exists, especially in the amount of cocaine detected in the hair. In controlled studies with Caucasians and Asians, Asians generally exhibit much higher concentrations than Caucasians, even though both racial groups had received the same amount of cocaine (Nakahara and Kikura, 1994; Henderson et al., 1996).

In theory, at least, it is possible to calculate a cocaine half-life for hair (Garcia-Bournissen et al., 2009). Cocaine and BZE can be detected in hair of former drug users after several months of abstinence. The calculated half-life for cocaine is over 1 month, which means that approximately 3–4 months would have to pass for hair testing to become negative in the segment proximal to the scalp. Knowledge of this time sequence would facilitate the process of interpreting, or at least trying to interpret, compliance status of abstinence of drug users. It might also confirm histories supplied by suspects in criminal investigations.

All cocaine metabolites can be detected in hair samples, but the parent compound predominates. Like heroin, cocaine has some unique metabolites, the presence of which is pathognomonic for cocaine ingestion. In particular, AEME is indicative not only of cocaine ingestion but also of cocaine smoking (Scheidweiler et al., 2003). Whereas heroin ingestion can be proved conclusively by demonstrating the presence of the unique heroin metabolite, 6-monoacetylmorphine, cocaine smoking can be confirmed by the identification of methylecgonidine, which is only produced during cocaine pyrolysis. Cocaethylene was once thought to be a unique cocaine metabolite, present only when ethanol and cocaine were consumed at the same time but, in fact, cocaine smuggled in alcohol beverages (a common smuggling practice) can be converted to cocaethylene (Casale and Moore, 1994). Thus, the presence of cocaethylene cannot be considered diagnostic for the simultaneous consumption of cocaine and alcohol (Schaffer et al., 2005).

Cocaine concentrations in the hair of active users range from 1 to 5 ng/mg of hair, or even higher (Henderson et al., 1996). Once cocaine is deposited in hair, it is stable for an indefinite period (Baez et al., 2000), a fact that can be of considerable importance should questions about drug use arise after death. The question can be easily answered by exhumation. Cocaine has been detected in the hair of mummified Peruvian coca chewers who died more than 1000 years ago (Springfield et al., 1993). As a practical matter, hair storage requires very little space, and some medical examiners now routinely collect and store samples, analyzing them only should the need arise at a later date.

Determining the presence or absence of tolerance is one of the issues that consistently plagues forensic toxicologists and pathologists. This issue probably applies to all drugs but is particularly troublesome in the case of heroin and cocaine. If a person has been taking heroin for any length of time, heroin or its metabolites are detected in the hair. Tagliaro et al. demonstrated in 1988 that morphine concentrations in the hair of heroin overdose victims are comparable to hair concentrations in former heroin users enrolled in rehabilitation programs and that hair from individuals in both groups contained substantially less morphine than hair from living, active heroin users (Tagliaro et al., 1988).

The results of a recent study suggest that the same can be said for cocaine. When head hair samples from the heads of known cocaine abusers were subjected to an external wash and still remained positive, the concentrations of cocaine and its metabolites

were determined in the hair: concentrations of cocaine, BZE, and EME were 19.7, 8.9, and 1.4 ng/mg, respectively. The mean concentrations of cocaine, BZE, and EME in pubic hair were 19.4, 6.1, and 1.0 ng/mg, respectively. In head hair, the median concentrations were 4.0 ng/mg for cocaine (range < 10 ng total, 384.7), 2.5 ng/mg for BZE (range < 10 ng total, 142.2), and 0.5 ng/mg for EME (range < 10 ng total, 39.5). In 23% of the positive specimens, only cocaine was detected (Cordero et al., 2010). In a 2014 study of 58 chronic cocaine abusers, levamisole was detected in 38% of all cases in concentrations ranging from 0.2 to 0.8 ng/mL (Fucci et al., 2014).

At nearly the same time, another study was published (Hess et al., 2014) using a liquid chromatography–mass spectrometry (LC–MS) method for the determination of levamisole and its metabolite aminorex in human plasma. The study was used to perform the first pharmacokinetic study of levamisole in humans. Both levamisole and aminorex could be detected in the plasma and urine of human volunteers given a 100 mg oral test dose of levamisole, but the observed concentrations of levamisole never exceeded the test's limit of detection.

It is tempting to suppose that reference ranges derived from the study of multiple hair samples could give an indication of the amount of cocaine actually being used by the decedent, classifying the lower quartile as light or occasional users (once or twice a week), the middle two quartiles as regular users, and the upper quartile as heavy users. The lower ranges corresponding to low or occasional use would, of course, imply lack of cocaine tolerance. The middle range corresponding to regular or habitual use (e.g., daily) would suggest but not prove tolerance, while the upper range would correspond to heavy or excessive use and presumed drug tolerance.

Segmental hair analysis can be used to demonstrate external contamination in postmortem cases; however, standard decontamination procedures do not completely remove external contamination in postmortem material. The use of a single hair for analysis is to be discouraged, although homogenous segmental analyses can be probably be used to discriminate between external contamination and long-term exposure, but only with considerable caution, because metabolites can also be present in putrefactive material (Kintz, 2012a; López-Guarnido et al., 2013).

1.10.4 Heart

PET scanning of humans demonstrates a very high uptake of cocaine by the heart. Within 2–3 min after injection, 2.5% of the dose administered appears in the heart, clearing rapidly over the next 10 min. When pharmacologic doses of cocaine are given to baboons, the pattern of uptake and washout is similar to that seen in humans. Even though the cocaine rapidly disappears, inhibition of the NE transporter persists for some time after the cocaine is gone (Volkow et al., 1996). This finding suggests that toxic levels of NE may persist in the heart for some time after the cocaine has been cleared. It may also explain the typical patterns of catecholamine-induced necrosis seen in the hearts of some abusers.

The relatively high uptake by the heart, in spite of the rapid rate at which cocaine is cleared, makes it possible that cocaine could be detected in myocardium at autopsy, especially in chronic users or those who have ingested large amounts of drug. Poklis described a case where death occurred after an intravenous dose of an unspecified amount of cocaine, where the concentration of cocaine in the heart was 6 mg/L while that in the blood was only 1.8 mg/L (Poklis et al., 1985). In a later report, the same author described

two other cases where myocardial cocaine concentrations exceeded 5 mg/L (Poklis et al., 1987). Neither of these reports specifies the postmortem interval, so it is not clear whether myocardium actually accumulates and/or concentrates cocaine or if the values measured are merely the result of redistribution.

1.10.5 Kidneys

In human studies of radioactive cocaine uptake, kidney uptake is greater than that of the heart. Kidney uptake occurs in the renal cortex only and, as in the heart, peak uptake occurs at 2–3 min; after 10 min, half of the dose will have been cleared (Volkow et al., 1992). Autopsy measurements of renal cocaine levels are rarely reported: those that have been ranged from 1 to 28 mg/kg (Lundberg et al., 1977; Di Maio and Garriott, 1978; Poklis et al., 1985), so widely as to be of no probative value. Comparison of the results from different case reports is also impossible because, in most cases, the postmortem interval is not even mentioned. A recently published case report described cocaine concentrations in liver and kidney of the same individual. Unfortunately, the body had been severely traumatized and neither blood nor urine was available for measurement and comparison. Cocaine and BZE measured in the kidney were 127 and 2477 ng/mL, respectively, while concentrations of EME were 2308 ng/mL. Concentrations of the same drugs in liver were 316, 2477, and 2308 ng/mL, respectively (Margalho et al., 2011).

1.10.6 Liver

Hepatic cocaine receptors are present in higher concentrations and have greater affinity for cocaine than those located in the brain (Calligaro and Eldefrawi, 1987). In Volkow's PET studies, hepatic accumulation of drug was very high. Uptake occurred after most of the other organs, but still within 10–15 min after intravenous injection. More than 20% of a given dose reaches the liver and, once there, concentrations remain stable for more than 40 min. The findings of these dynamic studies are generally in agreement with autopsy studies that have also shown high concentrations in the liver.

In the autopsy study reported by Spiehler and Reed (1985), the mean hepatic cocaine concentration in patients dying of cocaine toxicity was 6.7 mg/L, and the BZE concentration was 21.3 mg/L (Spiehler and Reed, 1985). An earlier retrospective study of 15 cases gathered from several centers yielded slightly different results. More cocaine was detected in the blood than in the liver, with a blood/liver ratio of 1.4 (Finkle and McCloskey, 1977). The high concentrations of BZE reported are hardly surprising given that the major pathways of cocaine metabolism involve plasma and hepatic esterase activity. Cocaethylene is also synthesized in the liver, and hepatic cocaethylene levels are much higher than hepatic cocaine levels (Hearn et al., 1991a). Whether or not this concentration difference explains why cocaethylene appears to be more hepatotoxic than cocaine remains an open question (Odeleye et al., 1993).

Hepatic oxidation of the nitrogen atom in cocaine's tropane ring also occurs. The resulting products are *N*-hydroxynorcocaine and the free radical norcocaine nitroxide. Norcocaine can also be found as a contaminant in illicit cocaine, where it is a by-product of the refining process. When potassium permanganate is added to crude cocaine mixtures, norcocaine can also be formed. Norcocaine is believed to be responsible for the hepatotoxicity observed when cocaine is given to experimental animals (Thompson et al., 1979).

Mice pretreated with phenobarbital, which activates their P450 microsomal systems, develop a specific type of hepatic necrosis similar to that induced by norcocaine (Kloss et al., 1983).

Norcocaine can also be detected in humans, possibly as a cocaine contaminant, but only in very small amounts, and it would appear that most norcocaine is formed within the human body. Cocaine is rapidly metabolized to its major metabolites, BZE and EME, and minor metabolites, such as norcocaine, *p*-hydroxycocaine, *m*-hydroxycocaine, *p*-OH-BZE, and *m*-hydroxybenzoylecgonine, in only very small amounts. When 18 healthy volunteers were given subcutaneous injections of cocaine (either 75 mg/70 kg or 150 mg/70 kg), cocaine was detected in plasma within 5 min, with mean ± SE peak concentrations of 300.4 ± 24.6 ng/mL (low dose) and 639.1 ± 56.8 ng/mL (high dose) 30–40 min after dosing. BZE and EME generally were first detected in plasma, 15 min postdose; 2–4 h after dosing, BZE and EME reached mean maximum concentrations of 321.3 ± 18.4 (low dose) and 614.7 ± 46.0 ng/mL (high dose) and 47.4 ± 3.0 (low) and 124.4 ± 18.2 ng/mL (high), respectively. Minor metabolites were detected much less frequently for up to 32 h, with peak concentrations ≤ 18 ng/mL for all analytes except *p*-OH-BZE, which was found in concentrations of up to 57.7 ng/mL. Under normal circumstances, liver production of norcocaine may be considered negligible (Kolbrich et al., 2006).

Interestingly, when human volunteers are given both cocaine and alcohol, they produce more norcocaine than controls given only cocaine. An explanation for this phenomenon is still wanting. It has been suggested that, given enough alcohol, blood pH will drop slightly and PCE will become less efficient, leaving more cocaine to circulate through the liver. No matter how the norcocaine gets into the body, its relationship to hepatic injury in humans is unclear (Inaba et al., 1978). Histologically, lesions similar to those seen in mice have been described in man but are quite rare (Marks and Chapple, 1967; Teaf et al., 1984; Perino et al., 1987; Charles and Powell, 1992).

1.10.7 Skin and Nails and Adipose Tissue

Nails can prove a good forensic matrix for the detection of abused drugs. Indeed, the international literature reports the use of nail analysis in a number of settings, including postmortem drug detection, drug testing in the workplace, and drug screening to detect prenatal exposure. Basic drugs, including those that are not thought of as being very lipid soluble, accumulate in the epidermis, dermis, and subcutaneous fat (Tables 1.7 and 1.8). Even the stratum corneum, the outermost layer of the skin, which consists entirely of keratinocytes that have lost their nuclei, still binds drugs.

Table 1.7 Concentrations of Cocaine and Its Metabolites Measured in Adipose Tissue in a Cocaine-Related Death

	Adipose 1	Adipose 2	Adipose 3	Adipose 4
Weight (g)	0.90	0.92	1.93	2.1
Cocaine (ng/g)	446	411	409	469

Source: Data adapted from Levisky, J. et al., *Forensic Sci. Int.*, 110, 35, 2000. With permission.

Note: The decedent was an unrestrained 37-year-old female driver involved in a single-vehicle rollover accident. Toxicologic examination revealed the presence of cocaine and BZE in the blood (no urine was available). Heart blood (right side) cocaine and BZE concentrations were 198 and 2734 ng/mL, respectively. Cocaine (931 ng/g) and BZE (2093 ng/g) were identified in the adipose layer of the skin removed from the abdominal region.

Table 1.8 Values from a Typical Cocaine-Related Death

	Adipose (ng/g)	Skin (ng/g)	Blood (ng/g)
Cocaine	220	259	341
Cocaethylene	348	188	209
Benzoylecgonine (BZE)	111	Negative	2903
Ecgonine methyl ester	Present	Negative	Present

Source: Data adapted from Levisky, J. et al., *Forensic Sci. Int.*, 110, 35, 2000.

Cocaine can enter the skin either via the circulation or as a result of external contamination. In a controlled study of cocaine and heroin users, all of whom were housed in a locked metabolic ward where no drugs were permitted, cocaine and BZE remained present in most stratum corneum specimens collected over a 3-week "washout" period, even though the volunteers were not receiving any drugs at all. When drugs were given, low concentrations quickly appeared in the stratum corneum and remained there for at least 2 weeks (Joseph et al., 1998). In other studies, cocaine, BZE, and THC (or 11-*nor*-delta-9-tetrahydrocannabinol, THC-COOH), when applied directly to the skin, remained detectable there for up to 3 days and were not removed by simple cleansing with isopropanol (Kidwell et al., 1997).

Most cocaine metabolites, including anhydroecgonine, can be extracted from the nails (Ragoucy-Sengler and Kintz, 2005). Several methods have been used and all can simultaneously resolve cocaine, BZE, and norcocaine (Ragoucy-Sengler and Kintz, 2005). This methodology seems complementary to hair testing but, like hair testing, it is subject to outside contamination and false-positive tests. Toenails provide a much better testing matrix than fingernails, because positive results from drug handling are unlikely.

Fingernails and toenails contain melanin (exactly the same structure as keratin found in hair). The results of preliminary studies suggest that nail drug testing may prove to be a more sensitive postmortem detector of cocaine use than either urine or blood. When fingernail and toenail specimens obtained from 18 suspected cocaine users were extracted in methanol and then purified by solid-phase extraction, nail analysis demonstrated past cocaine use even when other methods did not. Cocaine or one of its metabolites was present in 14 (82.3%) of the suspect cocaine users, but only 5 (27.7%) were found positive by conventional postmortem drug analysis. As is the case with hair and sweat, cocaine itself is the main analyte detected in nails, but smaller quantities of BZE are also found in all specimens positive for cocaine. Other metabolites, including AEME, EME, cocaethylene, norcocaine, and norbenzoylecgonine, may, or may not, be detected (Garside et al., 1998).

When the toenails of 46 decedents were tested for cocaine, BZE, norcocaine, and cocaethylene concentrations of cocaine and BZE ranged from 0.20 to 140.17 ($n = 20$) and 0.30 to 315.44 ng/mg ($n = 21$), respectively. Norcocaine concentrations of 6.78 and 0.66 ng/mg and cocaethylene concentrations of 2.60 and 0.73 ng/mg were detected in two specimens (Engelhart et al., 1998). Cocaine can also be detected in the finger- and toenails of infants and children. One case report described finding cocaine in the nails of a 3-month-old with SIDS (Skopp and Pötsch, 1997). It is now established that nearly 20% of SIDS deaths are associated with, and probably the result of, genetic mutations (Wilders, 2012), so very little can be inferred about causation based on these results.

Basic drugs can also be extracted from the underlying dermis. The dermis is composed of fibrous, vascularized connective tissue, hair follicles, and sweat and sebaceous glands.

The number of sweat and sebaceous glands and the output of sweat and sebum found in the dermis vary by location. The hands are the most richly supplied but sweat glands in any location are capable of excreting drug-containing sweat and sebum onto any part of the skin. In autopsy studies, drug concentrations measured in the dermis were comparable to those measured in blood (Levisky et al., 2000).

The adipose layer beneath the dermis consists of lobules of fat separated by fibrous connective tissue septa. Lipophilic drugs including diazepam, nordiazepam, meprobamate, and alprazolam all have been detected in skin and adipose tissue. In the case of heavy marijuana smokers, THC has been shown to remain detectable in fat for up to 28 days after smoking (Johansson et al., 1989). With other drugs, the detection time may be even longer. Measurable amounts of the drug terbinafine (an orally administered fungicide) can be found in plasma, serum, sebum, hair, nails, dermis/epidermis, and stratum corneum for more than 6 weeks (Faergemann et al., 1991). Persistence of THC in fat stores makes the interpretation of low postmortem blood THC concentrations very difficult, because the THC may have been released into the blood only after death.

Special considerations apply to the fetus. Human newborns rely on energy that is stored in brown fat, and that energy is liberated when catecholamines bind to the receptors on the fat. The brown fat of experimental rats avidly takes up cocaine. The high uptake is probably explained by rat nuchal (neck) brown fat that is richly supplied with sympathetic nerve terminals (Som et al., 1994). The children of cocaine-using mothers no doubt have detectable levels of cocaine in their fat stores, but measurements have not been reported, or at least not in humans.

The mechanism of uptake is not completely understood, but leptin, which is produced in several organs in addition to white adipose tissue, brown fat, the placenta, and fetal tissues (such as heart and bone/cartilage), almost certainly plays a role. The production of leptin by white fat is at least partly controlled by inhibitory β-adrenoceptor agonists that bind to β_3 adrenoceptors, and cocaine does not do that. White fat production is also controlled by cocaine–amphetamine-regulated transcript (CART), a neuropeptide that also serves as a neurotransmitter. In animals, it produces the same behavior as cocaine and amphetamine (Trayhurn et al., 1999). CART peptides, in particular CART (55–102), seem to have an important function in the regulation of energy homeostasis, which no doubt explains the connection with fat mobilization. CART expression is regulated, in turn, by several different peripheral peptide hormones, including leptin (Murphy, 2005), cholecystokinin, and ghrelin.

1.10.8 Biofluids

1.10.8.1 Amniotic Fluid

When cocaine and its metabolites were measured in amniotic fluid and umbilical cord tissue taken at birth, from 32 cocaine-abusing women, the main analyte detected was BZE. It was found in 28.1% and 18.5% of the amniotic fluid samples and umbilical cord tissue specimens, respectively. Measurable concentrations of EME and *m*-hydroxybenzoylecgonine were also present in amniotic fluid specimens, while umbilical cord tissues were found to contain mainly EME, norcocaine, and *m*-hydroxybenzoylecgonine (Winecker et al., 2001). In the only case report yet published on the subject, cocaine and BZE were quantitated in amniotic fluid, umbilical cord blood, and neonatal urine from children of cocaine-using mothers. BZE concentrations were 290 ng/mL and cocaine levels were 70 ng/mL

in the amniotic fluid. Levels of BZE and cocaine were much higher in the newborns' urine, although cocaine levels were roughly similar. Neither cocaine nor BZE was detected in umbilical cord blood (Jain et al., 1993).

1.10.8.2 Breast Milk

An estimated 5%–10% of American women use cocaine during pregnancy, yet the issue of cocaine in mother's milk is poorly studied. Cocaine certainly can be transferred to infants via mother's milk (Chasnoff et al., 1987) but even after 25 years the kinetics in humans are unclear and the relevance of experimental models, at least in terms of pathology and pharmacokinetics, has not been demonstrated. This may be because so many factors can alter final concentration measurements (Table 1.9). In human milk, both cocaine and cocaethylene are bound mainly to albumin. In fact, up to 55% of cocaine and up to 61% of cocaethylene found in milk is protein bound. It has been speculated that protein binding and the lower pH of milk relative to serum (6.9 vs. 7.4) may enhance the mammary secretion of cocaine and cocaethylene into the milk, exposing the child when it nurses (Bailey, 1998). Cocaine binds only weakly and nonspecifically to milk lipids.

Chasnoff et al. (1987) studied kinetics in a single case after a 0.5 g dose of cocaine. The peak cocaine concentration in the milk was 40 ng/mL, occurring 12 h after administration, and the drug was completely cleared from the milk by 36 h (Chasnoff et al., 1987). In a second, more recent case report, the milk cocaine concentration was 8 ng in one woman 6 days after injecting an unknown amount of cocaine (Sarkar et al., 2005). Presuming that the measurements reported by Chasnoff were typical for all women, a mother who consumes half a gram of cocaine per day while producing 1 L/day of milk would be delivering 0.48 mg/day to her child (0.09% of the dose consumed by the mother). Most experts agree, and clinical evidence confirms, that such a low dose poses little risk to a child (Sarkar et al., 2005). Similar measurements have been reported for other psychotropic drugs (Koren

Table 1.9 Factors Controlling Drug Transfer into Breast Milk

General factors

a. Molecular weight of drug: The lower the molecular weight, the more likely it is that a drug will appear in mother's milk; thus cocaine is readily taken up.

b. Acid–base dissociation constant (pKa): The higher the pKa (i.e., the more basic the molecule), the more likely it is that a drug will appear in mother's milk; thus, cocaine is readily taken up. Methadone and heroin have a higher pKa than cocaine and are more likely to be present in large amounts than cocaine.

c. Protein binding: The more a drug binds to a mother's plasma proteins, the less there is to enter the milk. Cocaine is >90% bound to maternal plasma proteins, making entry into milk unlikely.

d. Lipid solubility: Drugs that are highly lipid soluble will diffuse into milk. Cocaine is lipid soluble and so will diffuse into milk.

Maternal factors

a. Amount ingested: The more drug consumed, the more will end up in the milk.

b. Maternal liver or kidney disease: Disease may affect the mother's ability to metabolize or excrete the drug properly. Drug will accumulate and enter the milk.

c. Milk fat content: The higher the fat concentration of hind milk, the more fat-soluble drugs can be expected to appear.

d. Enzyme polymorphisms: If the mother is an abnormal metabolizer, either too much drug may accumulate or not enough form. Polymorphisms affecting cocaine metabolism are recognized, but there is no clinical evidence.

Source: Adapted from D'Apolito, K., *Clin. Obstet. Gynecol.*, 56(1), 202, 2013.

et al., 2006) but as a recent case report makes clear, the existence of genetic polymorphism may alter the picture (Madadi et al., 2007).

Winecker et al. (2001) reported finding milk concentrations of 12 μg/mL—100 times higher than earlier studies (Winecker et al., 2001). If correct, this would amount to the delivery of much more substantial amounts of cocaine—a total dose of nearly 50 mg/day, enough to raise legitimate questions about toxicity—but the observation has not been repeated, the study is more than a decade old, and it may well be that methodology accounts for the differences. At the time, it was not appreciated that drug concentrations in the first milk produced tend to be much higher than in the "hind" milk. The only way to get an accurate concentration measurement is to collect and analyze all of the milk produced over a 24 h period.

The consequences of such exposure, whether to 5 or 50 mg in 24 h, have not been established and will be difficult to characterize and to separate from the use of other drugs and environmental contaminants. Women who use cocaine during their pregnancy also use ethanol, cigarettes, and other drugs, all of which may be excreted in the milk. Indeed, one case report describes finding nicotine, codeine, and oxycodeine along with cocaethylene in the milk of one admitted cocaine user, and no drug in the milk of another woman who was also an admitted drug user (Dickson et al., 1994). Nursing mothers have been charged with assault, endangerment, and even manslaughter for the administration of drug-tainted milk, but in none of the cases that have come to trial has the mother, or her milk, ever been tested for the offending drug (Ariagno et al., 1995). Recently, when a reliable LC–MS–MS methodology was used to analyze milk samples obtained from a milk bank, and from a cocaine-addicted mother, cocaine was the only analyte found (concentration: 5 ng/mL), with BZE and other metabolites altogether absent (Marchei et al., 2011).

1.10.8.3 Fetal Gastric Aspirates and Meconium

The developing fetus swallows cocaine-containing amniotic fluid. Gastric aspirates taken from the newborn contain unpredictable amounts of cocaine and/or cocaine metabolite. In one study, cocaine was detected in nearly half (45.5%) of gastric aspirate samples collected from infants in whom meconium was positive for cocaine. This approach would seem to have little to offer over meconium testing, though it will provide a specimen that is easier to handle (O'Connor et al., 1996). Meconium testing itself is probably the most reliable matrix for fetal screening. In a recent study, cocaine concentrations were measured in placenta, meconium, and thrice-weekly second and third trimester urine specimens from 19, 15, and 17 participants, respectively. Only one matched umbilical cord and placenta specimen was positive for cocaine (7.3 ng/g in placenta) and/or its metabolite BZE (442.4 and 458.9 ng/g in umbilical cord and placenta, respectively), whereas prenatal exposure to cocaine was confirmed in 11 matched meconium specimens based on the presence of the cocaine metabolite, mOHBE. Urine tests were positive for cocaine in the second or third trimester in eight of these women; however, there was actually one confirmed cocaine user whose urine specimens were negative for cocaine on every test. In all matrices, cocaine metabolite concentrations were higher than the parent drug concentration (de Castro et al., 2011).

1.10.8.4 Oral Fluid (Saliva)

Saliva is also called oral fluid because it is a mixture of saliva and gingival products, crevicular fluid, debris of the oral mucosa, and bacteria. However, the bulk of the fluid consists of the output of the submandibular glands (approximately 70%) and the parotid glands (25%).

The total volume of fluid produced ranges from 500 to 1500 mL/day. Saliva contains very little protein, so unbound drugs in the plasma appear in almost the same concentrations in plasma as they do in saliva.

Saliva offers advantages over serum because collection is noninvasive, requires very little training, and is virtually risk-free. Gland-specific saliva can also be used for diagnosis of pathology specific to one of the major salivary glands. In addition, saliva harbors a wide spectrum of proteins/peptides, nucleic acids, electrolytes, and hormones arising from multiple local and systemic sources, and all of these can be measured for diagnostic purposes (Pfaffe et al., 2011).

Because cocaine is weakly basic and saliva is normally more acidic than plasma, the concentration of ionized cocaine in saliva may be as much as five times higher than plasma cocaine concentrations. For the same reasons, concentrations of BZE are two to three times higher in plasma than in saliva (Thompson et al., 1987; Cone and Menchen, 1988). Saliva cocaine concentrations correlate well with plasma concentrations. Concentrations in both saliva and blood correlate equally well with behavioral and physiologic effects. The half-life of cocaine in both fluids is also the same, on the order of 35 min. Five hours after a 40 mg intravenous bolus of cocaine, cocaine concentrations in both saliva and blood are near the limits of detection (29 ng/mL for saliva and 8 ng/mL for plasma). When cocaine is detected in saliva, it is very good evidence of very recent use (Cone and Menchen, 1988; Ferko et al., 1992).

Even though cocaine is the predominant analyte in saliva, BZE and EME have much longer half-lives (2.3–6.5 h), so both metabolites will accumulate in saliva with repeated dosing. The relative proportions and absolute concentrations of cocaine and its metabolites are highly dependent upon the method used to collect the saliva.

Stimulated saliva (saliva collected after the donor has been given a piece of sour candy) tends to contain much less drug than nonstimulated samples (Kato et al., 1993). Cocaethylene can also be detected in saliva (Cognard et al., 2006). In general, oral fluid testing seems to produce results equivalent to those obtained with urine testing and has the additional virtue of providing a longer window of detection (Cone and Weddington, 1989).

The detection of low concentrations of cocaine in the saliva is certainly consistent with past cocaine use, but it is not necessarily diagnostic of recent ingestion. During cocaine withdrawal, lipophilic storage sites in the brain continue to release cocaine. Small amounts of cocaine can appear in saliva and urine for weeks. Rats given 20 mg/kg of cocaine a day, twice a day for 2 weeks, have measurable cocaine levels in their fat. It is reasonable to assume the same occurs in humans. Chronic human cocaine users who are monitored during withdrawal will continue to excrete unmetabolized cocaine, detectable by radioimmunoassay, for 10 days or more after their last dose (Cone and Weddington 1989; Weddington, 1990).

1.10.8.5 Spinal Fluid

Measurements of spinal fluid might prove useful, especially since cocaine crosses the BBB so readily, but very few studies have been performed in humans. One case report described finding unmetabolized cocaine within the CSF at 24 h, but there has been no follow-up to the original study (Rowbotham et al., 1990). In 2013, Chilean researchers tested for cocaine and BZE in multiple matrices. While reasonable amounts of cocaine and BZE were detected in spinal fluid (537 and 3132 ng/mL, respectively), concentrations in femoral

blood were many times higher (3210 and 19,875 ng/mL, respectively) (Alvear et al., 2013). The very large concentration differential between matrices suggests spinal fluid can be useful, but not for screening purposes.

1.10.8.6 Urine

Cocaine is eliminated almost entirely by biotransformation. It has a renal clearance of less than 30 mL/min (Chow et al., 1985). The primary entities detected in the urine are cocaine and BZE. In a study of otherwise healthy drug addicts in treatment, the median concentrations of cocaine and BZE equivalents were 235 and 14,900 ng/mL, respectively, but in some instances, the maximum concentrations were many times higher (112,025 ng/mL of cocaine and 1,101,190 ng/mL of BZE in at least one instance) (Preston et al., 1998). Great intraindividual variation exists, and the route by which cocaine has been taken has an important effect on the proportion of metabolites produced and then excreted.

When single bioequivalent doses of cocaine were administered by intravenous, intranasal, and smoked routes to six volunteers, and all urine was collected for 3 days, peak cocaine concentrations occurred in the first specimen collected and thereafter fell to 1 ng/mL (the limit of detection) within 24 h. BZE was the most common metabolite detected (39%, 30%, and 16%, after intravenous, intranasal, and smoked routes, respectively) (Cone et al., 2003).

Other metabolites present in much smaller amounts were EME and six minor cocaine metabolites (norcocaine, norbenzoylecgonine, *m*-hydroxycocaine, *p*-hydroxycocaine, *m*-hydroxybenzoylecgonine, and *p*-OH-BZE). Taken together, the minor metabolites accounted for as much as 18% of the original dose when it had been given intravenously and as little as 8% when it had been smoked as "crack" (Weddington, 1990). When six volunteers smoked 40 mg of cocaine, maximum urinary concentrations for cocaine, BZE, and EME occurred at 2.2, 6.6, and 5.6 h, respectively. The last positive urine test for cocaine (10 ng/mL cutoff) occurred 55 h after smoking, but BZE (20 ng/mL cutoff) and EME (10 ng/mL cutoff) continued to be detectable for 106 and 164 h, respectively (Huestis et al., 2007).

Chronic abusers continue to excrete cocaine and its metabolites for much longer than occasional users. Cocaine's plasma half-life is longer in chronic users than it is in naïve ones, and the same holds true for urinary excretion. Most standard references indicate a 48 h excretion time for BZE, cocaine's principal metabolite, but several published reports have described different results. In one study, performed with three volunteers, BZE remained detectable in the urine at concentrations above the 300 ng/mL cutoff for more than 120 h, while cocaine itself was found in the urine for up to 24 h (Preston et al., 2002).

In a second study from the same group (Moolchan et al., 2000), volunteers who had used cocaine daily for at least 1 month were observed on a closed metabolic ward. On average, the group had been regularly using cocaine for more than 7 years and consuming, on average, 1 g/day. All were "crack" smokers who neither injected nor snorted cocaine. The mean age was 36.7 years. The group included 10 women and 8 men, with the last episode of cocaine use having occurred within 24 h of admission to the closed unit. All urine samples (n = 953) were collected, frozen, and batch analyzed. As would be expected, all the participants tested positive for urine BZE at the start of the study, with concentrations ranging from 630 to 277,709 ng/mL urine at Time 0. After the first 24 h had elapsed, BZE concentrations had decreased to only one-third (33.5%) of the

concentrations at Time 0. At 48 h, concentrations had dropped to 7.7% of the original value, and at 72 h, BZE concentrations had fallen to 3.6% of the Time 0 levels.

Unlike cocaine, BZE excretion fluctuates and does not decrease in a linear fashion. For example, at 48 h, four of the volunteers in the second study discussed earlier had urine concentrations that were above the standard 300 ng/mL cutoff, but only two of these same individuals tested positive 24 h earlier. Sixteen of the study participants remained in the locked ward for the completion of the study. For those individuals, the elapsed mean time from admission until the first urine that tested negative for BZE was 43.6 ± 17 h (range 16–75 h). The mean time until the last positive test (i.e., when all further tests were negative) was 57.5 ± 31.6 h.

While the numbers cited earlier are no doubt accurate, it may prove that none of the analytes discussed is as sensitive or as accurate as measurement of ecgonine. Urinary excretion of ecgonine peaks later than all the other metabolites. Following low doses of 10–45 mg cocaine (results with all routes of administration seem the same), detection times extended up to 98 h (using a 50 ng/mL cutoff concentration). The T_{max} was independent of dose, and route of administration did not have a significant impact on C_{max} or T_{max} for metabolites. This suggests that perhaps it might be better to test for ecgonine, rather than one of the other metabolites, as it is stable and can be detected in the urine, at above cutoff levels, for a much longer time (Huestis et al., 1992).

Chronic cocaine users who stop taking cocaine will continue to excrete cocaine metabolites for at least 2 days, and even after they finally do test negative for BZE, the next day they may well test positive again, and they will almost certainly remain positive for ecgonine.

In cases of cocaine-related sudden death, urinary cocaine concentrations may well exceed concentrations of BZE or other metabolites (Ramcharitar et al., 1995; Karch et al., 1998). Most, but certainly not all, commercial screening tests are designed to detect the cocaine metabolite BZE and not cocaine itself. Some cross-reactivity may exist, but antibody-based screening tests generally do not detect cocaine or other metabolites such as EME in the urine, even if they are present, which explains the false general impression that cocaine does not appear to any significant degree in the urine. In fact, detection of cocaine in the urine is a sign of recent ingestion, especially in occasional users. The absence of cocaine, on the other hand, is only evidence that the drug has not been taken within the last few hours (Jatlow, 1988).

Hospitalized patients undergoing detoxification continue to excrete metabolite for weeks after their last dose of cocaine (Weiss and Gawin, 1988; Burke et al., 1990; Weddington, 1990; Preston et al., 2002). The same caveats that apply to saliva and blood testing also apply to urine. It should also be apparent that no conclusion can be drawn about the degree of impairment, if any, at the time of urine testing. The presence of metabolite indicates only that the drug was used in the past.

The same is true for the newborn. The elimination half-life of cocaine and BZE in the newborn has been measured. The half-life of BZE during the first day of life, based on plasma measurements in 13 subjects, was found to be 16 h (95% CI, 12.8–21.4 h). The half-life of BZE during the first week of life, based on urine data from 16 subjects, was 11.2 h (95% CI, 10.1–11.8 h) (Dempsey et al., 1998). It should go without saying that there is no fixed relationship between plasma cocaine or cocaine metabolite concentrations and the amount that appears in the urine. The blood/urine ratio may be much greater or much less than 1, and the concentrations measured in the urine may vary greatly depending on the

individual's state of hydration and renal function. Attempts at relating urinary concentrations to impairment or toxicity have been characterized as "pure folly" (Jones, 1998).

1.10.8.7 Vitreous Humor

When cocaine is measured simultaneously in blood and vitreous, good correlation has been demonstrated. With reasonably short postmortem intervals, cocaine concentrations are higher in vitreous fluid in nearly three quarters of the published cases. Blood concentrations of cocaine were higher in cases where acute intoxication was apparent. On the other hand, nearly 80% of the time, BZE was higher in blood than in vitreous, sometimes by a factor of 10. Table 1.10 shows values in more than a dozen cases where cocaine and BZE were measured simultaneously in vitreous and blood (Antonides et al., 2007). In the most recent report, both cocaine and BZE were easy to detect in vitreous, but at concentrations only 1/10 as high as femoral blood (Alvear et al., 2013).

1.10.8.8 Sweat

Cocaine and its metabolites are excreted in sweat. Cocaine first appears in sweat within 1–2 h of administration (Huestis et al., 1999), and concentrations of up to 100 ng/mL of cocaine have been recorded. Sweat testing is a noninvasive technique for monitoring drug exposure and it is used increasingly in an assortment of medicolegal settings. Proprietary sweat-collection devices are available for this purpose. Cocaine is the main analyte found in sweat, but EME and BZE can also be detected, with EME generally detected in higher concentrations than BZE. In experimental studies, cocaine and EME were found to be detectable within 2 h of drug use; however, BZE is not detected until 4–8 h after low doses and only slightly sooner after high doses. The majority of the drug is cleared from the sweat

Table 1.10 Cocaine and Benzoylecgonine Concentrations (in µg/mL) in Blood and Vitreous Humor

Cocaine		Benzoylecgonine	
Bld Cal	Vit Cal	Bld Cal	Vit Cal
0.01	0.01	0.30	0.29
0.01	0.01	0.37	0.37
0.04	0.05	0.41	0.41
0.05	0.06	0.42	0.42
0.10	0.11	0.61	0.64
0.21	0.23	0.87	0.94
0.23	0.25	1.10	1.20
0.26	0.28	1.30	1.40
0.29	0.27	1.65	1.38
0.32	0.29	1.70	1.40
0.33	0.31	2.00	2.20
0.53	0.58	2.40	2.60
		2.60	2.20
		3.90	3.30
		5.20	5.90

Source: Antonides, H.M. et al., *J. Anal. Toxicol.*, 31(8), 469, 2007.
Abbreviations: Bld Cal, blood calibration curve; Vit Cal, vitreous calibration curve.

within 24 h (Kacinko et al., 2005). Of course this option is not available at autopsy, but swabbing the skin with saline-soaked cotton pledgets may be productive. Using LC–MS–MS, cocaine was demonstrated in 92% of the sweat patches applied to pregnant cocaine users. However, only 15 women were tested, and the intraindividual variation was enormous (1–1420 ng/patch), which, for all intents and purposes, makes estimating time of abuse impossible (Concheiro et al., 2011).

1.10.8.9 Semen

Cocaine appears in semen and may enter the circulation of both mother and child. There is evidence that the fetus may be exposed to chemicals in semen, either (1) by access of chemicals to the maternal circulation after absorption from the vagina or (2) by delivery to the egg of chemicals, including cocaine, that have become bound to the sperm cell. Direct chemical exposure following transport from the vagina to the uterine cavity has not been demonstrated in humans. Seminal fluid chemical concentrations are typically similar to, or lower than, plasma concentrations. Assuming total absorption of a seminal dose of a chemical with a high semen/blood concentration ratio, blood concentrations in the woman would be many orders of magnitude lower than in the man. The presence of cocaine in or on human sperm cells has been demonstrated. Based on in vitro studies, cocaine oocyte concentrations would be five orders of magnitude lower than blood concentrations associated with cocaine abuse (assuming of course that cocaine-laden sperm were capable of fertilizing) (Klemmt and Scialli, 2005).

The forensic value of semen testing is questionable. Prostatic fluid is more acidic than plasma and is likely to cause ion trapping of cocaine. On the other hand, vesicular fluid is more alkaline than plasma and the effects may cancel each other out. Only one controlled study has ever been performed and it is now over a decade old. Five volunteers were given variable amounts of cocaine (from 1 to 42 mg) intravenously and via insufflation. In most cases, the semen cocaine to plasma cocaine ratio approached unity. Ejaculate volumes ranged from 1.8 to 2.7 g, while the semen cocaine content ranged from 0 to 81 ng/g. In no case did the total of cocaine and BZE in the ejaculate exceed 0.001 mg at 1 h (after which the value would be declining because of cocaine's short half-life). Thus, it is extremely unlikely that enough cocaine and BZE could be transferred to a sexual partner for them to test positive. Other studies have shown that positive tests can be expected after a dose of 1–2.5 mg (Jenkins et al., 1996). Nonetheless, even using liberal assumptions about transmission of chemicals in semen or sperm, predicted exposure levels for a pregnant woman are three or more orders of magnitude lower than blood concentrations in the man whose semen is the putative vehicle for chemical transport (Klemmt and Scialli, 2005).

1.11 Electrophysiology of Sudden Death in Cocaine Abusers

Most cocaine-related deaths can be attributed to the occurrence of a lethal arrhythmia. Before arrhythmia can occur, two elements must be present: (1) a susceptible myocardial substrate and (2) a triggering mechanism. Collectively, the different anatomic changes providing the substrate are referred to as myocardial remodeling, a complex cascade of interconnected events (Burchfield et al., 2013). The term seems to imply that these changes are visible, but the remodeling process also involves invisible changes of the molecular structure of cellular receptors as well (Swynghedauw, 1999). Triggering mechanisms are provided by

environmental perturbations, either internal or external (Lippi et al., 2010). The substrate/trigger concept applies to most cases of sudden cardiac death, even in nondrug users.

Common substrates include chemical toxins, diseases (such as myocarditis), and genetic polymorphism, where single nucleotide polymorphisms occur in key enzymes and receptors. The occasionally fatal channelopathy called Brugada syndrome occurs when there are mutation(s) in the *SCN5A* gene encoding the cell membrane channels that control sodium flow (Remi, 2008). If key portions of the ion channel are distorted, the ionic currents constituting the action potential may be disrupted, although many other abnormalities can disrupt the action potential as well. Not only is cocaine a sodium channel blocker (which explains its anesthetic effects), but it also interacts with other types of ion channels including one region of the potassium channel known as hERG and also L-type calcium channels (Premkumar, 1999; Karle and Kiehn, 2002; Premkumar, 2005). The most prominent visual manifestation of acute cocaine toxicity is CBN (see below). A decade ago, most cases of long QT syndrome (LQTS) were thought to be due to genetic aberration. In fact, most instances are a consequence of drug interaction with the hERG potassium channel, that is, they represent an acquired form of LQTS (Bauman and DiDomenico, 2002). Cocaine cardiotoxicity cannot be understood without some appreciation of possible substrates and triggers and the way in which they interact (Shah et al., 2005). A brief introduction follows. Specific types of cocaine-related heart disease are then discussed in Section 1.11.4.2.

1.11.1 QT Dispersion and QT Prolongation

The electrocardiogram can provide at least two surrogate markers for the presence of a proarrhythmic substrate. If present, these markers can be a useful diagnostic tool when confronted with unexplained sudden death in a cocaine abuser. The first and least understood surrogate is QT interval dispersion (dispersion is defined as the time elapsed between the start of the Q-wave and the end of the T-wave, as illustrated in Figure 1.32). The QT interval is different in each lead of the cardiogram. QT dispersion is calculated by subtracting the shortest QT interval from the longest; the greater the degree of dispersion, the greater the chance of arrhythmia (Gamouras et al., 2000). QT dispersion is defined as the time difference between the longest and the shortest QT interval measured on the same 12-lead EKG, but only after each interval has been corrected for heart rate. At a glance, it should be apparent that the higher the heart rate, the shorter the r–r interval, which in turn alters the QT interval by making it shorter. If the QT interval were simply measured without accounting for heart rate, it would always be underestimated at higher heart rates, and cocaine use accelerates the heart rate (Saadeh, 2004).

The second, and much better characterized biomarker, is QT interval prolongation. The presence of dispersion implies the existence of actual physical alterations within the heart, especially concentric hypertrophy of the left ventricle. QT prolongation implies the existence of an abnormal polarization–repolarization cycle that may, or may not, be accompanied by myocardial hypertrophy. Both surrogates are important and powerful predictors of sudden cardiac death. A third marker, the presence of which cannot be inferred from the EKG, is called repolarization reserve (Roden, 2006).

At least four different QT correction formulas are in use. Unfortunately, the most widely used is the Bazett formula. It is incorporated into the software of most hospital EKG machines and it consistently overestimates the length of the QT interval when the

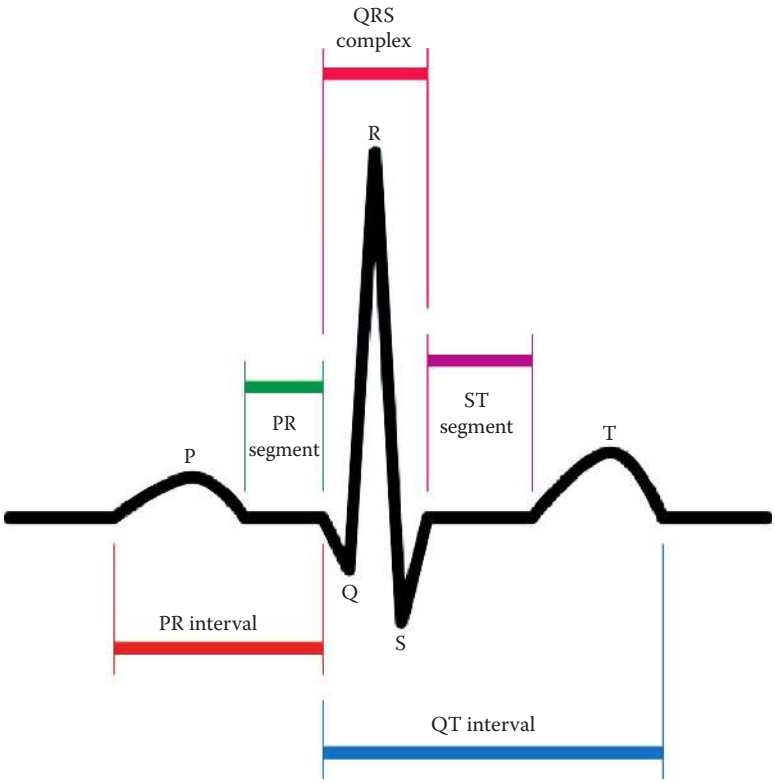


Figure 1.32 The QT interval is a measure of the time required for repolarization to occur. When the QT interval exceeds 440 ms, the probability increases that a lethal arrhythmia may be induced. Myocardial hypertrophy can lead to QT prolongations, as can some inherited diseases and some drugs, including cocaine. Basically, any drug that inhibits CYP450 causes the QT interval to exceed 500 ms, which means there is a serious potential for interaction with other drugs being given at the same time (Armahizer et al., 2013). Note that the blue line represents the QT interval in this normal EKG. See also Kannel et al. (1969) and Cheng et al. (1991). This idealized drawing of the QT interval was taken from Wikipedia.

heart rate is high. No matter which correction formula is used, QT dispersion is markedly increased when the left ventricle is enlarged (Ichkhan et al., 1997). Pathologists seldom hear the term QT dispersion mentioned because it is a metric used by clinicians. That is mainly because the methodology for determining QT dispersion varies significantly among researchers and partly because the clinical utility of this measurement has not been conclusively proven.

The weighted mean degree of QT dispersion in normal volunteers, participating in the largest study yet published, was 33.4 (±20) ms, rising to 65.6 ± 38.5 ms in patients with left ventricular hypertrophy, and 83.2 ± 71.9) in patients with known channelopathies (LQTS) (Malik and Batchvarov, 2000). But because cocaine binds to both the hERG potassium channel (Karle and Kiehn, 2002) and the sodium channel and induces myocardial hypertrophy via a different mechanism, it is very likely that QT dispersion would be detectable in symptomatic cocaine abusers. If dispersion is present, the depolarization wave will take longer to traverse the thickened ventricle, and the action potential will be further delayed by hERG interaction, sodium channel blockade, and myocardial hypertrophy (Guo et al., 2006; O'Leary and Hancox, 2010).

Dispersion of more than 80 ms is associated with 3.5 times the normal mortality rate, and a QTc > 440 ms is associated with 8 times the normal mortality rate (Antzelevitch, 2005). When confronted with an unexplained cocaine-related death, examination of a recent EKG with a cardiologist, should one be available, might provide valuable diagnostic clues as to the cause of death.

Technical reasons make consistent measurement of QT interval difficult, but an accurate determination of QT duration is important because QT prolongation is associated with a lethal form of ventricular tachycardia called torsades de pointes. QT prolongation may be congenital or acquired (Kapa et al., 2009). In the acquired form, prolongation is the result of systemic medical disorders such low potassium, low magnesium, or even HIV infection. More often than not, however, QT prolongation is the result either of a drug–hERG channel interaction, or of left ventricular hypertrophy, or both (Malik and Batchvarov, 2000).

The heritable form of QT prolongation is relatively uncommon, though not rare. It arises from mutation of one of several genes that tend to prolong action potential duration and lengthen the QT interval. The most common causes of LQTS are mutations in the genes *KCNQ1* (*LQT1*), *KCNH2* (*LQT2*), and *SCN5A* (*LQT3*). Without genetic resequencing, it is impossible to distinguish death from cocaine–hERG interaction or underlying genetic anomaly. However, tests for LQTS genes are now available from commercial laboratories and, with the advent of "NextGen" resequencing, the costs of these tests have been radically reduced.

Just what constitutes normal QT duration is not entirely clear. In one very large study of male soldiers (n = 41,767), the mean corrected QT interval (using the Bazett formula) was 394 ± 22 ms. One percent of the soldiers had a corrected QT shorter than 347 ms, and one percent had a corrected QT longer than 445 ms (Kobza et al., 2009). If the corrected QT interval is prolonged, then cardiac repolarization is, by definition, abnormally delayed. As a result, the action potential is propagated to neighboring cells at different rates due to the differences in the length of the refractory period of each individual cell, increasing the probability of myocardial arrhythmia (Kauferstein et al., 2009).

1.11.2 Myocardial Remodeling

The phrase "myocardial remodeling" was first used to describe changes that occur within the myocardium after myocardial infarction; however, all of the changes seen in remodeling are also seen in the hearts of chronic stimulant abusers, even if they have not sustained an infarction. The remodeling process involves individual cardiomyocytes, their supporting network of collagen fibers, and the ion channels that penetrate into the cardiomyocytes. Remodeling is the method by which the heart adapts when it is confronted with changing volume or pressure overload (Burchfield et al., 2013).

The remodeling process is initiated after myocardial infarction has occurred, largely with the goal of maintaining cardiac output even though viable muscle has been lost. As a consequence, hypertrophy of remaining cardiac muscle results. At first, hypertrophy is an adaptive process, an appropriate way of compensating for loss of heart muscle. At some point, however, the remodeling process becomes destructive, myocardial collagen content increases, cardiac function deteriorates, and the ventricle becomes fibrotic (Figures 1.33 through 1.36) (Abbate et al., 2006; Lafontant and Field, 2006). Myocardial fibrosis is very strongly associated with the generation of reentrant tachyarrhythmias and the occurrence

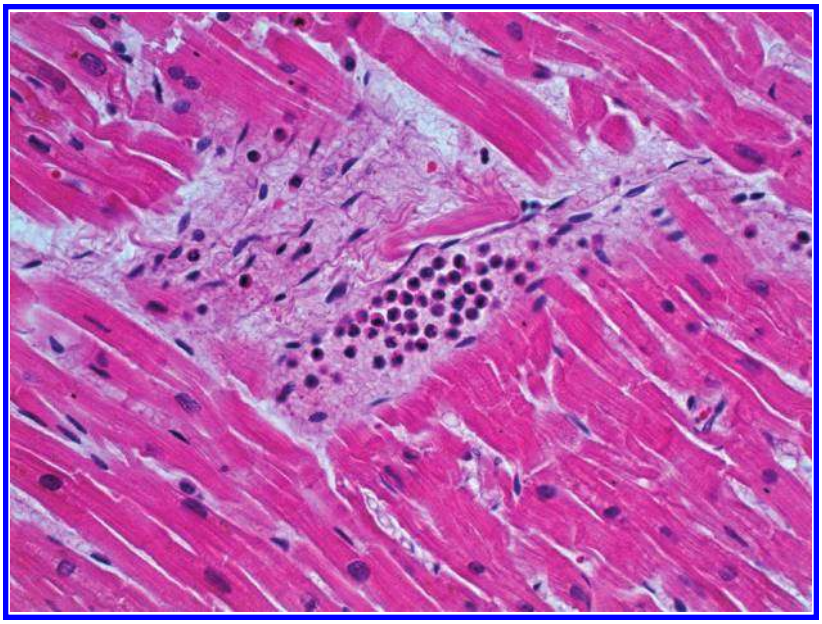


Figure 1.33 Active myocardial remodeling in a cocaine user with sudden death. Cytokine-damaged myocytes release cell contents attracting fibromyocytes that secrete other cytokines resulting in further cellular destruction. Here, invading macrophages are removing some of the debris.

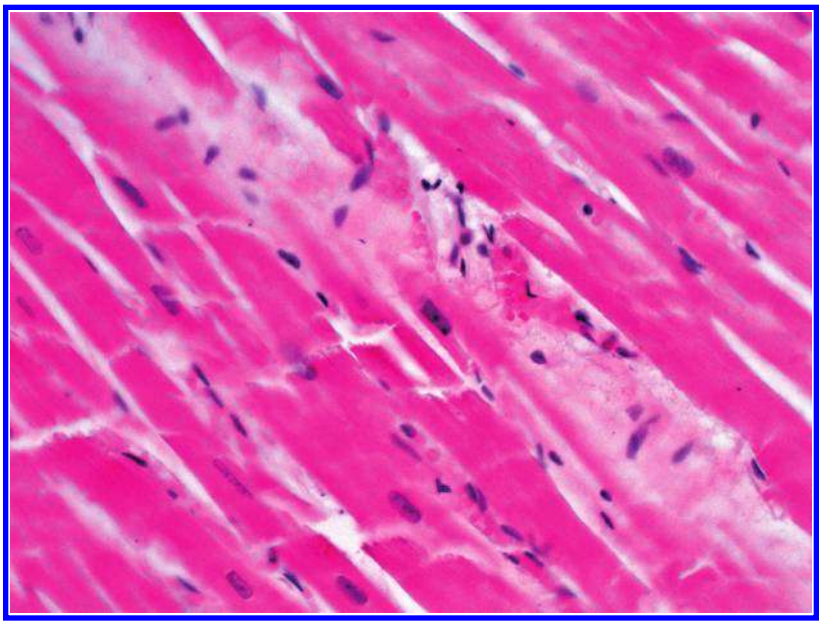


Figure 1.34 Early stages of myocardial remodeling/fibrosis showing thickened cardiomyocytes with "boxcar" (squared off)-shaped nuclei. All of the nuclei are cigar shaped and elongated. There are already areas of replacement fibrosis toward the center of the field. The myocytes are also thickened and there are scattered areas of autophagy throughout. Some of the fibers damaged are undergoing dissolution and new interstitial fibers are forming.

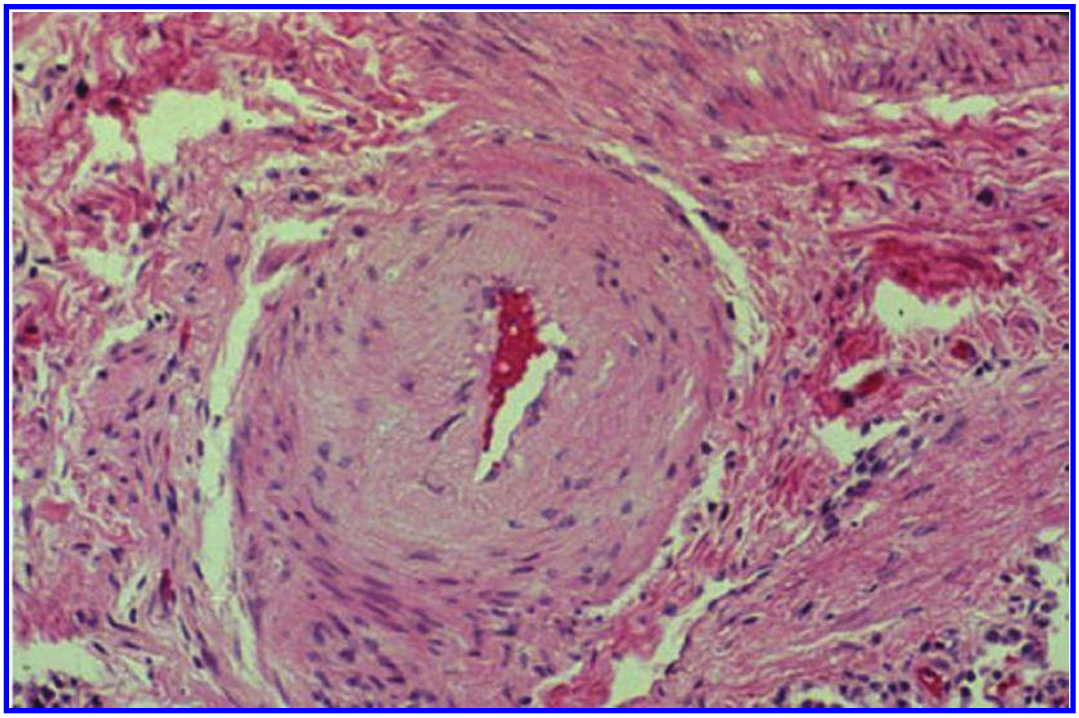


Figure 1.35 Perivascular fibrosis. The slide shows appearance with Luxol fast blue stain. Reparative fibrosis can occur either between cells or around blood vessels as seen here. The bluish areas of fibrosis may cause reentrant arrhythmias, and they may also restrict myocardial blood flow by preventing flow-mediated vessel dilation.

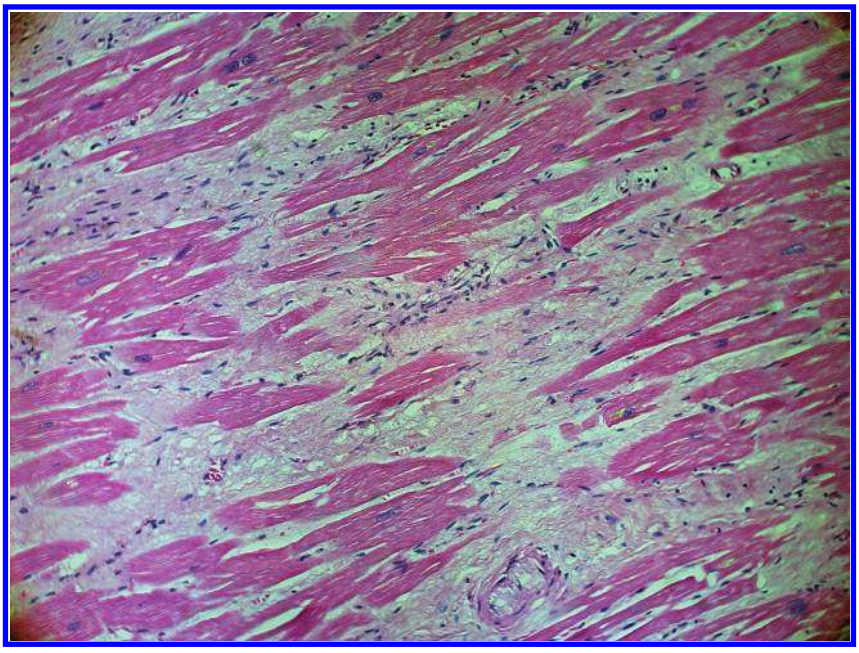


Figure 1.36 Massive interstitial fibrosis. Areas of fibrosis envelop the remaining intact cardiomyocytes. The interface between normal cells and nonconducting fibers in the interstitium provides the substrate for generation of reentrant arrhythmias.

of sudden death (El-Sherif et al., 1977; Weber, 1997), particularly along the boundary between the surviving muscle and forming scar. One practical consequence of this established cause–effect relationship is that "mild" interstitial fibrosis should never be dismissed as a possible cause of death. Its presence must be noted and duly considered as a possible cause of death.

All three of these processes occur normally in healing infarcts. However, chronic exposure to cocaine produces exactly the same changes within the heart, in addition to causing disease of the small myocardial arteries (see below and John et al., 2004).

1.11.2.1 Myocardial Hypertrophy

There are only two ways that the heart can enlarge: concentrically or eccentrically. Concentric hypertrophy occurs when stimulated cardiomyocytes synthesize new sarcomeres and then add them side to side to existing sarcomeres. The end result is an increase in wall thickness (most accept 1.3 cm, measured at midleft ventricle, as the upper normal limit of normal thickness). If, instead, the new sarcomeres are added end to end, rather than side to side, eccentric hypertrophy results and myocytes elongate instead of thickening (Grossman et al., 1975).

Concentric hypertrophy is the classic change associated with hypertension and aortic stenosis, two conditions that lead to pressure overload and decreased cardiac output; wall thickness increases, but heart chamber size remains unchanged. Concentric hypertrophy is also associated with the occurrence of apoptosis (self-induced cellular autodestruction). An extreme example of this phenomenon is shown in Figure 1.37.

Eccentric enlargement comes about for a completely different set of reasons. It is the result of volume overload (chiefly mitral regurgitation) or intense aerobic activity that

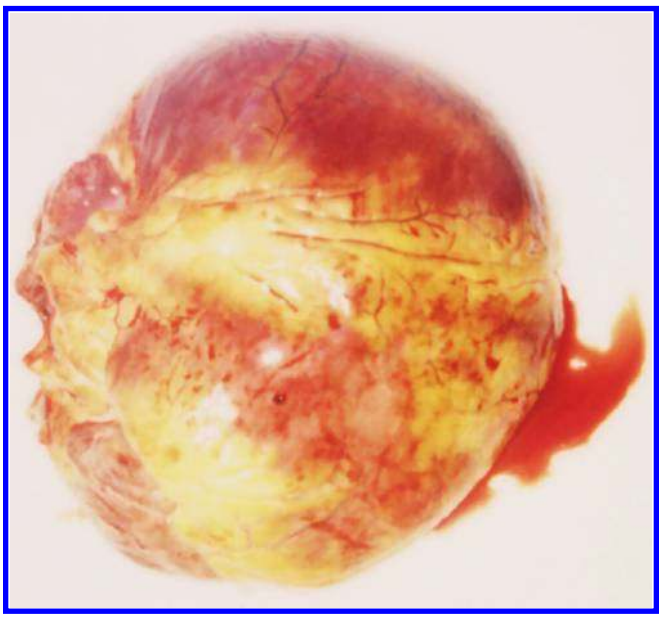


Figure 1.37 Cor bovinum. The occasional cocaine user is found to have massive myocardial hypertrophy, sometimes referred to as "cor bovinum." The heart shown here weighed over 1200 g. The decedent succumbed during his second episode of excited delirium. (Courtesy of Dr. Kari Blaho, University of Tennessee, Memphis, TN.)

requires increased cardiac output (de Simone, 2004; Osborn et al., 2007; Bernardo et al., 2010; Scharf et al., 2010). Concentric hypertrophy confers an increased risk for sudden death (Haider et al., 1998), but eccentric hypertrophy does not (de Simone, 2004). A 500 g heart in a competitive athlete is unlikely to be a cause of death. A 500 g heart in a sedentary hypertensive 45-year-old woman is very likely to be the cause of death.

There are conflicting theories as to why this state of affairs should exist, but two most important factors seem the most likely: (1) Concentric hypertrophy leads to QT dispersion and (2) concentrically hypertrophied myocardium is ischemic. Indeed, it has been demonstrated that all concentric hypertrophy is ischemic, partly because the enlarged myocytes are relatively underserved by their arterial supply and partly because the small vessels themselves are diseased (Nadruz, 2012).

In general, the process of angiogenesis lags behind the process of hypertrophy. This lag in vessel production is often apparent in the subendocardium. Morphometric studies have shown that the distance between myocytes and the nearest blood vessel is increased in various forms of cardiomyopathy, including ischemic and hypertensive cardiomyopathy, and that the distance between vessels and myocytes is much greater than in a normal heart (Mosseri et al., 1986; Yarom et al., 1990; Mosseri et al., 1991). There is simply less blood to supply more muscle. It is also becoming increasingly obvious that the myocardial remodeling process also involves dysplasia of small, intramyocardial vessels (Figures 1.38a and b, and 1.39). This change can lead to myocardial ischemia from endothelial disease, even in the absence of overt epicardial coronary artery disease (CAD) (Mosseri et al., 1986).

Methamphetamine abuse also causes concentric left ventricular hypertrophy (Karch et al., 1995, 1999a,b), even in the absence of risk factors such as hypertension. Hypertrophy occurs because an enzyme called calcium/CMKII becomes activated. Once activated, a signaling cascade is initiated, which eventually leads to the addition of more sarcomeres until sufficient muscle mass is created (Figure 1.40).

Myocardial hypertrophy often goes undiagnosed during life, particularly in people who are significantly overweight (body mass index [BMI] > 30). Larger people have larger hearts, and men's hearts are larger than those of women, while many different forms of heart disease are associated with some degree of cardiac enlargement. The hearts of the obese are enlarged because obesity brings about hemodynamic changes; both stroke volume and cardiac output must increase to compensate for added body weight. Obesity also limits the sensitivity of the EKG voltage criteria normally used to make the diagnosis of left ventricular enlargement (Jennings, 2010). It is critically important to determine and record heart weight at autopsy because heart weight is an independent risk factor for sudden cardiac death (Kannel and Abbott, 1986; Messerli and Soria, 1994).

A 10% increase in heart weight is likely to go unrecognized at autopsy, and probably even on echocardiography. In life, absent serial studies, an increase in heart weight of only 40–50 g (myocardial mass measured ultrasonically) is unlikely to lead to significant change in left ventricular mass index (LVMI—heart weight in grams/height in meters). This index is not sensitive to modest increases in weight, nor is ultrasonography likely to detect a small increase in wall thickness. Even if wall thicknesses were fastidiously measured at several sites during the autopsy, which is seldom the case, the increase would most likely go undetected. Weighing of the heart could disclose smaller heart weight increases, but because there is no universally accepted way to determine heart weight, differences in methodology are likely to obscure the presence of small increases (Zaglavara et al., 2005). Indeed, it is not possible to speak of a single "normal" weight, only an acceptable range.

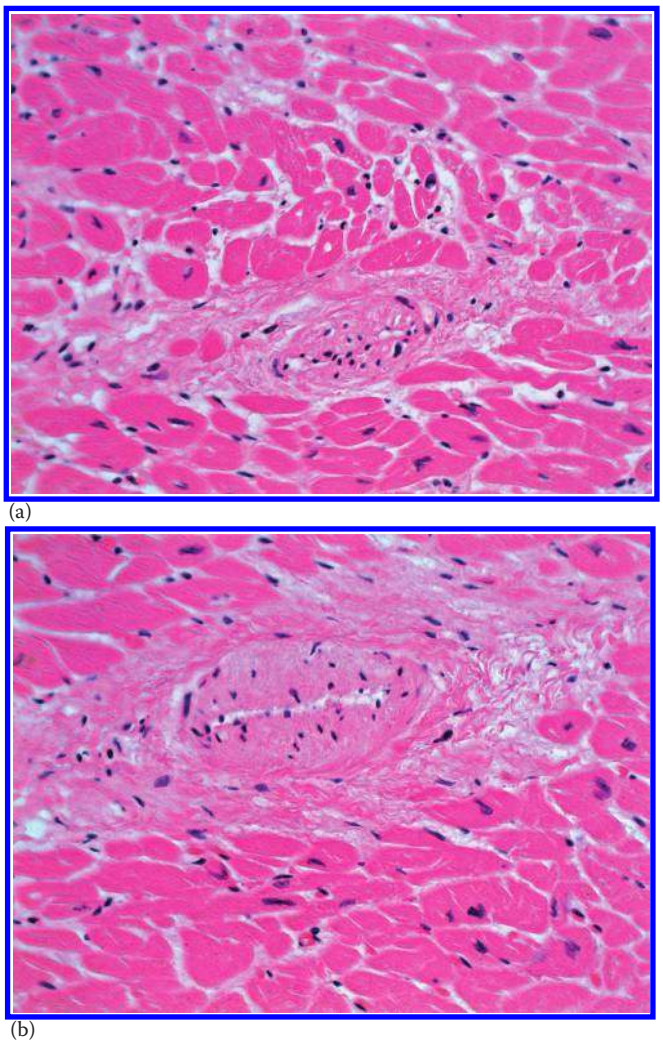


Figure 1.38 (a and b) Disease of the small intramyocardial vessels is particularly common in cocaine abusers and can result in ischemia, even when the epicardial vessels are normal. Generally, flow is reduced because of medial hypertrophy (only three cases involving intimal hypertrophy have ever been reported) within the small vessels, but rarely may be secondary to intimal hyperplasia, or both. Both illustrations are from a chronic cocaine user. Note also the vacuolization of many of the muscle cells. This is a common finding in many different varieties of cardiomyopathy.

Nearly a dozen autopsy studies have addressed the issue of normal heart weight measured at autopsy (Smith, 1928; Zeek, 1942; Reiner et al., 1959; Eckner et al., 1969; Hutchins and Anaya, 1973). In the past, pathologists often used arbitrary cutoffs: 380–400 g for men and 350 g for women, but that practice has become less and less frequent. More often than not, pathologists simply state the heart weight without comment, and only rarely compare measured weight to any of the published nomograms. For the present, the most reliable way to determine whether the heart is, or is not, normal is to use the Mayo Clinic nomogram. It relates heart weight to body height (see Appendix D; Kitzman et al., 1988), but its value is somewhat limited by the fact that wide deviations from the means are apparent

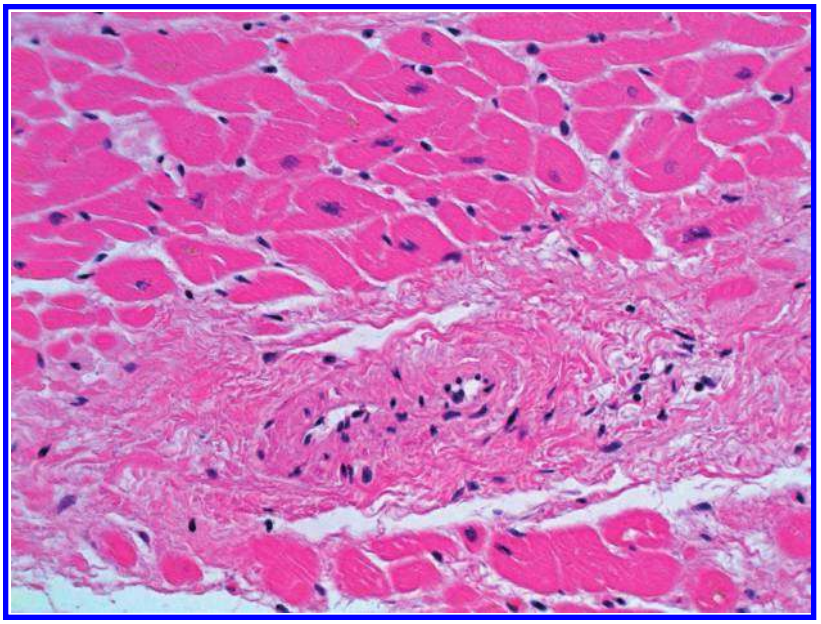


Figure 1.39 Smaller intramyocardial vessels develop medial hypertrophy, sometimes nearly occluding the vessel lumen. When this change is combined with perivascular fibrosis, there is decreased flow-mediated vasodilation, resulting in ischemia. Infarction in the face of normal coronary arteries is possible.

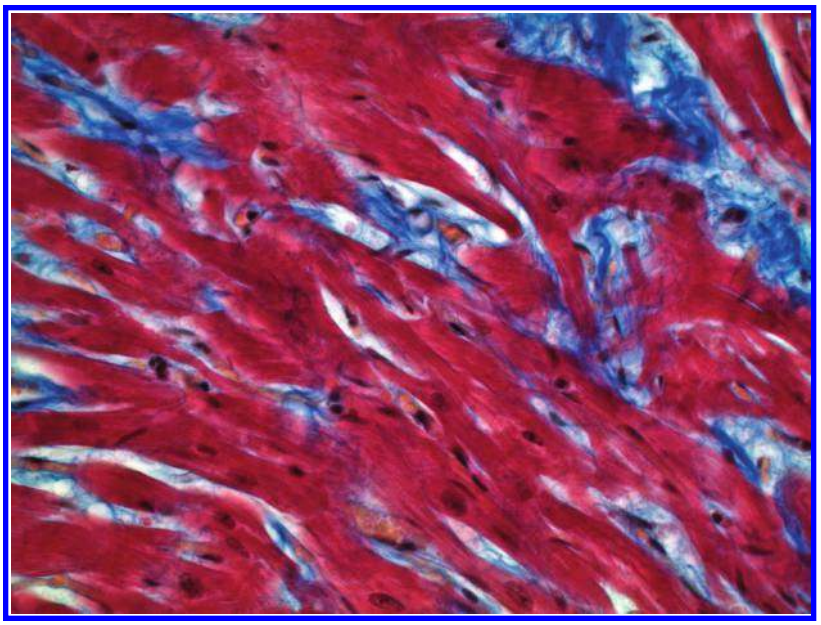


Figure 1.40 Myofiber disarray. This anatomic change, though classically associated with hypertrophic cardiomyopathy, can also be seen when there is catecholamine excess.

at every height. Some prefer to use the much older Zeek nomogram because the standard of deviation is smaller (Appendix E; Zeek, 1942). In a study of 232 cases, mostly trauma deaths, Molina and DiMaio (2012) found the average age was 29 years, the average height 173 cm, and the average weight 76.4 kg (168 lb). Regression analysis disclosed no relationship between body weight, body mass, and body length to allow for predictions. They suggested a reference range (95% inclusion) of 233–383 g for the adult male human. This does seem reasonable, but the total number of patients studied was smaller than the Mayo study and the study may be underpowered.

There are a number of reasons for supposing that nothing like a "normal" heart weight will ever be established. Particularly in the case of chronic stimulant abusers, the heart weight will have been altered by chronic drug exposure. A heavy cocaine abuser who had not indulged for several days might have a negative toxicology screen, and the pathologist would have no way of knowing that the observed heart weight reflects underlying disease. Not a single one of the nomograms takes toxicology into account. It seems clear that whoever makes the next attempt at establishing "normal" will be required to include hair testing in the protocol.

1.11.2.2 Electrical Remodeling

The second component of the remodeling process is electrical. It begins with the activation of genes in the cell nucleus and extends to the level of the ion channel. Changes occur in the number and function of β receptors. To compensate for the decreased cardiac output, more β receptors are made available to respond to catecholamines (Perrino et al., 2005). At the same time, potassium channels, which control myocardial depolarization, begin to function less efficiently (Swynghedauw, 1999; Furukawa and Kurokawa, 2006). Together the electrical and structural alterations favor the occurrence of sudden cardiac death, because ion channel alterations can also lead to QT prolongation, reentry, and sudden death (Kang, 2006; Charpentier et al., 2010), even if there are no apparent alterations in the myocardium.

Electrical signals in all biological systems are transmitted by the flow of inorganic ions (Na^+, K^+, Ca^{2+}) through pores that penetrate the cell membrane. Each pore is composed of proteins that rapidly change shape so that they can open and close the pore in response to biological signals (usually a change in transmembrane voltage or interaction with a neurotransmitter such as NE). This process of pore opening and closing is referred to as "gating," literally because the pore acts as a gate that opens and closes, allowing the ingress and egress of different ions during the various phases of electrical depolarization. When a gate remains open, ions continue to flow and the gate is described as activated. The term "inactivation gating" is applied when the channel remains closed (Swynghedauw, 1999). Failure of the pore to reopen, for whatever reason, may disrupt electrical conduction. The end result is indistinguishable from any heritable LQTS syndrome.

The function and expression of heart ion channels continuously responds to the hemodynamic state of the cardiovascular system and epinephrine and NE exert additional effects on the channels. These homeostatic forces act through multiple mechanisms at transcriptional, translational, and posttranslational levels, but malfunction at any of these levels can also cause arrhythmias. A great deal is known about the way ion channels work, but much less about the underlying controlling mechanisms and how they malfunction during remodeling and disease. One reason that so little is known is that all protein structures, including proteins that comprise the substructure of ion

gates, contain numerous polymorphisms, and deciding which polymorphism caused which abnormality can be difficult or impossible. In heritable LQTS, gating defects can prolong the time it takes cardiomyocytes to recycle after each heartbeat, leading to prolongation of the QT interval and possibly even TdP. Or they may cause the opposite ("short QT syndrome"); when the QT interval is shorter than normal, the risk for TdP is increased (Figure 1.41) (Shah et al., 2005).

The most studied component of the myocardial repolarization cycle is called I_{kr}, an abbreviation for "the rapid repolarizing inward potassium channel," also known as the delayed potassium repolarization current. The gene governing this is called hERG (a contraction of "human ether à-go-go related channel," so called because when the gene was inserted into fruit flies and the flies anesthetized with ether, their legs made motions that appeared, to the experimenters at least, like go-go dancing) (Figure 1.42). I_{kr} interactions between hERG and cocaine have been recognized for several years, but there appear to be unique differences between the way cocaine interacts with hERG and what happens when

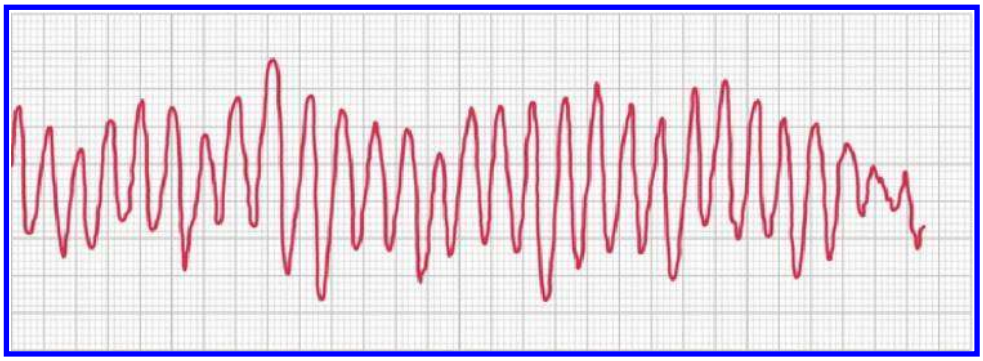


Figure 1.41 This EKG strip illustrates the arrhythmia associated with QT interval prolongation, either acquired or inherited. The pattern is called TdP (literally twisting of the points) as the constantly changing amplitude of the waves gives a sinusoidal appearance.

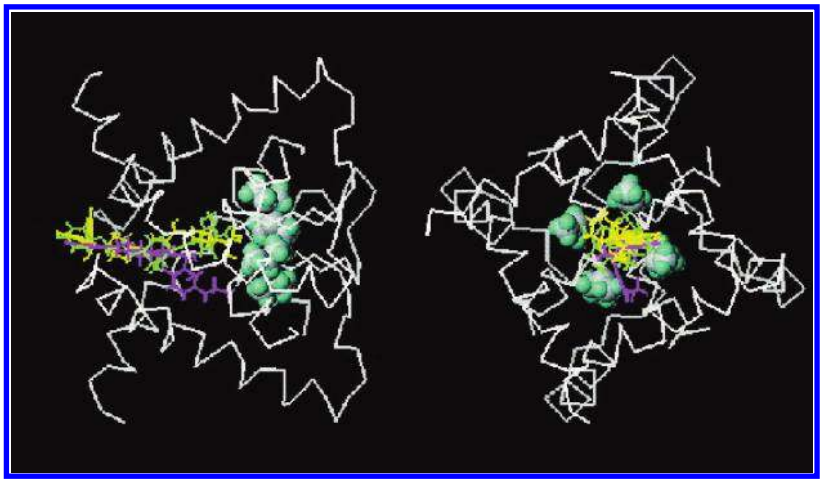


Figure 1.42 hERG is an abbreviation for the "human ether-à-go-go"-related gene. It is a gene that codes for a protein known as $K_v11.1$, which forms the alpha subunit of potassium ion channels. This figure is reconstructed from x-ray diffraction studies and shows the channel in three dimensions.

other drugs interact with the same channel. Most other drugs can interact with hERG only when the channel is open, but cocaine's ability to bind to the hERG channel has little if anything to do with whether the channel is open or closed (O'Leary 2001; Guo et al., 2006). Most drugs that have been withdrawn from the market in recent years have been withdrawn because of unexpected hERG reactions leading to QT prolongation and sudden cardiac death.

1.11.2.3 hERG Components

The late repolarization phase of the action potential is mainly influenced by the rapid component of the delayed rectifier potassium current, I_{kr}. Drugs that inhibit this current prolong the action potential and lead to QT prolongation. However, in cardiomyocytes, but not necessarily other myocytes, the hERG current has a second component, I_{ks}, that exerts exactly the opposite effect to hERG, limiting increases in action potential duration. This ability of I_{ks} to compensate partially for prolonged repolarization caused by I_{kr} inhibition is referred to as repolarization reserve. The greater the "reserve," the less likely it is that QT prolongation and TdP will occur (Roden and Yang, 2005). Drugs that do not cause TdP do nothing to deplete this reserve. It has only recently been discovered that testosterone shortens I_{ks} and therefore is potentially antiarrhythmic (van Noord and Rodenburg, 2011).

In experimental studies, the I_{ks} current is increased by beta stimulation. Heart preparations perfused with isoproterenol (a pure beta agonist) manifest little change in the QT interval, meaning that sufficient repolarization reserve exists to prevent QT prolongation. But, for reasons that are not understood, cocaine caused blockade of I_{kr} also decreases I_{ks}. The net result is that the QT interval is, indeed, prolonged (Overholser et al., 2008). Cocaine use causes plasma catecholamine concentrations to increase with concomitant alpha- and beta-adrenergic receptor activation. The result should be an increase in I_{ks}, but this effect is diminished because, at the same time, cocaine blocks I_{kr}. Cocaine, like some other drugs, decreases repolarization reserve, favoring QT prolongation and QT dispersion.

1.11.2.4 Fibroblasts and the Interstitium

The final component of the remodeling process involves changes that occur in the space between myocytes, called the interstitium. The interstitium of normal hearts contains mostly type I collagen fibers, which serve to tether muscle cells, fibers, and blood vessels while, at the same time, supporting these same structures. When blood pressure overloading leads to concentric hypertrophy, collagen in the interstitium must remodel in order to enhance tensile strength. At first, remodeling is adaptive, but eventually the process can set off a train of damaging tissue responses.

Many of the functional effects of cardiac fibroblasts are mediated via differentiation to form a myofibroblast phenotype expressing contractile proteins but also exhibiting increased migratory, proliferative, and secretory properties. Cardiac myofibroblasts respond to proinflammatory cytokines (e.g., TNFα, IL-1, IL-6, TGF-β), vasoactive peptides (e.g., angiotensin II, endothelin-1 [ET-1], natriuretic peptides), and hormones (e.g., noradrenaline), the levels of which are all increased in the remodeling heart. Their function is also modulated by mechanical stretch and changes in oxygen availability (e.g., ischemia and reperfusion). Myofibroblast responses include changes in cell proliferation, cell migration, extracellular matrix metabolism, and the secretion of various bioactive molecules including cytokines, vasoactive peptides, and growth factors (Porter and Turner, 2009) (Figure 1.43).

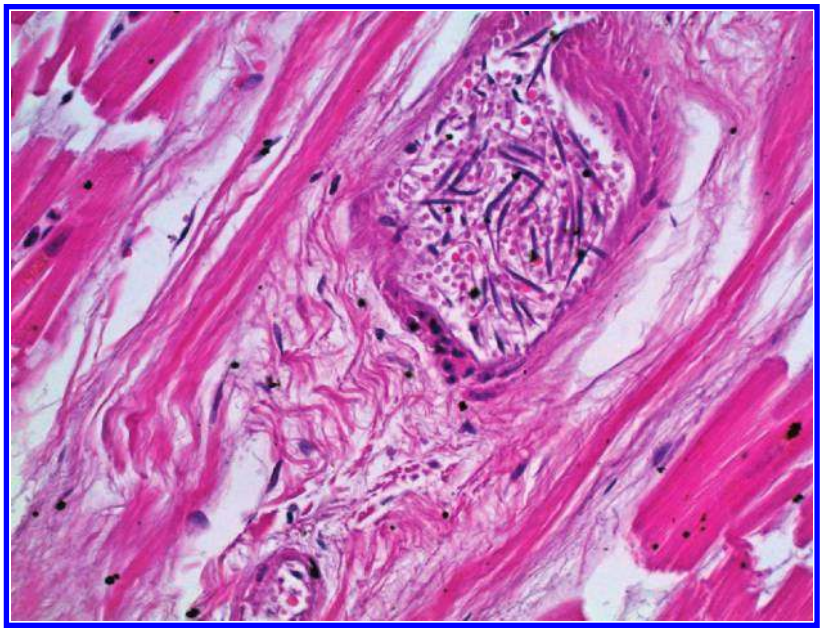


Figure 1.43 Cardiac fibroblasts are the most prevalent cell type in the heart (approximately 70% of cells), and they play a key role in normal myocardial function as well as in the adverse myocardial remodeling that occurs with hypertension, myocardial infarction, and stimulant abuse. During the remodeling process, fibroblasts differentiate to form contractile cells called myofibroblasts. These cells can migrate, proliferate, and, perhaps most importantly, secrete numerous cytokines and hormones that in turn lead to fibrosis within the interstitium.

The most distinctive histologic change seen in the hearts of stimulant abusers (and hypertensives) is interstitial fibrosis, where collagen is deposited between cells. This is not an "all or nothing" process, and the transition from normal myocardium to interstitial fibrosis is gradual and progressive. At some point in the process, however, sufficient fibrosis will be produced to supply the substrate for a lethal arrhythmia. Progressive fibrosis is illustrated in Figure 1.36.

After myocardial infarction, neoangiogenesis within the infarcted area is one component of the remodeling process, but the capillary network that arises is unable to support the greater demands of the hypertrophied myocardium. The result is progressive loss of viable tissue, infarct extension, and even more fibrous replacement. Collagen begins to accumulate between the cells, especially in the inferior wall of the left ventricle. As a consequence, myocardial stiffness increases, cardiac output declines, and there is shift in collagen structure from type 1 to type 3 (Kocher et al., 2001). Chronic stimulant abuse leads to the same sequence and an ever-accelerating cycle of cellular self-destruction.

It had always been presumed that the remodeling of stimulant abusers' hearts was a consequence of excessive catecholamine stimulation (Trouve et al., 1990; Chen et al., 2005), and it is true that the pattern of stimulant-induced injury looks nearly identical to the cardiomyopathic changes seen in pheochromocytoma. But, in fact, catecholamine excess is not solely responsible for the tissue destruction. Catecholamines influence the extracellular matrix, causing collagen deposition that ultimately leads to fibrosis around the arterial wall and within the myocardium (Galetta et al., 2010). But, the lowly fibroblast may be the key to much of the tissue damage that occurs during the remodeling process (Porter and Turner, 2009).

1.11.3 Electrical Vulnerability

Millions of people use cocaine every month. Given that cocaine interacts with the hERG channel, it is reasonable to ask why more deaths do not occur. The answer is clear: more than simple hERG dysfunction is required for death to occur. There must also be abnormal signal dispersion, implying the existence of myocardial remodeling. Most individuals who use cocaine do not consume enough cocaine on a regular basis to initiate the remodeling process. If a myocardial infarction does occur, it is most likely the consequence of preexisting, undiagnosed coronary artery disease combined with cocaine-induced coronary artery spasm, as cocaine induces modest, but not extreme vasospasm. In Lange's controlled human studies, where cocaine was injected directly into the heart's circulation, vasospasm did occur but never exceeded 15% (Lange et al., 1989; Pitts et al., 1998; Vongpatanasin et al., 1999).

Cocaine causes a predictable increase in myocardial work, as measured by double product (systolic blood pressure × heart rate). As double product increases, so does oxygen demand. Infarction may occur in the absence of either thrombus or plaque rupture (Figure 1.44) (Kobayashi et al., 2009), simply because cocaine use can cause coronary vasospasm even in the very occasional user (Lange et al., 1989). However, it may also occur as a consequence of ischemia within the hypertrophic muscle mass. Because the myocardium is hypertrophic and ischemic, ventricular dysfunction, ventricle and structural remodeling, and abnormal impulse propagation all take place at the same time, thereby providing the necessary substrate for arrhythmias to occur, especially if a hERG blockade is in place. If that was not sufficient explanation, it is also necessary to consider cocaine's central effects, which can be expected to lead to more stimulation of the peripheral sympathetics, further catecholamine release, and even greater oxygen requirements (Kies et al., 2011).

Other scenarios are also possible. An electrolyte imbalance (e.g., diuretic therapy for hypertension) may exist, or the neurohumoral system might already be activated.

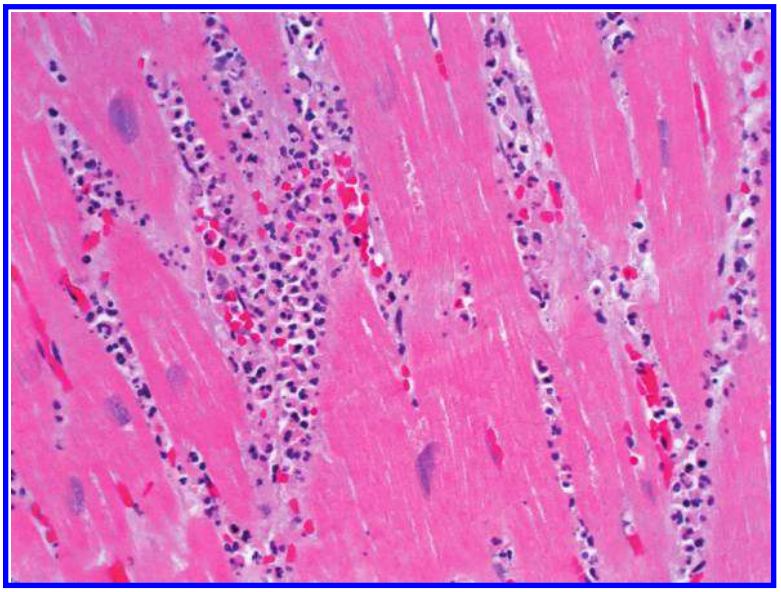


Figure 1.44 An area of microinfarction within the left ventricular free wall. Areas of microinfarction are often found in the hearts of stimulant abusers, presumably a result of increased sympathetic tone and vasospasm. They are not, however, diagnostic, as any patient maintained on vasopressor support may display similar findings.

Both mechanisms are thought by many to account for the sudden death of chronic stimulant abusers who develop the ExDS. If enough stimulant, either cocaine or methamphetamine, has been abused to cause excited delirium, it is highly probably that remodeling is already well established. Sudden death may then occur, which would not occur in a structurally normal heart, when catecholamine concentrations become elevated secondary to an intense physical encounter or altercation; remodeling is the substrate and catecholamines excess, perhaps together with hERG blockade, the trigger (Shah et al., 2005). This is, however, only a hypothesis. There are no experimental data that would confirm or refute this scenario. Death is also more likely if the hERG channel itself is abnormal in some way (polymorphic), a situation that turns out to be more common than had previously been thought (Koo et al., 2006; Opie et al., 2006; Chung et al., 2007).

1.11.4 Catecholamines and Action Potentials

When the sympathetic nervous system has been activated, both epinephrine and NE are released into the coronary sinus. The immediate result is an increase in heart rate and myocardial contractility and prolongation of the action potential (Smith et al., 2010). In patients with certain forms of LQTS, this combination of effects can be sufficient to cause TdP (Zhou et al., 2008). All cocaine abusers have elevated plasma concentrations of catecholamines (Dixon et al., 1989; Kiritsy-Roy et al., 1990), and to the extent that cocaine binds to and inhibits the hERG channel, cocaine abusers can be thought of as having an acquired form of LQTS. In the presence of cocaine, catecholamines induce an even greater increase in the action potential duration than they do alone (Overholser et al., 2008). This combination makes the occurrence of lethal arrhythmias even more likely.

1.11.4.1 Adrenergic Receptors

Catecholamine excess very likely is what triggers arrhythmias in users whose heats have already undergone remodeling. Both α and β receptors are G-protein coupled. $Alpha_1$ receptors cause coronary artery vasoconstriction but do not appear to have any direct arrhythmogenic properties. $Alpha_2$ agonists also cause coronary artery vasoconstriction (Woodman and Vatner, 1987), but probably not enough to cancel out β-receptor-induced vasodilatation. Except for decreasing the release of NE, α_2 agonists have no direct effect on impulse transmission.

The principal catecholamine of the heart is NE. Within the heart, NE functions as a neurotransmitter. It is released into cardiac synaptic clefts each time an impulse is transmitted. Impulse transmission stops only when NE is pumped back out of the synaptic cleft back into the presynaptic nerve ending. Because cocaine prevents the reuptake of NE, which in turn allows unmetabolized NE to overflow into the systemic circulation, NE is correctly classified both as a neurotransmitter and a circulatory hormone acting at α_1, α_2, and β_1 receptor sites. Epinephrine and NE bind to both α and β receptors, but they differ in their relative affinities for each type of receptor (see below). Epinephrine elicits a greater response at β_2 receptors than does NE.

Human β receptors belong to a group of receptors known as seven-transmembrane receptors (because the structure of the receptor traverses the plasma membrane seven times). Genes for these receptors are located on human chromosome five. They are classified into three main groups: β_1, β_2, and β_3. $Beta_1$ receptors increase heart rate by stimulation of the sinoatrial node (referred to as the chronotropic effect) and also increase myocardial contractility (the inotropic effect). Their mechanism of action involves the activation of intracellular

cyclic AMP, allowing the phosphorylation of the Ca^{2+} channel and thereby promoting calcium influx (Lefkowitz et al., 1983). When plasma concentrations are greatly elevated, the amount of calcium in the cytosol becomes elevated, sometimes to myotoxic concentrations.

There are several reasons why dangerous, or even lethal, elevations of calcium are likely to occur within the cytosol when cocaine is present. Cocaine binds to L-type calcium channels, allowing the excessive influx of calcium ions. This action, combined with sodium channel inhibition, is common to all local anesthetics and is proarrhythmic, further increasing the likelihood that sudden cardiac death will occur, especially when it can be taken as a given that cocaine-induced ischemia will be exacerbated by enhanced cocaine-induced sympathomimetic stimulation of coronary vasculature.

Sympathetic stimulation also causes calcium to be released from within the cell. Cocaine causes the phosphorylation of the ryanodine receptor (RyR2) by protein kinase A (PKA). When RyR2 is hyperphosphorylated, it is associated with sudden cardiac death; the RyR2 channel becomes "leaky," allowing calcium ions to leak from the endoplasmic reticulum, where they are normally stored, into the cell. Calcium leaked from the endoplasmic reticulum during diastole can generate "delayed after depolarizations" (DADs) capable of triggering fatal cardiac arrhythmias, which may manifest as ventricular tachycardia, ventricular fibrillation (VF), or TdP (Marks et al., 2002). It has recently been demonstrated that cocaine binds with a sarcoplasmic protein called calsequestrin, which leaves that much more calcium to "leak out," making sudden death all the more likely (Sanchez et al., 2013). At autopsy, the occurrence of this sequence is suggested by the presence of CBN within the media of arterioles (see Section 1.11.4.2) but is virtually impossible to prove—it is now possible to quantitate intracellular calcium, but whether these measurements would have any postmortem significance is impossible to say.

$Beta_2$ receptors are found mainly in the respiratory tract, particularly in airway smooth muscle. Cyclic AMP, which is formed when any β agonist binds to a β_2 receptor, causes airway relaxation, which is why this group of compounds is so widely used to treat asthma (Hoffman and Taylor, 2001). Cocaine use exacerbates asthma—whether by some as yet uncharacterized type of direct toxicity or via crack combustion products is not known (Restrepo et al., 2007). Downregulation of β_2 receptors, which occurs in diabetes and some other medical disorders (Mishra et al., 2010), makes cocaine abusers more susceptible to sudden cardiac death. At autopsy, there is no way to measure β_2 receptor status, but it may well be that what appears to be a fatal case of asthma is actually the result of inadequate β_2 stimulation. This situation may be resolved in the near future as it is now possible, using confocal microscopy, to visualize β receptors (Turillazzi et al., 2008).

It had been believed that β_3 receptors were found primarily in fat and not in the heart where they are, in fact, abundant. $Beta_3$ stimulation decreases cardiac contractility (Gauthier et al., 1996). More importantly, in experimental animals, β_3 stimulation can prevent the incidence of ventricular tachycardia (Zhou et al., 2008). In humans, β_3 adrenoceptor stimulation decreases both action potential amplitude and duration. There is also evidence that activation of this receptor directly inhibits calcium from transiting outside the cell and also prevents the release of calcium from the endoplasmic reticulum (where it is stored) via ryanodine channels.

Chronic catecholamine excess is usually associated with downregulation of β_1 and β_2 receptors but not of β_3 receptors (Conlee et al., 1991). Failure to downregulate has also been observed in infants born to substance-abusing mothers, in spite of the fact that they, too, have increased circulating levels of NE. Increased numbers of β_3 receptors are also seen in

human hearts with ischemic or dilated cardiomyopathy (Moniotte et al., 2001) and in heart failure. Upregulation of β_3 receptors is usually seen when catecholamine concentrations are elevated (as they are in cocaine users) or when intracytosolic calcium is elevated secondary to elevated catecholamines, a setting in which it appears that increased β_3 expression prevents arrhythmias (Zhou et al., 2008).

Defective handling of Ca^{2+} is central to the development of heart failure, because inotropes are effective only to the extent that they increase free cytosolic calcium. Factors leading to the faulty regulation of calcium include phosphorylation of L-type calcium channels (which has been documented in methamphetamine use); abnormal release of calcium from within the endoplasmic reticulum, partly because of ryanodine polymorphisms; and other polymorphisms of the calcium reuptake inhibitor known as phospholamban. Another possible explanation for abnormal Ca^{2+} handling would be the presence of a mutation in calsequestrin, the protein that holds calcium within the sarcoplasmic reticulum against the normally existing calcium concentration gradient, where cytosolic calcium is low. If calcium concentrations within the cytosol become pathologically elevated, the result will be the occurrence of delayed after depolarizations (DADs), reentry, and bidirectional ventricular tachycardia (Watanabe and Knollmann, 2011; Sanchez et al., 2013).

Excessive stimulation of adrenergic receptors sets off the process of myocyte apoptosis (autodestruction), further exaggerating the remodeling process. In fact, adrenergic signaling causes at least two separate types of myocardial damage—(1) activation of destructive mitogen-activated protein kinases and (2) increased mitochondrial toxicity—both of which result in the generation of ROS (Singh et al., 2001). It matters very little whether the remodeling is secondary to catecholamine excess or direct gene activation. The result is the same: apoptosis, cell death, and ever more remodeling.

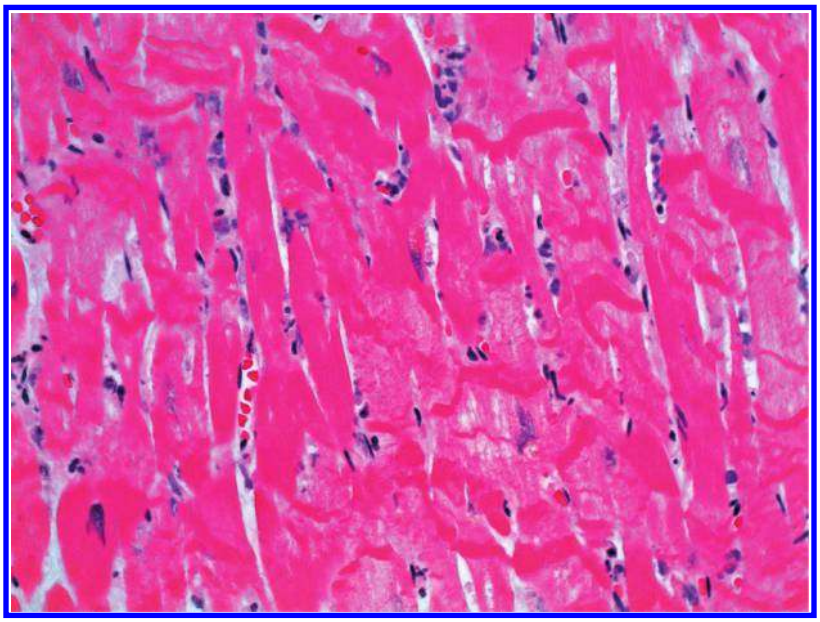
Finally, it has become clear that catecholamine gene variation is associated with NE and epinephrine concentrations both at rest and during exercise. This variation no doubt accounts for the large degree of interindividual variability seen in resting NE and epinephrine concentrations. Most of the variation seems to be attributable to four genes (*CYB561, VMAT2, CHFA*, and *PNMT*) (Ghimire et al., 2012).

1.11.4.2 Histologic Manifestations of Catecholamine and Cocaine Toxicity

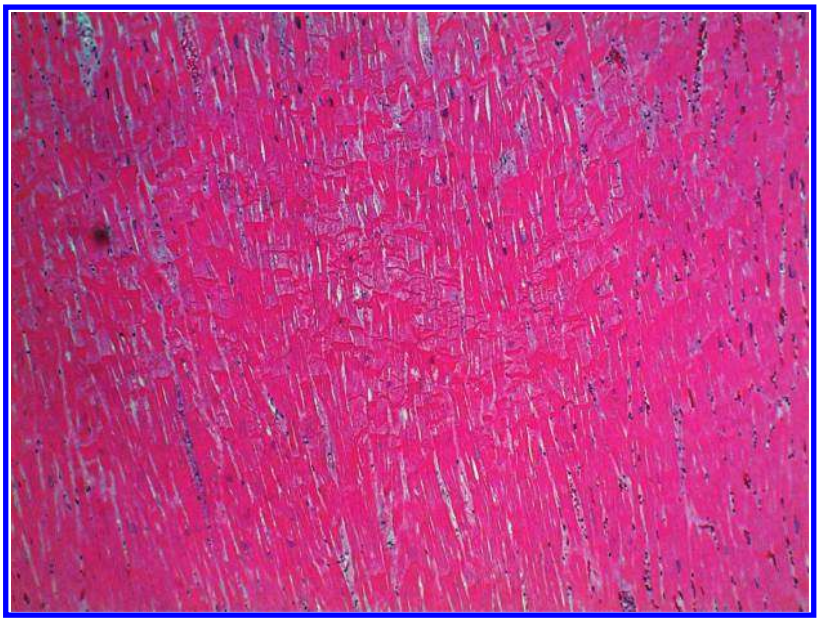
The following sequence of events leads to cardiac myocyte contraction. At the beginning of the action potential, as calcium rushes into the cell, calcium is also released from storage sites within the myocyte. Specifically, calcium is released from the endoplasmic reticulum via the ryanodine channel (there are several types of ryanodine receptors, but only type 2 is found in myocardium). Once the level of calcium within the cell has risen 100-fold, myofilaments are able to contract. If cytosolic calcium concentrations become too high, for whatever reason, irreversible damage occurs to the myofilaments, a condition known as contraction band necrosis (CBN) (Figures 1.45 through 1.47, Table 1.11).

The specific morphologic changes induced by catecholamine excess are essentially the same as those associated with cocaine and methamphetamine (Bravetta and Invernizzi, 1922; Karch and Billingham, 1988). The changes associated with cocaine and catecholamine toxicity are exactly the same as the morphologic changes associated with intracellular calcium overload, although anything that disrupts membrane integrity, including ischemia, can result in calcium overload (Rona, 1985).

The intracellular changes induced by catecholamine excess were first described more than 50 years ago (Szakacs and Cannon, 1958; Szakacs et al., 1959), and they are easily



(a)



(b)

Figure 1.45 Intense CBN observed in the heart of a 45-year-old chronic cocaine abuser who died suddenly. His heart weight was 600 g. Although the presence of CBN is associated with catechol excess and stimulant abuse, it can occur in any condition where membrane function is compromised, especially ischemia and reperfusion (a) is a high-power view from within (b). (Photographs from the authors' collection.)

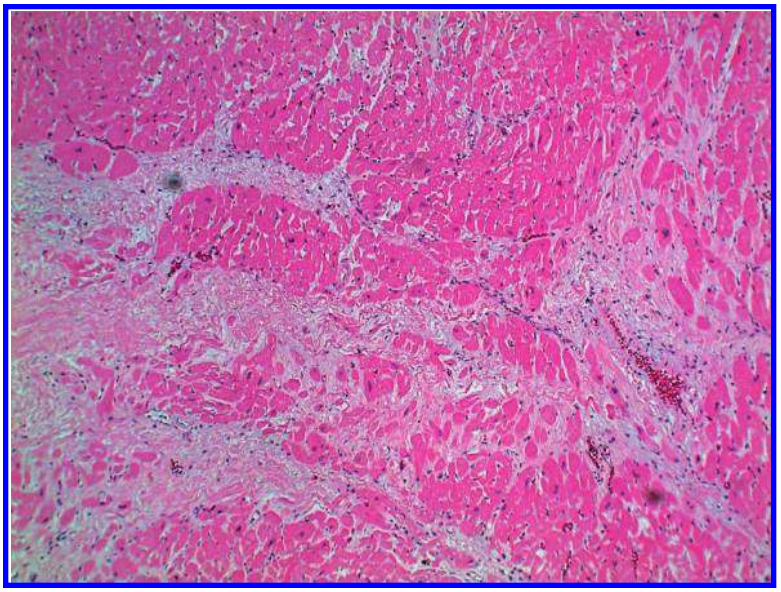


Figure 1.46 Microfocal fibrosis. CBN can be distinguished from ischemic necrosis by its extremely focal nature; small numbers of damaged cells are surrounded by normal myocytes (H & E stain). If CBN is irreversible, the zones of necrosis are replaced by fibrous tissue in a microfocal, interstitial distribution.

distinguished from those produced by ischemia. The most widely recognized change is CBN, sometimes called coagulative myocytolysis, and sometimes myofibrillar degeneration. CBN is a nonspecific finding and its presence is not necessarily synonymous with catecholamine excess. In fact, all that can ultimately be inferred from its presence is some sort of loss of membrane integrity, allowing for intracytosolic calcium overload.

CBN is always present in the zone bordering an area of reperfusion (Basso et al., 2010). It also occurs in the hearts of patients who have been subjected to multiple defibrillation attempts (Karch and Billingham, 1984) and is present in almost every myocardial biopsy. The presence of CBN in myocardial biopsies is probably explained by the disruption of local NE-containing nerve terminals. CBN is also a common finding in (1) drowning and intracerebral hemorrhage (ICH) (Karch, 1986; Lunt and Rose, 1987), although when it is seen in those settings, it probably just reflects the catecholamine excess associated with drowning and (2) pheochromocytoma (an expected finding given that the tumor produces catecholamines) (Karch and Billingham, 1986). CBN is often visible in cases of sudden cardiac death.

Catecholamine-induced necrosis and ischemic necrosis can be distinguished by their pattern of distribution (Table 1.12). In cases of ischemic injury, all the cells supplied by a given vessel will be affected. When the injury is due to catecholamine excess, individual necrotic cardiomyocytes are found interspersed between normal cells. Distribution is, in fact, one of the principal diagnostic features of catecholamine injury. Another feature that separates the two types of necrosis is the arrangement of the myofilaments within the cells. When the insult is ischemic, the myofilaments within the cardiomyocytes remain in register. When the damage is due to catecholamine excess, the filaments are disrupted.

The underlying mechanism in CBN is always the same: calcium overload. Whatever the mechanism of entry, a continuum of morphologic alterations can be observed, ranging from hypereosinophilia to total cell disruption. CBN lesions have no apparent relationship

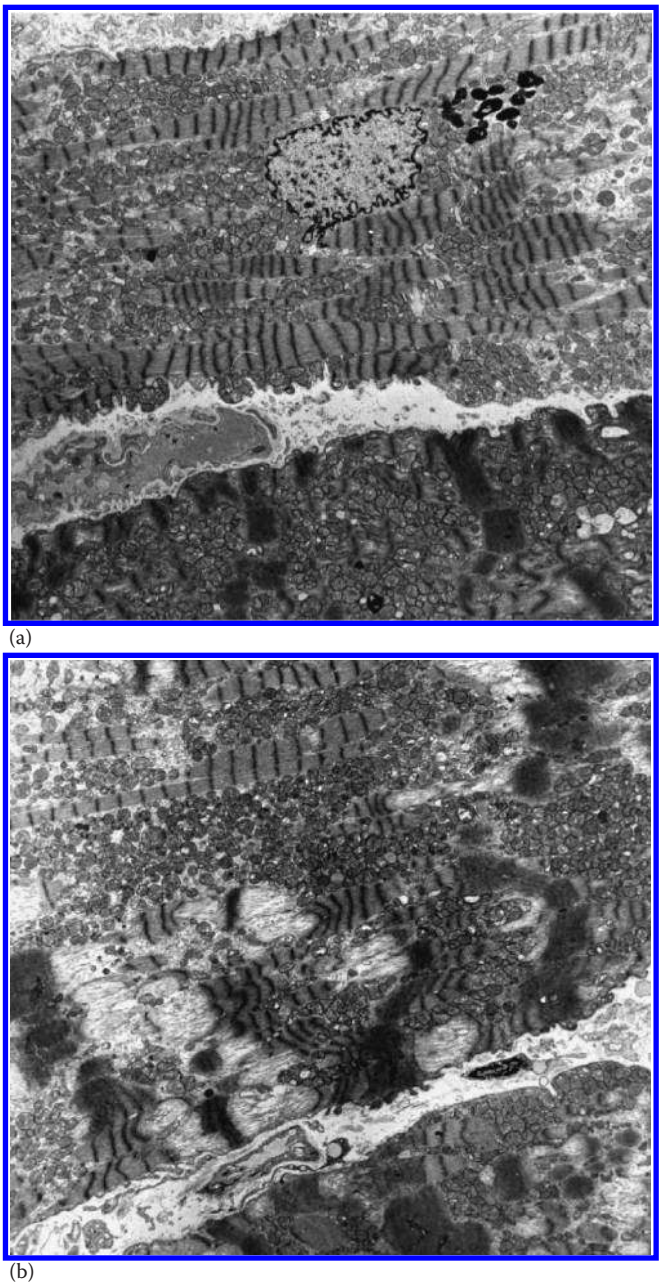


Figure 1.47 Effects of catecholamines on cardiac myocytes. The electron micrograph (a) shows normal human myocardium. The myofilaments are in register; the mitochondria are of uniform size and are neatly packed between the filaments. (b) The changes seen in CBN. The dark electron-dense material is all that remains of the myofilaments; the mitochondria are swollen and translocated. (Original magnification 4320×, (a and b).) (Micrographs courtesy of Dr. Margaret Billingham and Marilyn Masek, Stanford University School of Medicine, Stanford, CA.)

Table 1.11 Conditions Associated with Contraction Band Necrosis

Reperfusion	Norepinephrine
Steroid therapy	Cobalt poisoning
Electrocution	Starvation
Defibrillation	Myocardial infarction
Drowning	Free radical injuries
Cocaine	Brain death
Amphetamine	Phenylpropanolamine
Epinephrine	Intracerebral hemorrhage
Isoproterenol	MDMA

Source: Modified from Karch, S.B. and Billingham, M.E., *Hum. Pathol.*, 17, 9, 1986. With permission.

Table 1.12 Histologic Differences between Ischemic and Catecholamine Necrosis

Ischemic Necrosis	Catecholamine Necrosis
Involves many cells in area supplied by a single vessel.	Very focal; necrotic cell may be surrounded by normal cells.
Myofilaments remain in register.	Myofilaments are destroyed, forming eosinophilic clumps.
Mitochondria remain neatly packed, uniform size.	Mitochondria are translocated with distorted shapes.

to blood supply. In other words, the distribution of CBN is not confined to the areas supplied by any one blood vessel, and it can, and does, occur in the absence of significant coronary artery disease.

Because CBN is a prominent artifact in all myocardial biopsies, including biopsies taken from cocaine users, it is impossible to attribute the presence of CBN to cocaine (Adomian et al., 1978; Peng et al., 1989) based only on autopsy evidence. In some cardiac biopsies, Z-band remnants can be seen with electron microscopy. This particular finding is classically associated with dilated congestive cardiomyopathy and is not generally associated with catecholamine necrosis.

In ischemic infarction, the myofibrillar apparatus remains visible and in register, but in CBN, the sarcomeres become hypercontracted and distorted. The contractile apparatus may not even be visible. Milder forms of the lesion consist of eosinophilic transverse bands separated by areas containing fine eosinophilic granules. With electron microscopy, it is apparent that the myofilaments are completely out of register and the mitochondria translocated. The dense bands visible with light microscopy are seen as amorphous gray material under the electron microscope. This material is all that remains of both the thick and thin filaments.

Initially, and probably for at least 12 h, inflammatory cells are not in evidence around areas of CBN. Occasionally, a mononuclear infiltrate may appear. Eventually the injured cells are reabsorbed and replaced with fibrous tissue. The pattern is classically seen in patients (and experimental animals) with pheochromocytoma. Illustrating the progression of lesions in humans is quite difficult, but lesions corresponding to each stage in the evolution of catecholamine injury have been reported in experimental animals. For reasons explained earlier, the resultant fibrosis, which is often quite prominent in the hearts of cocaine users, may supply the substrate for lethal arrhythmias (Merx et al., 1977).

The impact of cocaine and chronic catecholamine excess on receptor physiology remains, for the most part, unstudied. The limited numbers of studies that have addressed this issue have all reached the same conclusion. In the heart, at least, there is no indication

of β-receptor downregulation in cocaine users (Conlee et al., 1991; Trouve and Nahas, 1991; Vitullo et al., 1993). This failure of downregulation distinguishes cocaine abuse from all other chronic hyperadrenergic states.

The incidence of CBN in various autopsy studies of other diseases, not involving cocaine abuse, is over 80% (Baroldi et al., 1979; Cebelin and Hirsch, 1980). In stimulant-related deaths, the incidence of CBN is much higher (Rajs and Falconer, 1979; Tazelaar et al., 1987). CBN heals by fibrosis, and postmortem studies of addicts in general, and stimulant users in particular, confirm the presence of reparative microfocal fibrosis (Rajs and Falconer, 1979; Oehmichen et al., 1990).

CBN never occurs as a fixation artifact (Adomian et al., 1978). Though unproven, it seems likely that during the process of myocardial biopsy, the bioptome will disrupt NE storage vesicles, releasing their contents into the cytosol. By themselves, contraction bands do not prove ingestion of cocaine or any other stimulant; their presence is merely consistent with it (Karch and Billingham, 1986). However, the presence of CBN does prove that an excess of calcium exists within the cytosol.

1.12 External Markers of Cocaine Abuse

1.12.1 Perforated Nasal Septum

Septal perforation is the best-known external manifestation of cocaine abuse. The first cases were described in the early 1900s, shortly after the practice of snorting cocaine became popular (Hutant, 1910; Maier, 1926). The presence of this lesion, however, is not pathognomonic. Perforations of the nasal septum can also result from chronic abuse of vasoconstricting nose drops.

Septal perforation occurs as a consequence of cocaine's ability to constrict blood vessels. Cocaine remains popular with ear, nose, and throat surgeons because cocaine-induced vasoconstriction controls bleeding, providing a dry operating field. With chronic use of intranasal cocaine, septal cartilage becomes deprived of its blood supply and breaks down (see Figure 1.48) (Kridel, 1999; Goodger et al., 2005; Trimarchi et al., 2006).

Other, much more severe, complications have also been reported as a consequence of chronic cocaine application to the nasal mucosa. These include nasolacrimal duct obstruction with orbital cellulitis (Alexandrakis et al., 1999), severe avascular necrosis of all of the nasal chambers (Braverman et al., 1999), and even perforation of the palate (Sastry et al., 1997). A type of central facial necrosis of the nasal septum, maxillary sinus, ethmoidal sinus, sphenoidal sinus, and soft palate also occurs (Sittel and Eckel, 1998; Caravaca et al., 1999; Rachapalli and Kelly, 2008). This constellation of symptoms is sometimes confused with Wegener's syndrome (Armstrong and Shikani, 1996; Helie and Fournier, 1997). After a brief hiatus, perhaps coinciding with the arrival of crack cocaine, new cases of Wegener's-like diseases continue to be reported (Smith et al., 2002; Trimarchi et al., 2006; Colasanti et al., 2010; Parker et al., 2010; Agusti-Mejias et al., 2012; Jiménez-Gallo et al., 2013). Some of these cases may be the result of impacted cocaine providing a nidus for infection (Tierney and Stadelmann, 1999), but others suggest an immunologic basis.

Occasionally, the reaction may be sufficiently extreme to simulate angiosarcoma. A recent case report described an exuberant ulcerative angiomatoid nasal lesion in a cocaine abuser. Microscopic examination showed polymorphous endothelial cells with

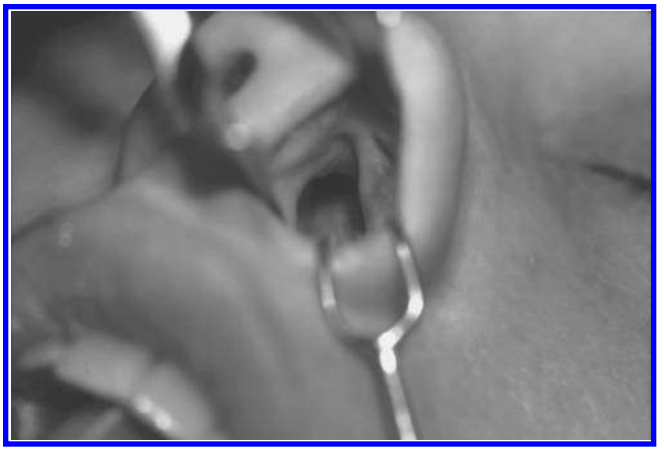


Figure 1.48 Perforated nasal septum. This lesion was first reported in conjunction with cocaine use in 1904. It is not absolutely diagnostic for cocaine abuse, as the same defect can be produced by the chronic use of vasoconstrictive nose drops. The first cases of cocaine-related septal perforation were reported just after the turn of the century. (Photo courtesy of Dr. Russel Kridel, University of Texas Health Sciences Center, Houston, TX.)

occasional mitoses, arranged in a lobular pattern with infiltrative-looking areas. Extensive areas of thrombosis with focal recanalization were also seen, but intravascular proliferation was not observed (Alameda et al., 2000).

The diagnosis of midline destructive lesions (MDLs) remains difficult but is aided by a systematic approach and familiarity with multiple diagnostic techniques. It is imperative to take multiple tissue specimens from various sites, send them to the laboratory fresh, and communicate suspicion of lymphoma. Despite diagnostic advances and improved understanding of the diseases underlying MDL, an etiology is often not identified (Parker et al., 2010).

1.12.2 "Parrot Beak" Nails

A pseudosclerodermatous triad of perniosis, pulp atrophy, and "parrot-beaked" clawing of the nails (Figure 1.49) is seen primarily in female chronic "crack" cocaine users. Some "crack" cocaine users develop coarsening changes in the appearance of their hands after prolonged use of the drug. The changes are thought to be the result of ischemia that results from the peripheral vasoconstriction induced by "crack" cocaine. Early changes may resolve with abstinence. The syndrome does not appear to be related to intravenous drug usage, nor is concomitant use of heroin a requirement for its occurrence. Since these changes only occur in a small subset of abusers, it is hypothesized that individuals with a vasoreactive circulation (i.e., those with vasomotor instability/perniosis) are more susceptible to this reaction pattern (Payne-James et al., 2007).

1.12.3 "Crack Thumb"

"Crack thumb" was first described in 1990. It is a repetitive-use type of injury. Crack smokers often use disposable cigarette lighters to heat their crack pipes. They may do this many times a day, and a callus can result from repeated contact of the thumb with the serrated wheel that ignites the lighter. The callus is usually located on the ulnar aspect of the thumb (Larkin, 1990; Gatof and Albert, 2002). Constant handling of a heated crack pipe can lead

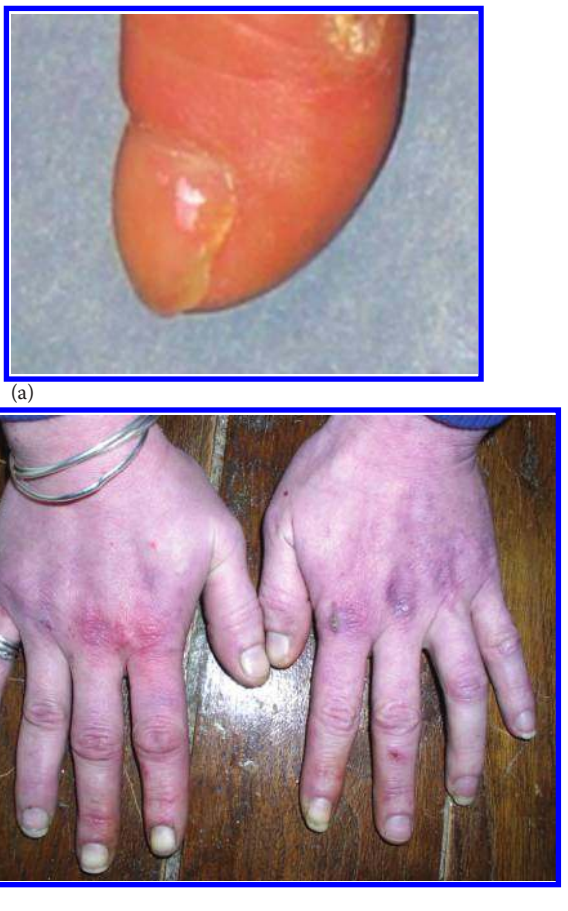


Figure 1.49 Some "crack" users develop coarsening changes in the appearance of their hands after prolonged use of the drug, more often in women than men. The changes include perniosis with cold, numb hands, finger pulp atrophy of the distal part of the pulps of some digits, and clawlike curvature of the nails. As the distal pulp is lost, it can no longer splint the nail straight and so the nail curves, clawlike, and reminiscent of a parrot's beak as it clings to the new contour. The syndrome consisting of the triad of perniosis, pulp atrophy, and parrot-beaked clawing of the nails should alert clinicians to the possibility of prolonged "crack" cocaine use. (Courtesy of Dr. Jason Payne James, London, U.K.)

to superficial burns on the palmar aspect of the hands. The same type of injury happens in methamphetamine smokers. A typical case is illustrated in Figure 1.50.

1.12.4 "Crack Lips"

"Crack" is often smoked with improvised pipes using pieces of steel wool to support the "rock." Hot steel wool may burn the lips or even be inhaled. Even the ubiquitous glass pipes may become so hot that they burn the lips. The resulting lesions are illustrated in Figure 1.51.

1.12.5 "Track" Marks

The adulterants most commonly found in cocaine are water soluble. Repeated injection tends not to produce the chronic inflammatory reactions and the type of granulomas

Figure 1.50 "Crack thumb." A repetitive-use injury from using disposable butane lighters to heat crack pipes. (Photo courtesy of Dr. Kari Blaho, University of Tennessee, Memphis, TN.)

Figure 1.51 "Crack lips."

associated with opiate abuse. Recent injection sites appear as salmon-colored bruises, sometimes with a clear central zone about the needle puncture site. Typical lesions are illustrated in Figure 1.52. As lesions become older, they turn blue and yellow, eventually disappearing without leaving any scar. In one fairly recent series of mixed drug deaths, nearly half the decedents were found to have track marks (Gatof and Albert, 2002).

Slowly healing cutaneous ulcers sometimes occur. The base of the ulcer may be red to gray, and the margins of the ulcers will have a pearly white appearance consistent with epidermal overgrowth (Yaffe, 1968). In experimental animals, healing of lesions is relatively rapid and complete. The histopathologic effects of subcutaneous cocaine injection have been studied on a limited scale (Bruckner et al., 1982).

In one study, subcutaneous injections of 0.1 mL of 2.0% cocaine solutions were found to cause blanching and hemorrhage. However, other workers have found no histologic damage, even after rats were repeatedly injected with large subcutaneous doses (32 mg/kg twice a day)

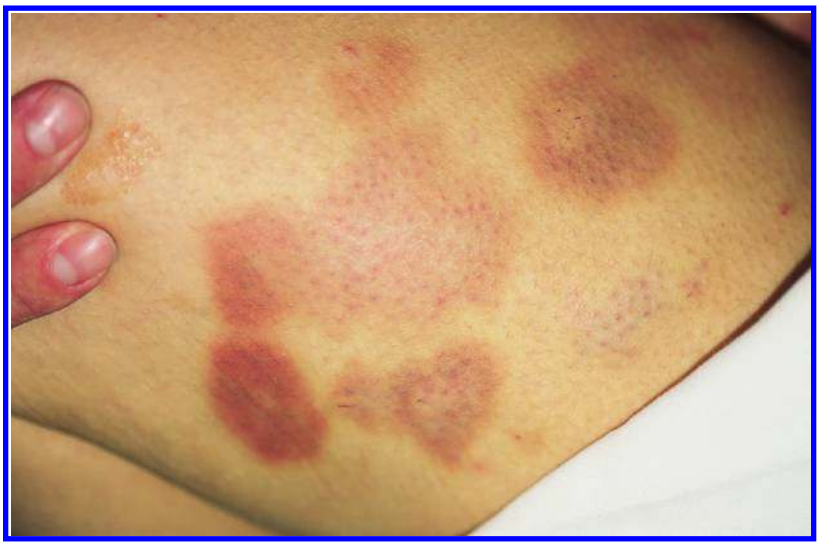


Figure 1.52 Cocaine injection sites. Injection with cocaine causes a distinctive salmon-colored bruise, sometimes with a clear zone around the needle puncture. (Photo courtesy of Dr. Kari Blaho, University of Tennessee, Memphis, TN.)

of cocaine over a 2-week period (Durazzo et al., 1994). If changes do occur, more is involved than cocaine-induced vasoconstriction and ischemia. Epinephrine is a more powerful vasoconstrictor than cocaine but does not produce similar lesions, and it seems likely that, at least in the case of the cocaine-related ulcers, an infectious component exists.

1.12.6 "Crack" Keratitis

"Crack" cocaine exposure has been reported to cause corneal disturbances ranging from subtle superficial punctate keratitis to perforation (Pilon and Scheiffle, 2006; Ghosheh et al., 2007). The corneas of "crack" smokers may inadvertently become anesthetized. When the smoker rubs his or her eyes, too much pressure may be applied and a sizable piece of the cornea may be rubbed off. This complication appears to occur with some frequency. In a 5-year review of cases of microbial keratitis at an urban county hospital in north Texas, nearly 5% of the patients were cocaine users (Pachigolla et al., 2007). This type of corneal abrasion/infection/injury is often referred to as "crack eye" (Figure 1.53) (Ravin and Ravin, 1979; Zagelbaum et al., 1991; Colatrella and Daniel, 1999; Parmar et al., 1999; Pilon and Scheiffele, 2006; Ghosheh et al., 2007).

1.12.7 Dental Erosions and Oral Lesions

Chronic intranasal cocaine users may have erosions on the enamel of the upper front teeth. The erosions occur because the teeth are bathed with acid cocaine hydrochloride trickling down from the sinuses and the posterior oropharynx (Krutchkoff et al., 1990). Methamphetamine abusers are more often edentulous than cocaine abusers and the cause appears to be poor hygiene and periodontal disease. Rapid gingival recession and dental erosion secondary to local cocaine application have also been reported (Kapila and Kashani, 1997; Ronderos et al., 2001; Vilela et al., 2002; Driscoll, 2003; Blanksma and Brand, 2005; Shibli et al., 2005).

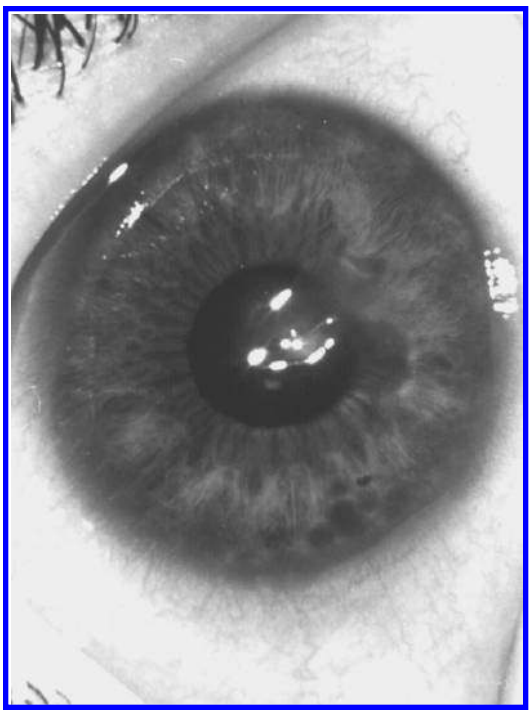


Figure 1.53 "Crack" keratitis. Volatilized cocaine anesthetizes the cornea so that abusers cannot feel how hard they are rubbing their eyes. Evidence indicates that crack smokers may be less able to resist corneal infection, and infected ulcers with corneal clouding may be the result. (From *Am. J. Ophthalmol.*, 111(3), 247, 1991. With permission; Photo courtesy of Dr. Peter S. Hersh, Chairman, Department of Ophthalmology, Bronx-Lebanon Hospital, New York.)

Whitish lesions of the oral mucosa are said to be common in "crack" smokers (Parry et al., 1996; Blanksma and Brand, 2005), and leukoplakia is a recognized consequence of coca leaf chewing (Hammner and Villegas, 1969; Negrete, 1992). HIV-infected patients who also use cocaine may manifest atypical ulcers of the mouth that may be difficult to identify (Mitchell-Lewis et al., 1994).

1.12.8 "Crack Hands"

This lesion has much in common with "crack thumb." Examination of chronic "crack" smokers may disclose blackened, hyperkeratotic lesions on the palmar aspect of the hands. The pipes used to smoke cocaine can become quite hot, and chronic users are likely to sustain multiple small burns (see Figure 1.50) (Feeney and Briggs, 1992). In some cases, they may be extreme (Dhawan and Wang, 2007).

1.12.9 Evidence of Terminal Seizures

Bite marks of the lips and tongue may occur. A minority of cocaine users may experience seizure activity as a terminal event (Wetli, 1987). However, because seizures do not always occur, even in conjunction with massive overdose, and because many other agents can cause terminal seizure activity, the usefulness of this sign is somewhat limited.

1.12.10 Marks and Mutilation

The most likely reason to see superficial, or even moderately deep, skin lesions in a cocaine abuser is formication, where the abuser experiences a sensation that bugs are crawling on (or under) the skin. Susceptible individuals may fixate on the sensation and even use surgical instruments to remove "bugs"—the imagined irritant (Brewer et al., 2008). This can only be described as a form of cocaine psychosis. It was first described by the great French neurologist, Valentin Magnan, in the late 1800s (Karch, 1998).

In other settings, superficial skin lacerations may have a more sinister implication. Wetli et al. (1997) described a series of 10 heroin "body packers". In two of the cases, the drug couriers died after reaching their destinations, and their accomplices made abdominal incisions to remove the drug packets. A similar case was reported from the United Kingdom, where the back and buttocks of a deceased courier were marked with a number of superficial lacerations, corresponding to the number of drug-containing packets that the deceased had swallowed, a sort of living invoice.

1.13 Skin Toxicity

Subcutaneous drug injection (called "skin popping"—see Figures 1.54 and 1.55) is still more common among heroin than cocaine users, but it is losing its popularity. Street prices for heroin are so low, and purity so high, that insufflation is just as effective as injection and much safer. Subcutaneous injection in cocaine abusers usually produces fewer complications than in heroin users and far fewer than after intravenous ingestion.

Be that as it may, a radical shift in cocaine adulterants is occurring and it may have important health consequences. Today, roughly 80% of the U.S. cocaine supply and 50% of the European supply is adulterated with levamisole, an old anthelmintic drug. Levamisole adulteration was unheard of until the year 2000. The use of levamisole-adulterated cocaine

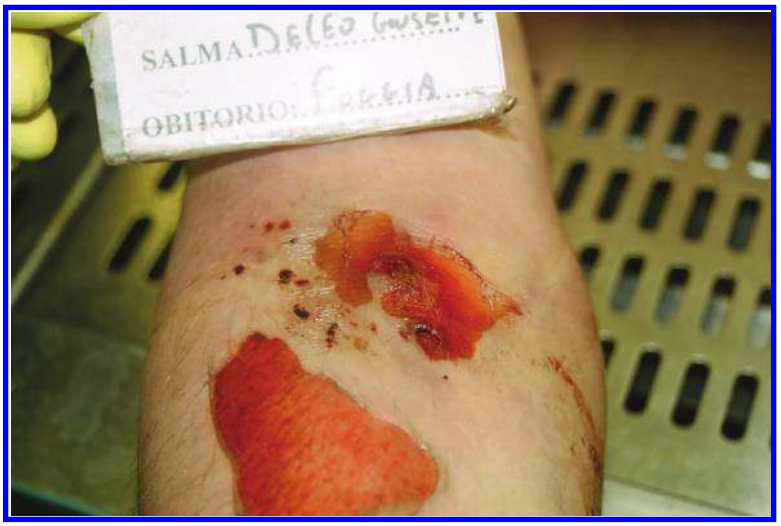


Figure 1.54 "Skin popping." The injection of cocaine into the skin rather than a vein, remains popular. This photograph illustrates the typical lesions that may result.

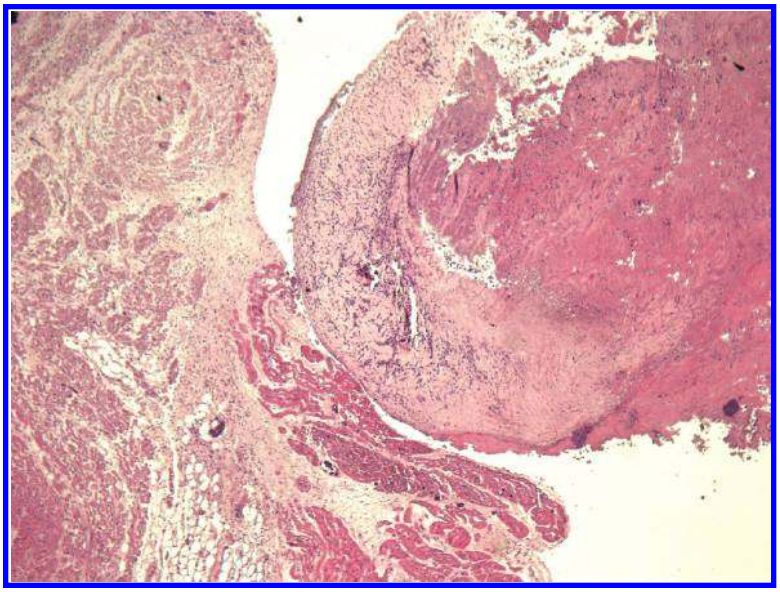


Figure 1.55 This micrograph illustrates the inflammatory response that injection produces in the underlying skin.

is associated with agranulocytosis, vasculitis, skin necrosis, and purpura (see Figure 1.29) (Rongioletti et al., 1999; Waller et al., 2010; Walsh et al., 2010; Click, 2011; Lung et al., 2011). When a young patient with purpura first presents for medical care, cocaine abuse, by no matter what route, should be high on the list of differentials. At autopsy, the obvious presence of purpura should direct the prosector's attention to possible cocaine-related disease, if not disease from levamisole itself. Indeed, levamisole may prove to be as important an issue as the subcutaneous skin abscesses. The skin changes associated with levamisole sometimes are accompanied by cellulitis, lymphangitis, and lymphadenopathy (Ben Diane et al., 2000).

Skin infection in drug abusers is generally the result of infection with oral flora that are sensitive to multiple antibiotics, but surgical debridement is often required. Necrotizing fasciitis has occasionally been reported in cocaine users (Jacobson and Hirschman, 1982). Depending on the infectious agents, there are two main types of fasciitis: type I, a polymicrobial infection, and type II, which is more invasive, serious, and fulminant and is mainly caused by group A *Streptococcus pyogenes*. Both the frequency and severity of these infections seem to be increasing (Herr et al., 2011; Loscar et al., 1998).

Streptococcal skin infections in cocaine abusers are increasing, but the connection between skin disorders and illicit drug injection is much stronger for heroin than cocaine (Smeets et al., 2007; Bellapianta et al., 2009). Soft tissue infection in drug abusers has become such a serious and widespread problem it is now the most likely reason for abusers to seek medical attention. Many hospitals have established diversion clinics in order to segregate cases of skin infections from other complications of drug abuse. Most of the infections are indolent and superficial (Sen et al., 2005), but infection deep within the neck, groin, and scrotum has also been reported (Lautermann et al., 2005). As evidenced by multiple outbreaks of necrotizing fasciitis secondary to *Clostridium sordellii* in black-tar heroin users, it is clear that the risk of serious infection is much greater with some drugs than others (see Chapter 5).

Scleroderma occurs in cocaine abusers, but not very often (Kerr, 1989; Attoussi et al., 1998); reports of this disorder remain uncommon. The estimated annual incidence is between 4 and 12 new cases of scleroderma per 1,000,000 persons year. Scleroderma is three times more common in females, but when it occurs in young people, it is 15 times more common in women than men (Poormoghim et al., 2011). The median age of onset for scleroderma is between 40 and 50. Less than a dozen cases of cocaine-related scleroderma have been reported in the English literature and all have involved male cocaine users, usually in their 40s (Kerr, 1989; Attoussi et al., 1998). New reports have been conspicuously absent in the last decade.

Scleroderma is characterized by the deposition of normal collagen in pathologic amounts. Excessive fibrosis, vascular injury, autoimmunity, and inflammation are the predominant features of the disease and they lead to irreversible organ damage (Kowal-Bielecka and Distler, 2010). Primary myocardial involvement is common in this disorder, strongly suggesting that the cardiac injury is in some way related to repeated focal ischemic injury leading to the development of irreversible myocardial fibrosis. Microcirculatory impairment accompanied by abnormal vasoreactivity, with or without structural vascular abnormalities, seems to be the primary mechanism. Clinically evident cardiac involvement portends a poor outcome. Pericardial involvement is frequent but usually asymptomatic. Conduction system abnormalities also appear to be common, and life-threatening arrhythmias may occur. Systemic sclerosis does not cause significant valvular disease. Treatment for myocardial involvement includes long-term systematic administration of calcium channel blockers and possibly angiotensin-converting enzyme inhibitors (Allanore et al., 2010).

Cocaine users and victims of scleroderma each may develop isolated cerebral vasculitis but, in the past, it was uncommon in either disorder. Now that cocaine is almost universally contaminated with levamisole, the situation is likely to change in the near future, as levamisole itself is known to cause vasculitis (Scheinberg et al., 1978; Bradford et al., 2011). In the two biopsy-proven cases of cocaine-associated vasculitis, the vessels were infiltrated with lymphocytes. In the one case of scleroderma-associated vasculitis, the biopsy was nondiagnostic (Pathak and Gabor, 1991; Andonopoulos et al., 1998). When clear evidence of vasculitis is seen in a cocaine abuser, the possibility of levamisole involvement must also be considered.

Numerous other dermatologic manifestations of cocaine use have been reported, but they are quite rare. The list of reported conditions includes, but is not limited to, skin necrosis (Hoeger et al., 1996), acute generalized exanthematous pustulosis (Lu and High, 2007), chronic skin ulcers (Abidin et al., 1990), Stevens–Johnson syndrome (Hofbauer et al., 2000), Churg–Strauss vasculitis (Orriols et al., 1996), and Raynaud's phenomenon (Balbir-Gurman et al., 2001).

1.14 Cardiovascular System: General Overview

Cocaine causes vascular disease, but then so do many other risk factors, and they all do so with some frequency. Each year, an estimated 785,000 Americans will suffer a new coronary attack, and 470,000 will have a recurrent attack. It is estimated that an additional 195,000 silent first episodes of acute myocardial infarction (AMI) occur each year. Approximately every 25 s, an American will have a coronary event, and approximately every minute, someone will die (American Heart Association, 2011). At the same time, the

most recent U.S. government estimates indicate that there are 1.9 million current cocaine users aged 12 or older, comprising 0.7% of the total U.S. population (Substance Abuse and Mental Health Services Administration, 2010). It is inevitable that there would be considerable overlap between events that are drug related and those that are not. In general, there is little to distinguish cocaine-induced vascular disease from naturally occurring vascular disease, probably because shared common pathways are involved. No single abnormality is absolutely diagnostic for cocaine, but many forms of disease have been attributed to its use.

1.14.1 Coronary Artery Disease

It has been suggested that cocaine users, even those without other risk factors, experience large, transient increases in the risk for acute coronary syndrome (ACS) immediately after using cocaine (Mittleman et al., 1999), but the most recent studies suggest that this risk may be confined to specific subgroups, specifically those 18–45 years of age who are classified as relatively frequent users (Aslibekyan et al., 2008), although this conclusion has been challenged. A study published in 2011 analyzed the arteriographic findings in nearly 1000 patients and concluded "cocaine use was not associated with an increased likelihood of coronary disease after adjustment for age, race, sex, and other risk factors for coronary disease" (Chang et al., 2011). However, it is also true that cocaine users tend to have more pronounced coronary atherosclerosis compared to patients who are not cocaine users (Ebersberger et al., 2013) (Figures 1.56 through 1.58). Obviously, many cocaine users have multiple preexisting risk factors, and it would be foolish to ignore them.

A great deal of attention has been focused on the sympathomimetic effects of the drug and its proven ability to induce coronary artery spasm (Lange et al., 1989), but other mechanisms are involved, including vasculitis, accelerated atherosclerosis, exacerbations of preexisting fixed lesions of the epicardial arteries, disease of the microvasculature, and drug interaction, to name the most obvious. In one study of 97 consecutive patients with

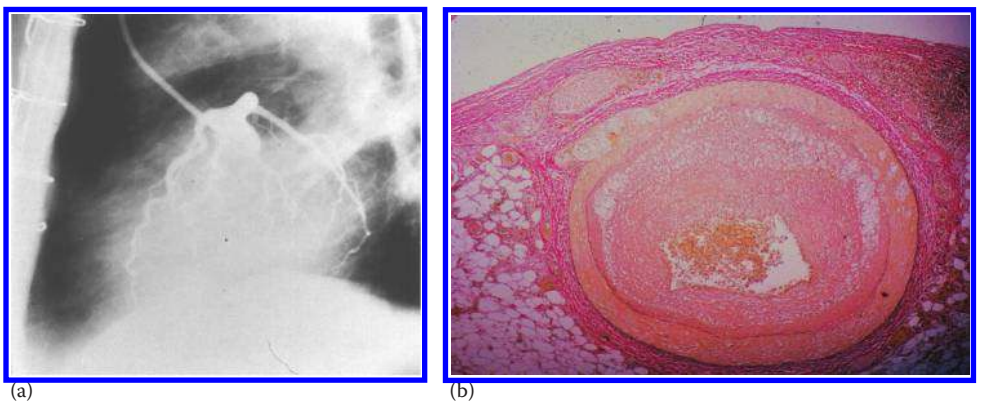


Figure 1.56 Coronary artery disease in cocaine users. The coronary arteries of cocaine users may undergo the same type of intimal hyperplasia as seen in transplant recipients. Because this sort of lesion concentrically involves the entire length of the involved vessel, obstructions may not be apparent unless earlier studies are available for comparison. (a) The normal-appearing study was obtained just 2 weeks before the patient died of myocardial infarction. (b) A cross section of the left anterior descending coronary artery from the same patient. Concentric intimal hyperplasia has almost entirely obstructed the lumen. (H&E stain.) (Courtesy of Margaret Billingham, Stanford University School of Medicine, Stanford, CA.)

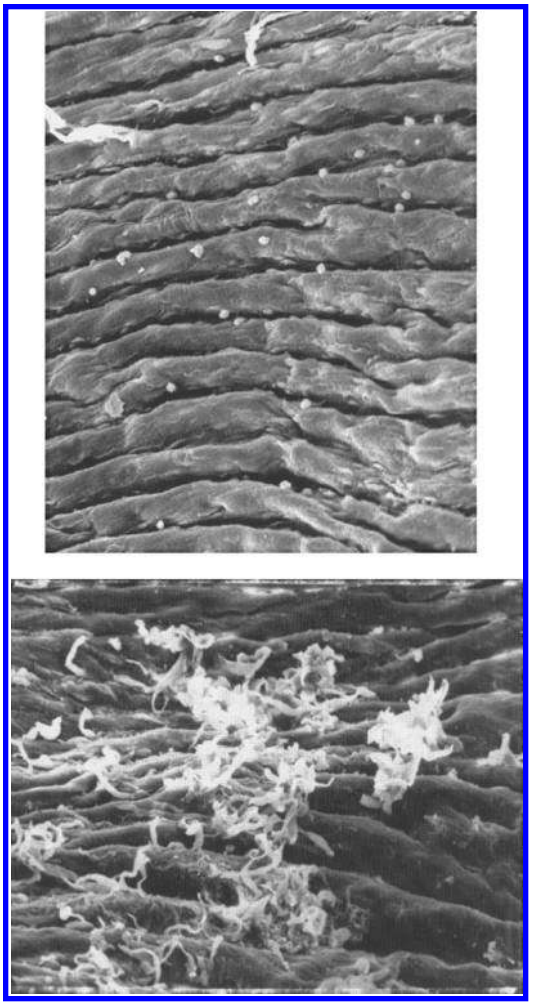


Figure 1.57 Effects of cocaine on the endothelium. Both scanning micrographs are of a canine coronary artery. The photograph on top is from a control animal (original magnification 312×). The lower photograph is from a dog that received 1 mg/kg/day of cocaine for 4 weeks; sloughing of endothelial cells is evident (original magnification 520×). (Courtesy of Dr. Randall L. Tackett, Department of Pharmacology and Toxicology, University of Georgia, Athens, GA.)

documented AMI and positive urine drug screen for cocaine metabolites, ST segment elevation was present in 32%. ST segment depression, T-wave inversion, left ventricular hypertrophy, conduction blocks, and/or old infarct were observed in more than 80% of the patients. The majority (82%) had obstructive CAD, and single-vessel disease was the most frequent finding (Ma et al., 2006). Neither left ventricular hypertrophy nor previous myocardial infarction develops overnight. Barring the occasional naïve cocaine user with serious, undiagnosed coronary artery disease, most cases of myocardial infarction in cocaine abusers appear to involve chronic cocaine abuse, and chronic cocaine abusers will have already undergone myocardial remodeling, placing themselves at risk for any number of reasons.

Only a handful of autopsy studies of AMI in cocaine users have ever been published. The findings of these studies tend to confirm that if vulnerable plaque is not present, at least some degree of remodeling is. Nearly all of the studies have demonstrated preexisting

Figure 1.58 Premature coronary artery disease is common in stimulant abusers. This section of the left anterior descending coronary artery is from an asymptomatic 32-year-old crack smoker who was murdered.

disease in either of the coronary vessels (epicardial or intramyocardial) (Isner et al., 1986; Simpson and Edwards, 1986; Stenberg et al., 1989; Karch et al., 1998). In the most recently published autopsy of cocaine-related deaths, left ventricular hypertrophy was observed in 57%, small vessel disease in 42.9%, severe atherosclerotic coronary artery disease in 28.6%, and coronary thrombosis in 14.3%. Taken together, these changes could easily account for ischemia-related sudden death (Lucena et al., 2010).

On occasion, cocaine users have coronary artery lesions resembling those seen in the hearts of transplant patients with chronic rejection. One case report described multivessel blockage due entirely to intimal hyperplasia. There was no sign of collagen or elastin deposition (Simpson and Edwards, 1986). This type of lesion is routinely seen in transplanted organs and also occurs in some connective tissue disorders (Dawkins et al., 1985). Similar alterations have been observed in the hearts of other cocaine users (Pamplona et al., 1990; Roh and Hamele-Bena, 1990); however, this complication remains so rare that it is still reportable, and its etiology remains unknown.

Cocaine users who do experience AMI often are treated with angioplasty. No controlled studies, let alone case series on these patients, have ever been published, but there is an emerging consensus that cocaine may be one possible cause of in-stent occlusion. If this suspicion proves correct, it is likely the result of cocaine-induced platelet interaction (Krylov, 2009; Yao et al., 2011) (see Section 1.14.2). Whether drug-eluting stent carry and equal risk is not known.

1.14.2 Microvascular Disease

Chronic cocaine causes disease in the smaller coronary vessels, and the changes seen are highly reminiscent of the changes seen in hypertension (Figures 1.59 and 1.60). The similarity is hardly coincidental, as both are a consequence of excessive neurohumoral

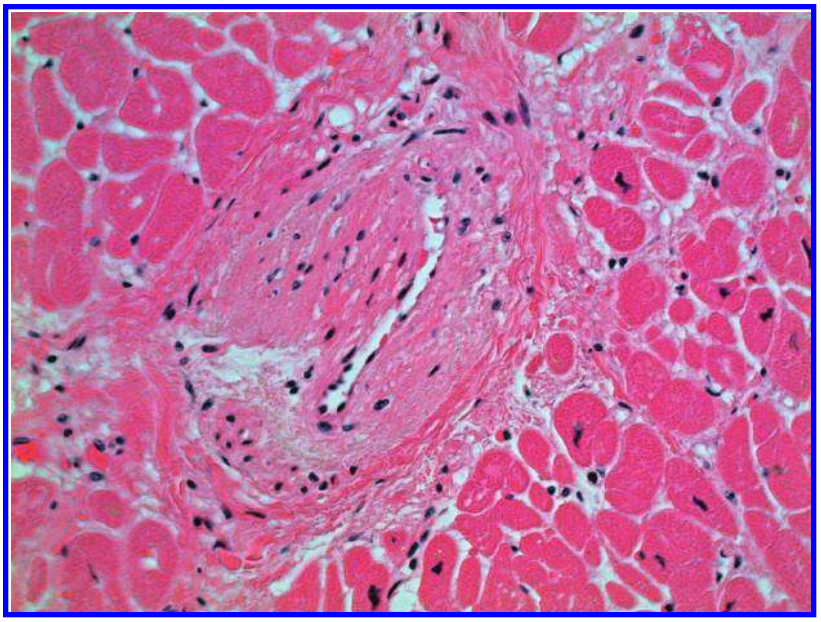


Figure 1.59 Part of the myocardial remodeling process includes medial and intimal hypertrophy of very small intramural vessels. Even in the absence of disease within large epicardial vessels, this abnormality could lead to ischemia even at rest as the photograph here demonstrates.

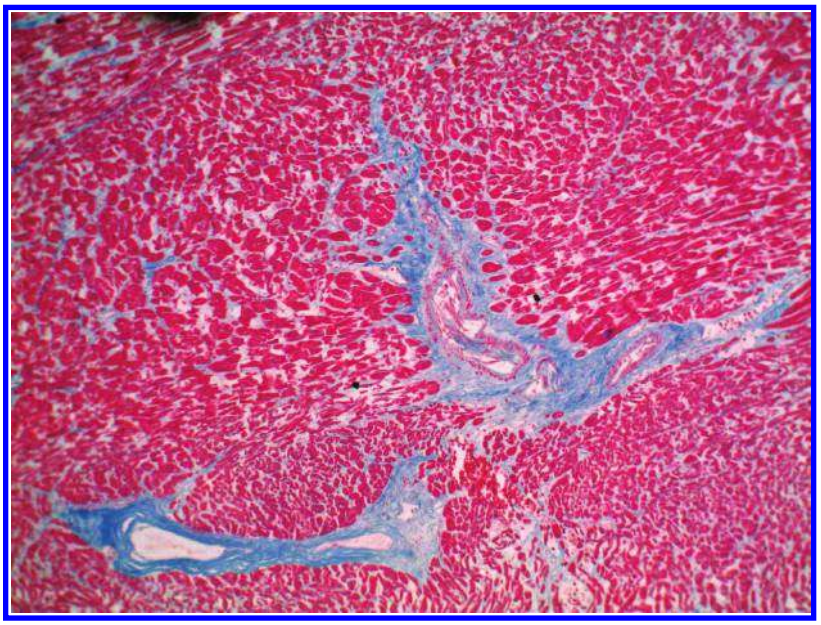


Figure 1.60 Disease of the small intramyocardial vessels is very common in cocaine and methamphetamine abusers, but it is also observed in individuals suffering from poorly treated hypertension. In this instance, perivascular fibrosis is present as well, further diminishing flow-mediated deviation.

activation. As a result of these changes, the lumen of the arterioles becomes decreased (a consequence of either vasoconstriction or wall thickening), and abnormal amounts of collagen are deposited within the vessel wall (Gavin et al., 1998). In the living, this means that microvascular tissue perfusion is impaired even when global hemodynamic or laboratory signs of hypoperfusion are absent (Lauten et al., 2011). The consequences are just as great for death investigators; ACS could easily occur in an individual with little or no epicardial disease. One might suppose that the same could be said of coronary spasm, but virtually all the human studies have demonstrated that cocaine causes only modest degrees of vasospasm—not sufficient to provoke ACS unless there is preexisting underlying disease. Endomyocardial biopsies from 11 cocaine users with symptoms of myocardial ischemia demonstrated marked medial thickening of small intramyocardial arteries (20–40 mm) in seven patients (Majid et al., 1990).

Increased circulating levels of ET-1, a potent vasoconstrictor peptide, have been found in patients with diabetes, just one among many disorders where endothelins (there are three, but ET-1 is the object of most intense interest) are elevated (Kalani, 2008). Endothelins constrict blood vessels and raise blood pressure. Their tendency to raise blood pressure is normally balanced by other mechanisms, but when they are overexpressed, they contribute to high blood pressure and heart disease. Nitric oxide (NO) and ET-1 are involved in endothelial cell activation and leukocyte recruitment (Pradhan et al., 2008), both consequences of cocaine abuse. It has been speculated that ET-1 itself damages artery walls.

In experimental preparations, cocaine exposure for long durations (24–72 h) causes a temporary decrease in both nitric oxide production and endothelial nitric oxide synthase expression. Furthermore, both short-term (24 h) and long-term (72 h) exposure to cocaine increases endothelial adhesion of monocytes by 20% and 40%, respectively. These data suggest that a concomitant increase in both ET-1 and endothelin receptor expression in cocaine-exposed tissue culture cells may cause decreased endothelial NOS expression and nitric oxide production, ultimately resulting in endothelial activation and leukocyte adhesion.

Thickening of the media together with intimal hyperplasia has also been observed in the nasal submucosal vessels of chronic cocaine addicts (Chow et al., 1990), suggesting that in susceptible individuals similar changes occur throughout the body. Such changes have been observed in the kidneys of chronic cocaine abusers where medial thickening, decreased luminal circumference, and vessel obstruction are nearly universal findings (Di Paolo et al., 1997). Presumably the same mechanisms operate when large abdominal and chest vessels are involved (Hoang et al., 1998).

1.14.3 Atheromatous Coronary Artery Disease

Cocaine is atherogenic. Its use is associated with the development of accelerated coronary atherosclerosis, even in individuals without conventional risk factors (Patrizi et al., 2006). One recent study found that accelerated atheroma formation was evident in 76% of all cases of cocaine-related sudden death (Lucena et al., 2010). In fact, this relationship has been known for a very long time—it became apparent almost as soon as scientists started doing systematic autopsy studies of cocaine users (Karch et al., 1998).

The loss of normal endothelial homeostatic function is one of the initiating and most important driving factors of coronary atherogenesis (Giannotti et al., 2010). Both in vitro and in vivo studies demonstrate cocaine damage to endothelial cells. In particular, cardiomyocytes grown in tissue culture undergo apoptotic-like changes and display increased permeability.

There are several different ways to measure endothelial damage. One increasingly popular approach is to measure impaired flow-mediated vasodilation (FMD) (Corretti et al., 2002), together with the resultant abnormal release of diverse molecules produced either by endothelial cells or cells of endothelial origin (cytokines, a class of regulatory proteins, such as the interleukins and lymphokines) released into the circulation. These compounds act as intercellular mediators and include the von Willebrand factor (vWF), interleukin-6, tumor necrosis factor-α, and cell adhesion molecules. All of these have been shown to induce vascular damage (Armstrong et al., 2006). Recently, the measurement of circulating endothelial cells (CECs) has emerged as a reliable and accurate marker of endothelial damage (Goon et al., 2005) and may prove to be the method of choice in postmortem investigations.

Chronic cocaine use is clearly associated with endothelial cell injury in human beings. Specifically, cocaine abuse results in endothelial cell detachment and the release of vascular reactive mediators and chemoattractants, and this abnormal release continues long after cocaine use is discontinued (Sáez et al., 2011). Cocaine also causes endothelial cells to release tissue factor (TF), alternatively referred to as platelet TF, factor III, thrombokinase, or CD142 (CDF is the general name for a family of platelet-derived microparticles implicated in ACS). TF is a protein present in subendothelial tissue, platelets, and leukocytes and is necessary for the initiation of thrombin formation. Under normal circumstances, the actions of TF are opposed by those of TF pathway inhibitor (TFPI). Cocaine both increases TF production and decreases production of TFPI, thereby favoring thrombus formation (Steffel et al., 2006).

In a study now more than two decades old, over 60% of the patients with cocaine-associated sudden death were found to have moderate to severe coronary atherosclerosis (the patients had a mean age of 47). In such a young age group, a much lower percentage of significant lesions would be expected (Dressler et al., 1990). Other autopsy studies have also found an increased incidence of significant atherosclerotic lesions (Karch and Billingham, 1988; Virmani et al., 1988).

When the coronary arteries of cocaine abusers dying of thrombosis were compared to those of cocaine users without thrombosis, and those dying of non-coronary-related disease, as well as other cases of sudden death not associated with cocaine use, the average age for the cocaine thrombosis group was only 29 years, and the degree of luminal narrowing was much higher than would be expected in this age group. In the patients with thrombosis, moderate to severe coronary atherosclerosis was seen, as well as increased numbers of adventitial mast cells (Figure 1.61) (Kolodgie et al., 1991).

One plausible explanation for the high incidence of cocaine-associated AMI is that its occurrence is in some way related to circadian, circaseptan, and circannual variation. Triggering of an AMI by heavy exertion, sexual activity, anger, mental stress, cocaine, marijuana use, and exposure to air pollution has been reported (Servoss et al., 2002). There is no reason not to suppose that stress, superimposed on preexisting lesions, may well trigger infarction (Servoss et al., 2002). Not infrequently, cocaine users die of AMI during sex play. This leaves medical examiners the nearly impossible task of determining whether death was accidental or homicidal (Kloner, 2006).

Mast cells are multifunctional cells containing various mediator molecules, such as cytokines, tryptase, and histamine. Large numbers can be identified in infarcted myocardium. This observation has led some to suggest that mast cells may, in some way, be causative. However, recent studies suggest quite the opposite. In the setting of

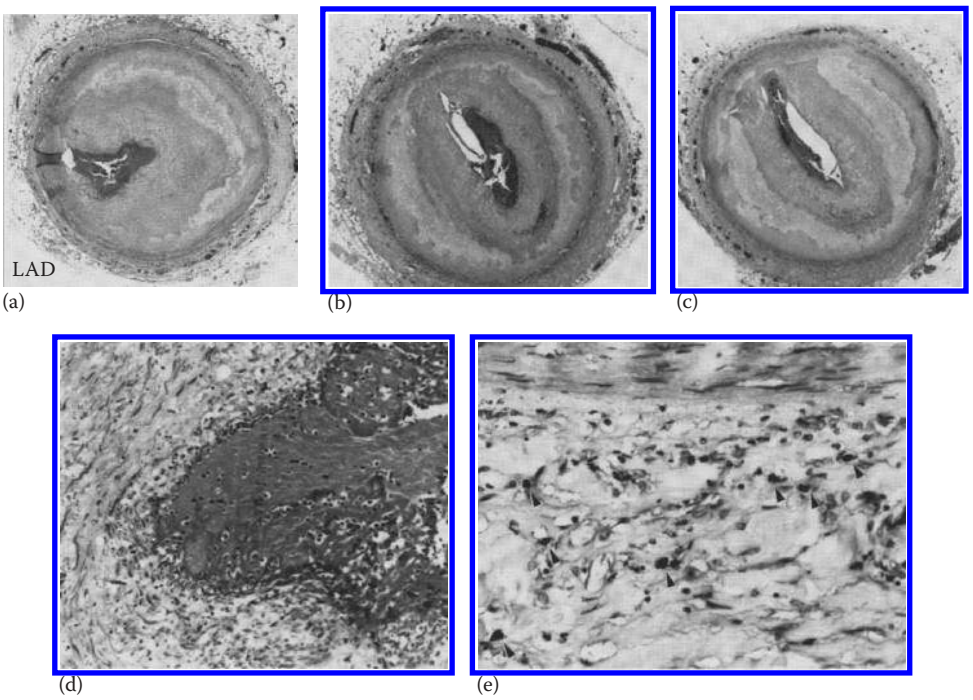


Figure 1.61 Adventitial mast cells in cocaine users. Compared to age- and sex-matched controls, more mast cells are present in the adventitia of the coronary arteries of cocaine users, and the degree of luminal narrowing correlates well with the number of mast cells present. (a–c) Severely narrowed cross sections from a decedent with severe coronary disease; (d, e) high-power views of the adventia of the same vessel. Toluidine blue staining demonstrates the presence of numerous mast cells. (Original magnification 150×.) (Courtesy of Dr. Rene Virmani, Department of Cardiovascular Pathology, Armed Forces Institute of Pathology, Bethesda, MD.)

experimental myocardial infarction, mast cells, or at least the granules they release, are cardioprotective, preventing apoptosis and prolonging cardiomyocyte survival (Kwon et al., 2011).

Uncomplicated cases of AMI can occur in cocaine users who already have extensive coronary artery disease. If underlying fixed lesions are present, cocaine use can make those lesions symptomatic by increasing myocardial work. In controlled human studies, intranasal cocaine (2 mg/kg body weight) increased arterial pressure and the rate pressure product. At the same time that the rate pressure product was rising, coronary sinus blood flow was falling significantly (Flores et al., 1990). The end result is that cocaine increases myocardial work and oxygen demand while, at the same time, decreasing blood flow (Lange et al., 1990).

Failure to demonstrate lesions with angiography does not necessarily mean that they are not there. Studies have shown that angiography usually underestimates the severity of arterial lesions, especially when preexisting obstruction is already in the 50%–75% range and when multivessel disease is present (Gruberg et al., 1999). The 2D silhouette image provided by coronary angiography has well-recognized limitations. Angiographic images do not accurately represent the true complexity of the luminal morphology in coronary disease and give no indication of the functional influence of luminal changes on coronary blood flow. These limitations are more pronounced in angiographically

intermediate stenoses and in patients in whom there is a clear discrepancy between the clinical picture and angiographic findings. In such cases, there is often poor concordance between the estimated percentage of angiographic stenosis and the corresponding intravascular ultrasound image or noninvasive functional data (Melikian et al., 2010). Dimensions measured at autopsy are certain to underestimate luminal diameter (Gallagher et al., 1978).

1.14.4 Coronary Artery Spasm

Cocaine induces human coronary artery constriction. In one of the few reported human trials, in which 2 mg/kg of cocaine was given intranasally to 45 patients undergoing cardiac catheterization for chest pain, the dose administered did not cause chest pain but it did reduce the diameter of the left coronary artery by at least 8%–12% (Lange et al., 1989). In related studies, it was observed that vasoconstriction is more intense in atherosclerotic vessels than in vessels without apparent underlying disease (Flores et al., 1990). Cocaine and cigarettes act synergistically on diseased arterial segments to produce even greater degrees of vasoconstriction: 19% constriction for cigarettes and cocaine combined versus only 9% constriction after cocaine and 5% after cigarette smoking (Moliterno et al., 1994).

Just the opposite happens when cocaine is combined with alcohol. When patients evaluated for chest pain were administered ethanol and cocaine in combination, significant increases in myocardial oxygen demand were observed. However, there were also concomitant increases in epicardial coronary artery diameter, suggesting that no net decrease in myocardial oxygen supply resulted (Pirwitz et al., 1995).

The etiology of coronary spasm in cocaine users is likely to be much the same as it is in cocaine-associated accelerated atherosclerosis. Preexisting coronary artery disease is associated with endothelial dysfunction and derangement of nitric oxide metabolism of endothelial cells. The question to be answered is how this derangement occurs. Cocaine causes increased production of vascular endothelial growth factor (VEGF) in the lung (Strong et al., 2003) but elsewhere exerts conflicting effects; it is impossible, at the moment, to say which effect predominates in coronary arteries. VEGF inhibition is thought to decrease NO production in arteriole walls and increase ET-1 production, which is a potent vasoconstrictor (Masumoto et al., 2002; Kamba and McDonald, 2007).

Increased rho-kinase, an enzyme that controls the production and orientation of actin filaments (ROCK) activity, is known to play an important role in coronary artery spasm, at least in animal models (Kandabashi et al., 2000). When ROCK inhibitors are given to patients with acetylcholine-induced coronary artery spasm, spasm is mostly abolished and a significant effect on suppressing coronary artery spasm in patients with vasospastic angina has been demonstrated (Masumoto et al., 2002). Autonomic tone is increased in cocaine users (Vongpatanasin et al., 1999; Mehta et al., 2004a), and even in normal coronary arteries, spasm is induced by the application of acetylcholine and blocked by the application of atropine. There is mounting evidence that central sympathetic control has a significant impact on coronary artery caliber (Menon et al., 2007).

Unless spasm occurs at the site of preexisting coronary artery narrowing, it would seem unlikely that it could account for very many episodes of infarction, at least in cocaine users. In humans, anything less than a 75% cross-sectional coronary obstruction is unlikely to produce symptoms, so constriction of an epicardial vessel of only 10%–12% should be

an asymptomatic event unless, of course, severe atherosclerotic disease is already present. In fact, in another human study, doses of 1.2 mg/kg of cocaine administered intravenously to 20 human volunteers (resulting in mean cocaine levels of 709 ng/mL vs. levels of only 120 ng/mL in the Lange et al. studies cited earlier) produced only nonspecific T- and R-wave changes with no changes in the left ventricular ejection fraction or wall motion score index (Eisenberg et al., 1995).

1.14.5 HIV-Related Myocardial Disease

Infiltrates in the hearts of drug users may indicate HIV infection. A variety of opportunistic infections occur as a consequence of HIV infection and, as more effective therapies have been introduced and survival times have lengthened, new complications of late-stage HIV infection have emerged. Almost any agent that can cause disseminated infection in patients with acquired immunodeficiency syndrome (AIDS) may involve the myocardium, but clinical evidence of cardiac disease is usually overshadowed by disease in other organs, primarily the brain and lungs. In the past, reports suggested that cardiac abnormalities could be found at autopsy in two-thirds of patients with AIDS but, in the age of aggressive medical treatment, the number of cocaine-using, HIV-infected patients seems to have decreased. An autopsy study published in 2008 found an increased frequency of coronary wall and adventitial infiltrates, myocarditis, and thickened intramyocardial vessels (14.5%, 17.4%, and 17.4% vs. 6.5%, 3.3%, and 0% in controls) in HIV-infected cocaine users coming to autopsy (Mosunjac et al., 2008). All of the reported findings are nonspecific and without particular diagnostic value.

1.14.6 Valvular Heart Disease

As intravenous drug users (IVDUs), cocaine abusers are at risk for the development of valvular heart disease. However, such events must be very rare, with only an occasional case report appearing in the literature. In theory, some of the amphetamine analogs should be injurious to heart valves. Very little has been written on the subject, but echocardiographic studies have failed to demonstrate increased risk with cocaine (Gullone et al., 2003).

1.14.7 Aorta and Peripheral Vessels

Acute aortic dissection as a consequence of "crack" cocaine use is common, presumably as a consequence of abrupt, transient, severe hypertension and catecholamine damage to the aorta itself (Hsue et al., 2002). It has been hypothesized that apoptosis of vascular cells in the walls of great vessels can lead to hemorrhage and ischemic stroke (Dabbouseh and Ardelt, 2011). According to this later theory, cocaine may cause apoptosis of cells in the aortic wall, leading to apoptosis of vascular endothelial and/or smooth muscle cells, thereby weakening the vascular wall and resulting in a dissection-prone state. While that may be the case, it has never been proven in an experimental model. Fewer than 60 cases of cocaine-related dissection have been reported since the mid-1980s (Mehta et al., 2004b). Most have been type I dissections with the process extending from the ascending aorta to the iliac vessels. Only one of these individuals was found to have the typical pattern of medial degeneration associated with Marfan's syndrome, and even then, none of the other stigmata of the disorder were present (Cohle and Lie, 1993).

The pattern more commonly encountered in cocaine-related dissection is a focal microcystic medial necrosis and a fragmentation of the elastic fibers in the arterial walls (Palmiere et al., 2004). Transient hypertension occurs in virtually all cocaine users, and some preliminary studies have shown damage to the media and elastic layers in the aortas of rats chronically treated with cocaine (Lagner and Bemet, 1991). A third, though seldom considered, predisposing factor is pregnancy, and dissecting aneurysm has been described in pregnant cocaine users (Madu et al., 1999). Like coronary artery aneurysms, aortic aneurysms in pregnancy are thought to be the result of progesterone excess. The high concentrations of progesterone associated with pregnancy may lead to destruction of elastic fibers and fragmentation of reticular fibers in the wall of the aorta, ultimately resulting in loss of structural integrity (Madu et al., 1999). Transverse tears in the aortic wall itself initiate the process of aortic dissection. For dissection to occur, tears must extend through the intima and at least halfway through the media (Houston et al., 2011). There are only two cases in the peer-reviewed literature relating cocaine use to abdominal aortic dissection (Madu et al., 1999; Guerot et al., 2002).

1.14.8 Eosinophilic Myocarditis

The presence of an eosinophilic infiltrate suggests a hypersensitivity phenomenon. Hypersensitivity myocarditis is distinguished from toxic myocarditis by several important features: (1) it is not dose related, (2) the lesions are all of the same age, (3) hemorrhages are rare, and (4) myocyte necrosis is not present. The list of drugs causing hypersensitivity myocarditis has become increasingly long (Billingham, 1985). When eosinophils have been observed in the myocardium of cocaine users, they are usually considered as an incidental finding, independent of whether the tissue was obtained at autopsy or from biopsy specimens obtained to evaluate chest pain, heart failure, or arrhythmia. Most of the time, the clinical manifestations of this disorder are so nonspecific that the diagnosis is rarely suspected during life (Taliercio et al., 1985).

None of the cocaine users with eosinophilic infiltrates have had signs of extracardiac involvement such as polyarteritis nodosa or eosinophilic leukemia. In general, these patients do not match the picture classically associated with acute necrotizing myocarditis (Herzog et al., 1984), but they may resemble patients with eosinophilic coronary arteritis (Churg–Strauss syndrome, also called allergic granulomatous angiitis) (Orriols et al., 1996). In one case report, a female "crack" smoker first presented with relapsing fever, bronchoconstriction, arthralgias, and weight loss. She then went on to develop pulmonary infiltrates, arthritis, microhematuria, skin rash, and mononeuritis multiplex. Both skin and muscle biopsies showed eosinophilic angiitis.

It is difficult to say where eosinophilic angiitis fits into the classification of this group of disorders. The inflammatory process involved only the small- and medium-sized vessels. Both skin and peripheral nerve involvement occurs, and in addition to eosinophilia, there may be extravascular necrotizing granulomas and eosinophilic infiltration of multiple organs (particularly the lungs but also the gastrointestinal tract, the heart, and the kidneys) (Basak et al., 2011).

Many agents can cause toxic myocarditis, and even though the purity of cocaine sold in the United States is increasingly high (generally >60%), a variety of adulterating agents are still in use, with new agents added to the list almost monthly. Of particular concern is the relatively recent introduction of levamisole as an adulterant. It is found in much of the world's cocaine supply (Bertol et al., 2011; Buchanan et al., 2011). Prior to the advent

of levamisole, a review paper published in 1988 listed sugars (lactose, sucrose, mannitol) as the most common cocaine adulterants, followed by stimulant drugs (caffeine, amphetamines) and local anesthetic agents. In Europe, and in many parts of the United States, caffeine and lidocaine were, and remain, popular.

After an initial flurry of reports in 1986 and 1987, recent mentions of eosinophilic infiltrates are uncommon. One explanation may be that most cocaine users are now "crack" smokers, and "crack," while it may contain large amounts of bicarbonate, is otherwise largely free of other chemical contaminants. Finally, it must be emphasized that the mere presence of eosinophilic cells in the myocardium does not necessarily mean that active eosinophilic myocarditis is present. Similarly, the lymphocytic infiltrates seen in the hearts of cocaine users are generally not accompanied by myocyte necrosis. According to the Dallas criteria, infiltrates without necrosis do not prove myocarditis (Aretz, 1987), though it is now generally accepted that DNA resequencing is required to make a definitive diagnosis. What these infiltrates represent is not clear, but similar infiltrates are also seen in experimental animals with catecholamine toxicity.

Confusing the issue even further is the recent discovery that many cases of myocarditis (proven positive by DNA analysis) are not accompanied by infiltrates (Baughman, 2006). Of course, such an occurrence would be irrelevant in a cocaine abuser. Since nothing would be seen at autopsy, the diagnosis would not be considered.

1.14.9 Coronary Artery Dissection

Spontaneous coronary artery dissection (SCAD) remains a reportable cause of ACS or sudden death. A connection between cocaine abuse and coronary artery dissection is recognized (Cohle and Lie, 1992; Eskander et al., 2001; Steinhauer and Caulfield, 2001; Bizzarri et al., 2003; Castro, 2003; Ijsselmuiden and Verheye, 2009; Sanchez-Recalde et al., 2009; Kanwar and Gill, 2010; Rasoul et al., 2010), but not really understood. Coronary artery dissection usually affects young women during the peripartum period and women using oral contraceptives. Unlike atherosclerotic intimal dissection, the dissection plane in spontaneous coronary dissection lies within the media or between the media and adventitia. Eosinophilic periadventitial inflammation has been commonly observed in such cases (Gowda et al., 2005).

More recently, evidence has been published raising the possibility that there may be a nexus between the fibromuscular dysplasia (FMD) present in the small intramyocardial arteries of the hearts of most chronic users and coronary dissection (Alfonso, 2012). The possibility of a connection was recently raised when one author reviewed 87 consecutive patients (mostly women) with SCAD seen at the Mayo Clinic over a 30-year period. In every case, a dissection plane was visible on angiography but there was no evidence of atherosclerosis (Tweet et al., 2012).

Tweet observed, "it seems reasonable to speculate that FMD (fibromuscular dysplasia) may be both present in the coronary arteries and mechanistically linked to the occurrence of SCAD (spontaneous coronary artery dissection) in a significant proportion of young patients with sudden death." Because SCAD is much more common in women, particularly women in the peripartum period, a direct hormonal effect on the coronary vasculature is possible, if not likely. Whether cocaine can trigger the same downstream events is pregnancy is not known. What we do know, however, is that cocaine can initiate events associated with myocardial remodeling—a downstream event initiated by an upstream stressor (Henning and Cuevas, 2006).

1.14.10 Nonatheromatous Coronary Artery Disease

Nonatheromatous coronary disease causes slightly more than 10% of sudden coronary deaths and is more frequent in young Black women. This type of disease is a relatively infrequent pathway for coronary plaque progression but still may lead to plaque erosion and sudden death. Cocaine use alters production of VEGF. As a consequence, the coronary arteries of an occasional cocaine user will display exactly the same histologic abnormalities seen in transplant patients (Simpson and Edwards, 1986; Nykänen et al., 2006); the reasons remain unknown.

1.14.11 Accelerated Coronary Artery Disease

Significantly elevated plasma levels for multiple markers known to be associated with atherosclerosis (plasma levels of stromal cell-derived factor-1, monocyte chemotactic protein-1, β-soluble intracellular adhesion molecule, high-sensitivity C-reactive protein, and ET-1) may be evident in many, if not all, cocaine abusers. These values tend to normalize when cocaine is discontinued, which is fortunate because most of these compounds are clearly prothrombotic. When present in excess, these factors would favor the occurrence of endothelial damage and thrombosis, which perhaps explains the high incidence of ST segment elevation myocardial infarction in cocaine users (Saez et al., 2011).

1.15 Excited Delirium Syndrome

1.15.1 History and Overview of Excited Delirium Syndrome

Luther V. Bell, an American physician, first described excited, or agitated, delirium more than 150 years ago. The *American Journal of Insanity* published his lengthy paper "On a form of disease resembling some advanced stages of mania and fever, but so contra distinguished from any ordinarily observed or described combination of symptoms as to render it probable that it may be an overlooked and hitherto unrecorded malady." All victims died suddenly after they had experienced a brief period of mania and fever (Bell, 1849). Fifty years after the publication of Bell's paper, similar reports began to reemerge in the popular press, but these reports were intended to do more than draw attention to a new, and potentially fatal, disease. The new reports were, in fact, deeply interwoven with elements of racist hysteria. They were published in part by opponents of the prohibitionist movement. They argued that people would just start using other drugs, such as cocaine, if they were unable to purchase alcohol. The prohibitionists, on the other hand, argued that cocaine users might become deranged—deranged enough to commit random acts of homicide.

A physician working for the *New York Times* (Williams, 1914) wrote the most racist of these reports. His article described a series of homicides, violent crimes, and sexual assaults allegedly committed by Black men under the influence of cocaine. Williams claimed that cocaine not only made the men crazed and resistant to bullets, but it gave them a temporary immunity to shock. He, too, attributed the new menace to the restriction of alcohol sales (Knopf, 1924). When prohibition ended, so did concerns about cocaine-related violence, and reports of new cases simply stopped appearing in medical journals and newspapers.

The first modern mention of cocaine-associated excited delirium was in a scientific paper published in 1981 (Fishbain and Wetli, 1985). Most of the cases reported in that paper ended in death. However, when a group of researchers at UCLA examined all cases of apparent excited delirium reported to the emergency medical service (EMS), they found that fatalities only occurred in approximately 10% of cases (Stratton et al., 2001). Anecdotally, it appears that ExDS is a recurring disease and many individuals suffer more than one episode. It is also clear that not all of the cases are due to cocaine (O'Halloran and Lewman, 1993; Kiely et al., 2009; Mash et al., 2009; Lusthof et al., 2011) and that different mechanisms leading to death may be involved. Several case reports suggest that occasional use of synthetic cannabinoids and the bk-amphetamines is capable of producing exactly the same symptoms as those seen in chronic stimulant abusers (Lusthof et al., 2011; Wiegand et al., 2013; Penders and Gestring, 2014). Having said that, it is important to note that the vast majority of reported cases have occurred as a consequence of stimulant abuse (mostly cocaine and methamphetamine). The mechanisms of cocaine-induced ExDS have been reasonably well characterized.

Fatal cases of ExDS have of four separate components, each occurring in sequence: hyperthermia, followed by delirium with agitation, followed by respiratory arrest, and death, although hyperthermia may be absent in some cases and drugs in others. As a rule, individuals who develop this disorder as a consequence of chronic cocaine abuse are found to have low to modest blood cocaine concentrations. Regardless of the drug or psychiatric disorder responsible, the sequence of symptoms appears to be the same.

The clinical course of patients with ExDS is entirely different from the clinical course of body packers suffering from massive cocaine overdose (continuous seizures, respiratory depression, and death). No government or international agency effectively tracks the incidence of ExDS. However, there is very little doubt that the number of cases has increased markedly since the late 1980s. Deaths from excited delirium now account for a significant number of in-custody deaths both in the United States and in Europe. There is good reason to think that the number of newly diagnosed cases indicates that the supply of cocaine is increasing, or at least remaining relatively stable. The increase might also be explained by the availability of other, equally dangerous stimulants, particularly drugs categorized as "bk-amphetamines, many of which remain legal" (EMCDDA, 2010), and which, after only one dose, can produce the same ExDS as seen in chronic stimulant abusers.

In the early stages of the syndrome, which rarely last for more than a few hours, the vast majority of victims are hyperthermic, sweaty (warm to the touch even in frigid weather), paranoid, grossly psychotic, and agitated to the extent that they may perform superhuman feats of strength, especially when attempts are made to forcibly restrain them. Sudden and unexpected death usually occurs shortly after the struggle has terminated. After a relatively short struggle, agitation ceases and the patient stops breathing. Death investigators are accustomed to hearing officers say that once "the suspect was restrained, he suddenly became quiet." Based upon numerous published descriptions and court testimony, another minute or so may elapse between the time the individual is subdued and the time it is noted that the individual is not breathing.

The ExDS victim may well have been shot with a TASER®* (conducted electrical weapon [CEW]) during the restraint process. Because death sometimes occurs soon after the victim has been shocked, a causal relationship has been suggested. Putting aside other issues, such as the

* TASER is a registered trademark of TASER International, Inc.

extremely low current delivered by these devices (less than half that of a cattle fence), such a relationship is unlikely for several reasons, the most obvious being that respiratory arrest, according to anecdotal reports, usually does not occur concurrently with delivery of the shock, though if it does, a nexus must be considered. The body does not store electricity; lacking that ability, there is no way for a CEW discharge to cause death minutes after it was applied. Of course these considerations eliminate any possible involvement when the device is used in the "drive-stun" mode, as no current ever goes through the body, just a localized area of skin (Figure 1.62), and this observation has been affirmed by the courts. In *Hoyt v. Cooks*, 672 F.3d 972 (C.A.11 (Ga.) February 27, 2012), the court found, among other things, "a stark contrast between the prong mode (which overrides the central nervous system and disrupts muscle control) and the much less serious [drive] stun mode (which results merely in pain, a burning sensation)."

It also has been suggested that CEW shocks can somehow cause the heart rhythm to deteriorate into VF. Against this possibility are two important observations, the first being that victims of ExDS are, for all intents and purposes, almost always found in asystole (Swerdlow et al., 2009), not VF; there is no way to induce asystole with an electric current. Another important consideration is that, even if a CEW's shocks are proven to be capable of causing VF or asystole in humans, there would be no way for that to occur unless the heart were actually a part of the electrical circuit created by the device. Should the barbs land in the leg, abdomen, or back (as is often the case), the heart would not be included in the circuit and, therefore, could not be affected by the electrical discharge. Lastly, it should be noted that fewer than 20% of ExDS victims have received a CEW discharge, leaving no explanation for the deaths of the other 80% (Mash et al., 2009).

In 2009, the U.S. Department of Justice commissioned a detailed evaluation of the effects of the Taser on humans (*An Independent Assessment of the Physiological and Cognitive Effects from the X-26 TASER® Device in Volunteer Human Subjects*) (Criscione 2009). The study was particularly relevant because it was a controlled study with human volunteers, specifically designed so that the heart would be included in the circuit when the

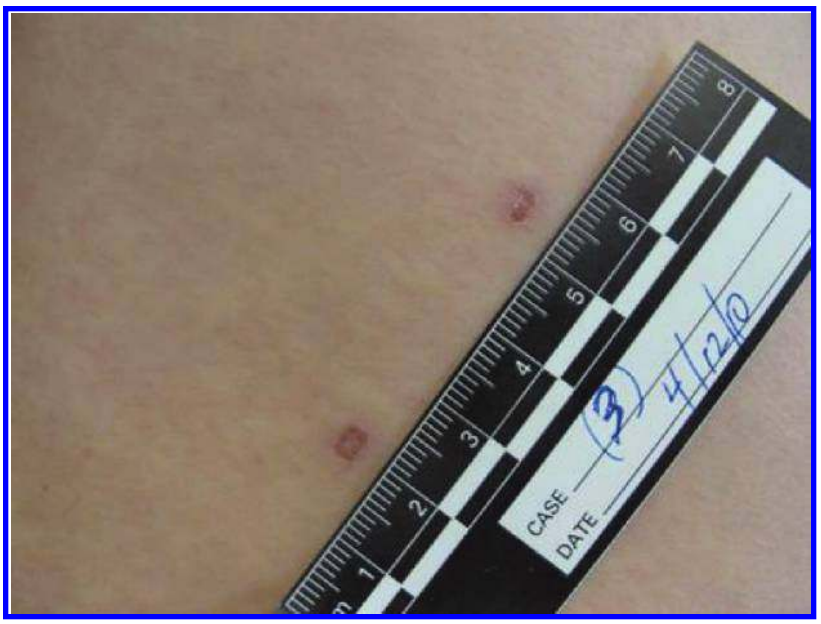


Figure 1.62 Burn from a conducted electrical weapon, 6 days post injury.

Taser was discharged. Multiple physiologic and hematologic parameters were recorded. The results showed no clinical effects of any consequence. Blood lactate increased to levels comparable to those seen in strenuous exercise, and plasma cortisol concentration increased by one standard deviation (Criscione 2009).

The panel's conclusion would seem to be confirmed by a case report published in 2006 that described a 51-year-old woman (height, 170 cm; weight, 75 kg) who had undergone placement of a single-chamber internal cardiac defibrillator (ICD; Guidant Prizm 2VR, model 1860, Guidant Inc., St. Paul, MN, USA) 5 years earlier for idiopathic VF (presumably from a channelopathy, though the paper never stated as much). The lead had been placed in the right ventricular apex. The patient was "Tased" because of uncontrolled violent behavior (the case report does not specify the circumstances). The CEW darts struck the sternum directly. When the CEW trigger was activated, current was delivered for 5.36 s. After the initial immobilization, the woman recovered and suffered no immediate adverse effects. Two months later, the patient presented for regular follow-up at the ICD clinic. Interrogation of the ICD device revealed one episode of what appeared to be VF, which corresponded to the time the CEW had been activated. What had happened was that the ICD interpreted the CEW discharge as an episode of VF, causing the capacitor in the ICD to charge so that the woman could be defibrillated. But, by the time the ICD had charged, the CEW was no longer firing and the ICD turned itself off without ever delivering a defibrillator shock. It was apparent from interrogation of the device that at no time during the CEW discharge had there been any change to the underlying rhythm (Haegeli et al., 2006).

ExDS victims who do not come to police attention are often found dead in their bathrooms, surrounded by wet towels and clothing, sometimes even with empty ice trays scattered about, suggesting a vain attempt to treat their fevers, which may be as high as 108°F. Epidemiologic studies of victims who actually die on scene, or at least die on the day of symptom onset, indicate that decedents are much more likely to be men than women. They are also more likely to die in custody and more likely to live for 1 h after onset of symptoms. In Miami, men with excited delirium account for 10% of all cocaine-related deaths. The syndrome is more common in summer, especially when the weather is warm and humid. Indeed, all types of cocaine-related death, not just excited delirium, seem to be more common when temperatures are elevated (Ruttenber et al., 1997; Marzuk et al., 1998).

In a case series of 90 ExDS victims collected from around the world, 38.9% were found to have died in hospital, 31.1% at the scene, 15.6% during EMS transport, and 14.4% during police transport (Mash et al., 2009). In a Canadian case study of 21 unexpected deaths associated with ExDS, it was ruled that death had been caused by a primary psychiatric disorder in 12 people (57%) and by cocaine-induced psychosis in 8 (38%). Eighteen of the victims (86%) were in police custody when they died. Four (19%) had been sprayed with capsicum oleoresin, and heart disease was found in another four at autopsy (Pollanen et al., 1998).

In 1985, Wetli and Fishbain described seven cases. All had fairly stereotyped histories, such as the 33-year-old man who was found pounding on the door of a house he had moved out of some time previously (Wetli and Fishbain, 1985). Since then, many more cases have been reported and the clinical presentation of most mirrors one of Wetli's cases described in the following.

> He was shouting that he wanted to see his wife and daughter. The occupants informed him that nobody by that name resided there, yet he pursued his actions. Four bystanders finally restrained him and assisted police units upon their arrival. The subject was handcuffed and

put into a police car, whereupon he began to kick out the windows of the vehicle. The police subsequently restrained his ankles and attached the ankle restraints and handcuffs together. He was then transported to a local hospital. While en route, the police officers noted he became tranquil (about 45 min after the onset of the disturbance). Upon arrival at the hospital a few minutes later, the subject was discovered to be in a respiratory arrest. Resuscitative attempts were futile. A postmortem examination was performed 1 h and 45 min later (about 3 h after the onset of the disturbance), and a rectal temperature of 41°C (106°F) was recorded. He had the needle marks typical of intravenous drug abuse and was also found to have pulmonary and cerebral edema. Abrasions and contusions of the ankles and wrists were also evident from his struggling against the restraints. Toxicologic analysis of postmortem blood disclosed 52.3 mg/L of lidocaine and 0.8 mg/L of cocaine. No lidocaine was administered to the victim during resuscitative attempts.

1.15.2 Excited Delirium and the Redefinition of "Positional Asphyxia"

If police restrain a violently agitated individual, who is almost always under the influence of drugs, and that person dies, questions will be raised about the type and level of force applied. If the force is deemed inappropriate, litigation will ensue. There is no question that some methods, such as the baton choke hold, are clearly harmful and can cause death (Reay and Eisele, 1982), but these obviously inappropriate applications of force are no longer permitted. There is, however, a general perception that any form of police restraint may be fatal, especially when prisoners have been bound while lying prone, with the arms behind their backs ("the hobble restraint" or "hog tying"). This belief is erroneous and runs counter to all established principles of medical physiology, but it endures nonetheless. The situation is not helped by the number of case reports published on the topic by well-meaning pathologists untrained in the general principles of exercise physiology.

Beginning in the late 1980s, pathologists first began publishing anecdotal case reports describing the deaths of drug users who were being restrained (O'Halloran and Lewman, 1993, 2000; Belviso et al., 2003; Otahbachi et al., 2010). Because they were only case reports, and not controlled scientific studies, they served little value except to draw the attention of the medical community to the fact that a problem did, indeed, exist. In general, these reports were incomplete, uncontrolled, retrospective, and lacked operational criteria for identifying when an adverse event had actually occurred (Kelly, 2003; Hollingsworth and Lasker 2004; Kelly et al., 2007).

In the late 1980s, some medical examiners began applying the term "positional asphyxia" whenever they were confronted with an agitated psychotic patient transported prone who died suddenly. If the autopsy was said to be unrevealing, the term "positional asphyxia" was applied (Reay et al., 1992). In fact, the autopsies were rarely unrevealing. The prosecutors had simply failed to recognize key anatomic and histochemical changes.

In most reported positional asphyxia deaths, autopsies are often incomplete: heart weights are not obtained or are not normalized for body size, the histologic examination may be omitted altogether, and the brain is examined without prior fixation or microscopic examination. Paramedics and even medical examiners fail to record the patient's temperature, either at the scene or at the time of postmortem examination. If the temperature has not been recorded, proving that a decedent suffered from excited delirium becomes that much more difficult.

The concept of positional asphyxia, at least as it was first applied in the 1980s and 1990s, has been thoroughly reviewed, and the underlying hypothesis, that death may occur simply as a result of restraint in a prone position, has been tested as required by the scientific

method and rejected. The results of controlled clinical studies simply do not support the idea that "hog tying" or similar measures can lead to a fatal outcome.

Many studies now have shown that the "hog tying" of normal-sized individuals (BMI < 30) has no significant effect on respiratory function (Chan et al., 1997; Schmidt and Snowden, 1999; Elfawal 2000; Meredith et al., 2005; Michalewicz et al., 2007), or even in the obese provided they have normal hearts and lungs (Cary et al., 1999; Sloane et al., 2014). It is now clear that the term should be reserved for those cases where alcoholics, or the otherwise infirm, fall into a confined space, unaware that their chests are not expanding enough to support respiration (DiMaio and DiMaio, 1989; Purdue, 2000). The mechanism in such cases is easily identified because the autopsy will disclose marked skin congestion, cyanosis, and showers of petechiae, findings conspicuously absent in decedents dying during the restraint process.

As a consequence of these controlled studies, and the admission by the originator of the theory of positional asphyxia that he had been mistaken (Reay and Howard, 1999), some medical examiners confronted with a decedent who dies while being restrained may now attribute death to an entity referred to as "restraint asphyxia" or, alternatively, "death during a restraint procedure." Most pathologists who chose to use these descriptors rarely articulate their reasons, but one presumes that they believe that pressure applied to the back in some way disrupts ventilatory mechanics, thereby asphyxiating the victim. As discussed earlier, it has been argued that obese individuals (BMI > 30), when placed in a prone position, will have upward pressure exerted on their diaphragm, basically asphyxiating themselves. This hypothesis has been tested and we now have Level I data that obesity is not a significant contributor to the problem.

1.15.3 Confounding Issues

It is not generally appreciated that attempts at endotracheal intubation and CPR can produce petechiae, contusions, and even damage to the tracheal mucosa and strap muscles of the neck (Raven et al., 1999; Hashimoto et al., 2007; Buschmann and Tsokos, 2009). Any one of these artifacts may wrongly be attributed to the effects of neck compression or application of a choke hold. It is also clear that rib fractures are often associated with CPR, and there is some evidence that newly adopted techniques for chest compression increase the likelihood of rib fractures (Reyes et al., 2011). If resuscitative attempts go undocumented, allegations will be raised that inappropriate levels of force were exerted.

1.15.4 Exercise Physiology of "Positional Restraint" and "Restraint Asphyxia"

In order for tissue to receive an adequate supply of oxygen, two things must occur: (1) The blood must be oxygenated (blood flowing through the lungs must absorb oxygen from, and release carbon dioxide into, the air being pumped through the bronchi) and (2) the oxygenated blood must flow normally to the tissues. At rest, a normal human being exchanges approximately 500 mL of air with each breath and does so 12–16 times a minute. Put another way, each minute the normal lungs of a normal person move between 6 and 8 L of air, just enough to supply all of the body's oxygen needs. If there were a greater need for air, for example, someone competing in a 1000 m relay, the normal body could easily increase the airflow to 160–180 L/min (Guyton and Hall, 2000).

The body has enormous reserves of oxygen, even at rest. In fact, the maximal breathing capacity is about 50% greater than ventilation during maximal exercise (Guyton and Hall, 2000). This excess is what protects athletes, because it supplies them with the additional oxygen delivered by the extra ventilation. This increased supply of oxygen can then be called upon for use as demand increases. Even someone exerting himself or herself as hard as they possibly can (this state is referred to as VO_2 max, or "maximal oxygen consumption") would be unable to use up all of the oxygen available to them.

Even if sufficient weight was placed on the back to impede the delivery of blood and oxygen to the tissues, loss of consciousness would hardly be immediate. The body contains a great deal of oxygen (both in the lungs and in the blood), and death would be delayed until all of these oxygen reserves were used up, a process that would take several minutes and would almost certainly be accompanied by florid cyanosis and VF—not asystole, which is usually observed in these situations (Stratton et al., 1995). Furthermore, the limiting factor in delivering oxygen to the tissues is not the ability of the lungs to exchange air but, rather, the ability of the heart to pump oxygen-containing blood.

If it were possible to place enough weight on the back to prevent respiratory exchange (short of preventing the chest from moving entirely, which was what the term "positional asphyxia" was originally meant to describe), how much of a reduction in ventilation would be needed to cause significant symptoms? Evidence from numerous sources suggests that a reduction in breathing capacity of nearly 80% would be required (Rochester and Esau, 1994; Neuman, 2006). This number is not arbitrary; rather it derives from observations made in numerous medical conditions where oxygenation/ventilation is disrupted.

The question then becomes, "How much weight would be required to cause asphyxia?" The simplest answer is "Whatever weight is sufficient to reduce ventilation by 80%" (the majority of patients with lung disease are not considered eligible for a lung transplant until 80% of their lung capacity has been lost). Can a policeman, or even two policemen, placing their feet on a prisoner's back, exert enough force to prevent chest movement, and, if so, how many pounds of pressure would that be? Controlled clinical studies have shown that placing up to 225 lb on the backs of restrained volunteers produces no significant decrease in maximum voluntary ventilation (Michalewicz et al., 2007). Whatever the real number is, it must be greater than 225 lb (100 kg) to produce any measurable, let alone clinically significant effect.

The newest variant on the "asphyxia" theme is that death during restraint is the result of tako-tsubo cardiomyopathy, the onset of which is precipitated by a surge of catecholamines. There is no dispute that high levels of catecholamines can precipitate coronary vasospasm or even infarction in an individual with preexisting coronary disease, especially in the case of stimulant abusers. Given that tako-tsubo disease almost always involves middle-aged or elderly women and is rarely fatal, tako-tsubo seems an unlikely candidate for causation. The pathophysiology of this disease is not well understood, but it is characterized by transient systolic dysfunction with apical ballooning. There is an emerging consensus that the prime cause of this disorder is an abnormal focus of cardiomyocytes triggered by the autonomic nervous system (Movaheda and Donohueb, 2008; Fineschi et al., 2010). It is important to understand that, although its initial manifestations may resemble AMI, the best way to make the diagnosis is via thorough history taking and the speed with which myocardial function recovers (very quickly in tako-tsubo) (Wybraniec et al., 2014).

1.15.5 Neurochemistry of Excited Delirium Syndrome

Within the brain, the chief abnormalities in ExDS have to do with the number and type of dopamine receptors and dopamine transporters present, the location of these structures within the brain, and their individual molecular structure. Cocaine users have increased numbers of D_1, D_2, and D_3 receptors (Staley and Mash, 1996). When compared to the brains of drug-free trauma victims, the brains of nonpsychotic cocaine users have more active dopamine-binding sites located in the striatum. That is not the case in cocaine users with excited delirium. In fact, the opposite holds true for the psychotic form of the disease. Patients with ExDS lack an effective way to clear dopamine from their brain synapses.

Disordered dopamine metabolism is, of course, a key component of Parkinson's syndrome, and any information that helps to explain the mechanism of one may go a long way toward explaining the mechanism of the other. A disorder known as "dopamine transporter deficiency syndrome" (dopamine transporters are abbreviated as DATs) is the first identified parkinsonian-type disorder known to be caused by genetic alterations of the dopamine transporter. It occurs in children with mutations in the gene that encodes the dopamine transporter (*SLC6A3*) (Figure 1.63).

Similar, but not identical measurements have been made in patients suffering from ExDS, and a comparison of autoradiographs and PET scans shows striking similarities as seen in Figure 1.64: the brain of a drug-free control, the brain of one individual who died of a cocaine overdose, and a brain from a patient with ExDS. The brighter the color of the structure, the greater the increase in the number of DAT present. Note that there is no increase in the control and no increase in the victim with ExDS.

The abnormalities causing ExDS involve more than just a DAT polymorphism. In nonpsychotic cocaine users, major increases also occur in the density of D_1 receptor subtypes throughout the striatal rewards areas. Similar increases are not seen in individuals with excited delirium. Tolerance is also explained by changes in the number of dopamine-binding sites.

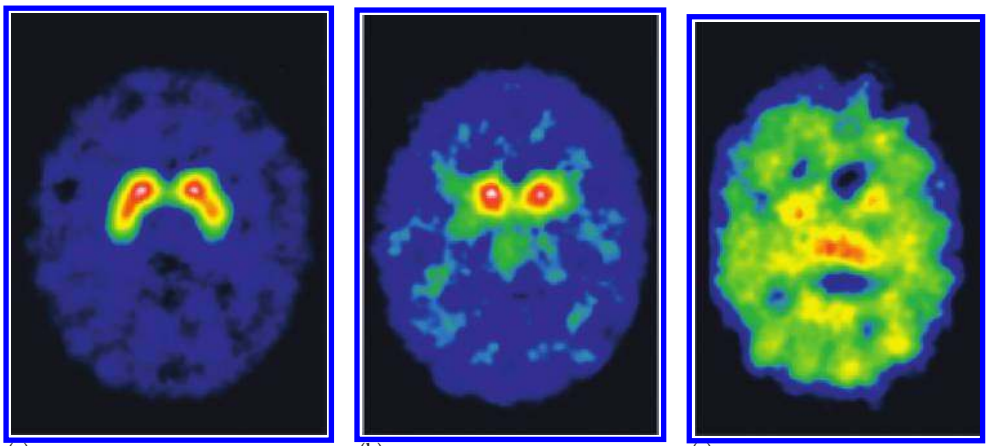


Figure 1.63 PET scans showing dopamine transporter activity in children: (a) The normal pattern of transporters in a control individual with normal function, (b) the pattern in a patient with juvenile parkinsonism of unknown etiology, and (c) the brain of a child with a genetically defective gene for the dopamine transporter. (c) A striking resemblance to autoradiograph studies in the ExDS victims (Kurian et al., 2011). Although Parkinson's disease and ExDS are clearly different disorders, it would appear that sufferers of both disorders share a common inability to clear excessive dopamine from certain critical areas of the brain. (Reproduced with permission from PUBMED.)

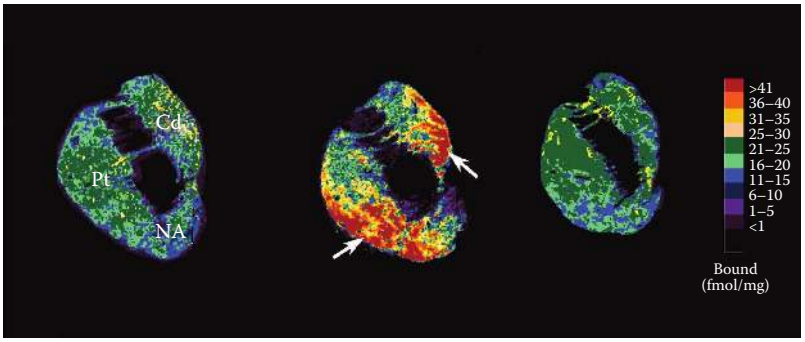


Figure 1.64 Excited delirium. Adaptive increases in the number of dopamine transporters can be seen in the brains of cocaine users dying of overdose but not in the brains of patients with excited delirium. Shown here are in vitro autoradiographic maps of [^{3}H]WIN 35,428 labeling of the dopamine transporter in coronal sections of the human brain from (a) a representative age-matched and drug-free subject, (b) a cocaine overdose victim, and (c) a cocaine-related excited delirium victim. Color codes are presented at the right and are matched for the range of density values across the groups (red = high densities; yellow = intermediate; blue = low to background densities). *Abbreviations:* Cd, caudate; NA, nucleus accumbens; Pt, putamen. (Courtesy of Debra Mash, University of Miami School of Medicine, Miami, FL.)

In nonpsychotic cocaine users, the number of D_2 receptors remains relatively unchanged, but in ExDS victims, there are almost always marked decreases. ExDS victims have temperature elevations because there are marked reductions in the number of D_2 receptors in the hypothalamus. These receptors mediate temperature control. With fewer D_2 receptors available, D_1-mediated temperature increases are unopposed (Staley et al., 1994). Whether or not hyperthermia occurs, and the magnitude of the temperature increases, depends upon the absolute decrease in D_2 receptors; if it is not very great, then hyperthermia may or may not occur.

The focal distribution of the D_3 dopamine receptor in brain regions implicated in emotional and cognitive functions has become a target of drug discovery efforts (Heidbreder and Hagan, 2005). Repeated exposure to drugs of abuse produces long-term molecular and neurochemical changes thought to be involved in the addiction process. Knowledge of the number of molecular and cellular targets for addictive drugs is steadily increasing. These include the D_3 receptor in both the rodent and human brain. There is hope that a selective D_3 antagonist might prove an effective treatment for cocaine addiction (Heidbreder and Newman, 2010).

The involvement of D_3 receptors only became apparent after it was discovered that, compared to drug-free controls, the brains of nonpsychotic cocaine users contain an increased number of D_3 binding sites (Mash and Staley, 1999), with a one- to threefold increase measurable in the nucleus accumbens (NAC) and in the ventromedial sectors of the caudate and putamen. The NAC is a collection of brain stem neurons deeply implicated in the process of addiction to all drugs.

By mechanisms yet to be determined, the increase in D_3 receptors is related to an increase in the number of κ-opioid receptors. Nonpsychotic cocaine users, when compared to drug-free controls, have twice the number of κ receptors in the NAC and other corticolimbic areas. Unlike nonpsychotic cocaine users, cocaine users who die of ExDS have a selective upregulation of κ receptors in the amygdala (Staley et al., 1997; Mash and Staley, 1999).

Proteins called α-synucleins are also elevated in patients dying with ExDS (Figures 1.65 and 1.66). The function of α-synucleins, encoded by the *SNCA* gene, is not really

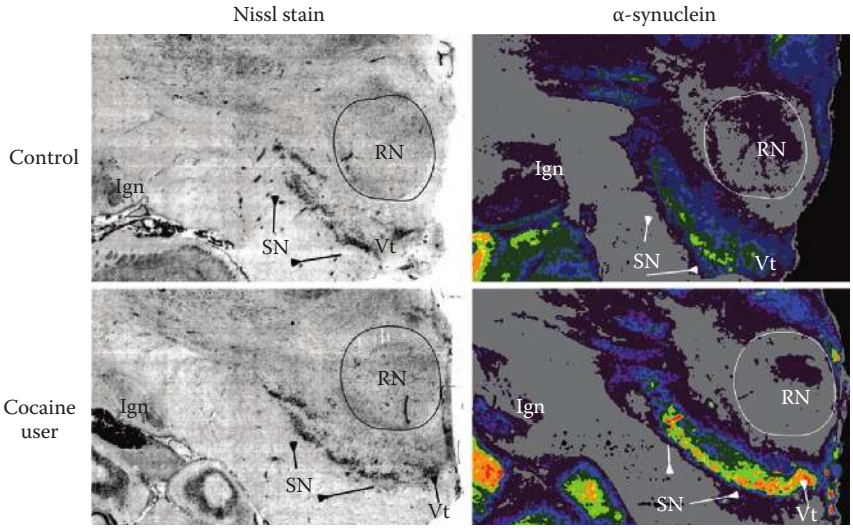


Figure 1.65 Alpha-synucleins, seen in the brain of a patient dying from excited delirium. Cocaine blocks dopamine reuptake, but its actions are modulated by α-synuclein. Cocaine causes concentrations of α-synuclein to increase, especially in the ventromedial sectors of the striatum other than in the dorsal caudate nucleus. Thus, overexpression of α-synuclein may play a role in cocaine-induced plasticity and regulation of dopamine synaptic tone. (Courtesy of Debra Mash, University of Miami School of Medicine, Miami, FL.)

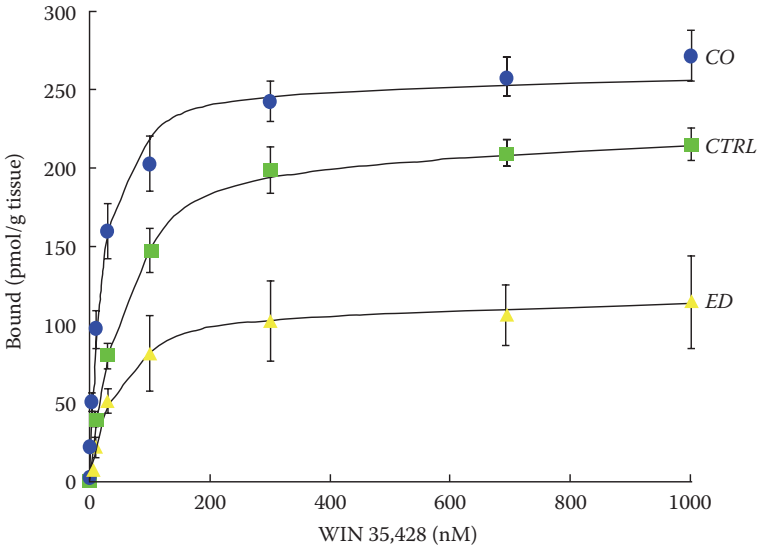


Figure 1.66 A graphical representation of the same information displayed in the autoradiogram shown in Figure 1.64. It is apparent that, compared with controls, WIN binding (a marker for dopamine transporters) is elevated in occasional cocaine users but is markedly reduced in patients with excited delirium. (Courtesy of Debra Mash, University of Miami School of Medicine, Miami, FL.)

known, but at least one fragment of the protein produced by this gene can be detected in the amyloid-rich fractions of brains from patients with Alzheimer's disease. Yet, for whatever reason, concentrations of α-synucleins are rarely if ever quantitated, even in diseases where alterations in their concentrations are well documented. Alpha-synucleins can aggregate to form insoluble fibrils in pathologic conditions characterized by the presence of Lewy bodies, especially Parkinson's disease. Their concentrations in other medical and psychiatric disorders remain a matter of speculation. The problem with these biomarkers is that they can only be measured in fresh, or rapidly frozen, brain. The testing itself is difficult and costly. Dopamine can be measured peripherally, but the relationship between central dopamine and peripheral concentrations is not known in normal controls, let alone in patients with ExDS, though there is no reason why these values could not be determined.

Serotonin (5-HT) regulation also plays a role in ExDS because it modulates dopaminergic neurotransmission. Chronic use of cocaine may cause a "serotonin deficit," resulting in a form of 5-HT dysregulation. In patients with simple cocaine overdose, levels of 5-HT transporters are elevated within the NAC and throughout the anterior and posterior sectors of striatum. A much different picture is seen in patients with excited delirium where there are increases in the number of 5-HT transporters in the anterior striatum, but not the posterior sectors. Chronic cocaine exposure upregulates 5-HT transporter densities in the substantia nigra in victims of cocaine overdose, but not in excited delirium victims. Some speculate that the lack of 5-HT upregulation in the substantia nigra and posterior striatum may identify a distinct phenotype for excited delirium victims (Mash et al., 2000).

Some of the neurochemical effects of cocaine seem to be gender related. Studies utilizing proton magnetic resonance spectroscopy have shown that the brains of cocaine users, when compared to those of non-drug-using controls, contain decreased amounts of *N*-acetyl compounds, an indicator of neuronal damage. At the same time, production of myoinositol, an indicator of glial activation, is increased. Both of these alterations are most prominent in the frontal lobes, and both changes are much more pronounced in men than in women. Whether these neurochemical alterations explain why nearly all ExDS victims are men is not known (Chang et al., 1999).

1.15.6 Autopsy Findings

In the past, the presence of petechiae, particularly conjunctival petechiae, was often cited as proof of death from "positional asphyxia" (Reay et al., 1992), but petechiae around the eyes are not infrequently seen in individuals with heart failure, for whom there is no question of drug abuse, and in cases of strangulation (Rao and Wetli, 1988). Petechiae can, and do, occur as a result of resuscitative attempts (Maxeiner and Winklhofer, 1999; Raven et al., 1999) and they may not become apparent until some time has elapsed after death (Kondo et al., 1997; Burke et al., 1998). Finally, petechiae may form simply because of the body's position. As stasis occurs, pressure increases within dependent vessels. Thus, photographic documentation of the absence of petechiae is just as important as documentation of their presence, as they may well first become evident only hours after death.

If appropriately examined, the heart will, invariably, be found to be abnormal: if not grossly enlarged, then at least it will be enlarged above the predicted weight (Mayo nomogram—see Appendix D, Kitzman et al. (1988), or any of the other nomograms in common use). Even if the heart is not enlarged according to weight criteria, microscopic examination may well disclose hypertrophy of individual myocytes and other evidence of early

myocardial remodeling (Frangogiannis, 2008; Miller et al., 2009). Cocaine activation of CMKII (Henning and Cuevas, 2006) causes myocyte hypertrophy and increased concentrations of calcium within the cytosol.

Taken together, all of these irregularities lead to elevated concentrations of cytosolic calcium, myocardial hypertrophy, and electrical irritability. Collectively, these changes very likely constitute the underlying mechanism that leads to "electrical storms" within the myocardium (recurrent bouts of VF) (Tsuji et al., 2011).

1.15.7 Toxicology of Excited Delirium Syndrome

There is a clear difference between cocaine and BZE concentrations in individuals dying from cocaine toxicity (where sometimes concentrations of the drug can be many milligrams per liter) and individuals with ExDS, where plasma cocaine concentrations are usually quite modest. The mean cocaine concentration in 45 cases of ExDS seen by the Miami Dade County Medical Examiner was 1.32 mg/L (range 0.05–11.8 mg/L, $n = 34$), while the BZE level was 3.78 mg/L (range 0.08 ± 14.75 mg/L, $n = 38$). In these same deceased individuals, the mean brain cocaine concentration was 1.90 mg/kg (range 0.05 ± 4 mg/kg, $n = 10$), while the mean BZE concentration was 2.69 mg/kg (range 0.85 ± 3.5 mg/kg, $n = 6$) (Wetli et al., 1996). In Mash's study of 90 patients, all of whom had clearly died from cocaine-induced ExDS, the results were comparable, the values are shown in Table 1.13.

1.15.8 Potential Biomarkers

Because cocaine alters the metabolism of so many different chemical cascades, it may well be that the presence of an altered compound (or normal compound in excessive amounts) might eventually prove to be diagnostic for the syndrome. Markers may be present within the CNS or in the periphery. The technology for measuring these compounds is available now, though costs are still high; all the indicators suggest that prices will decrease rapidly as new genetic techniques are introduced. The CNS changes have been discussed in detail in Section 1.15.5. Possible peripheral changes, which can be measured, are discussed below.

1.15.8.1 Neuropeptides

There is hope that another new class of potential biomarkers, called neuropeptides, can be used to confirm the diagnosis of ExDS. Neuropeptides are small, protein-like molecules used by neurons to communicate with other neurons, but in a manner quite distinct from the larger, better-understood neurotransmitters. They are neuronal signaling molecules

Table 1.13 Brain and Blood Concentrations of Cocaine and Benzoylecgonine in Excited Delirium Deaths

	Cocaine-Related Deaths		Excited Delirium	
	Blood (mg/L)	Brain (mg/kg)	Blood (mg/L)	Brain (mg/kg)
Cocaine	3.30 ± 0.74	450 ± 0.74	0.81 ± 0.11[a]	2.73 ± 0.66
Benzoylecgonine	4.00 ± 0.47	1.21 ± 0.14	3.29 ± 0.34	1.53 ± 0.15
Cocaethylene	0.39 ± 0.18	0.15 ± 0.04	0.08 ± 0.01	0.11 ± 0.02

Note: Values in the cocaine-related deaths column refer to mean values in 100 non-ExDS related deaths. Those in the excited delirium column refer to mean values in 81 ExDS deaths.

involved in particular brain functions such as analgesia, reward, food intake, learning, and memory.

Some of the neuropeptides are very much involved in the process of stimulant addiction and abuse, and a good deal is known about them. The best studied is called CART. CART appears to play different roles in reward, feeding, and stress (Kimmel et al., 2002). Similarly, the orexins (types 1 and 2, or A and B) have also become objects of interest. Orexin A controls responses to adaptive cardiorespiratory control mechanisms of stimuli—both internal and external (Shahid et al., 2012). Studies have shown that orexin-deficient mice develop sleep apnea and lose the ability to respond to hypoxia-induced ventilatory and phrenic nerve long-term facilitation. This observation suggests that the orexin system is one of the essential modulators required for coordinating the circuits controlling respiration (Kuwaki and Zhang, 2010) and could well play a role in the asystole observed in ExDS victims.

Neuropeptides can be measured peripherally (Jiang et al., 2012), raising the possibility that some recognizable pattern of changes might be identified in patients with ExDS, especially if a number of other biomarkers were measured at the same time (see Section 1.5.8.1). Before that can occur, normal ranges must first be determined in stimulant abusers, psychotic and nonpsychotic, as well as drug-free individuals; such measurements may yet prove diagnostic.

1.15.8.2 Heat Shock Proteins

In Mash et al.'s (2009) study, decreased numbers of dopamine transporters and elevated concentrations of heat shock protein (HSP) 70 were observed in the brains of 70 medical examiner cases, each case having died with symptoms of ExDS (Mash et al., 2009). Almost all had cocaine detectable in the brains but, unfortunately, such increases occur in other conditions besides ExDS.

However, Mash et al. also recorded increases in HSP 70. HSPs are functionally related proteins that are involved in the folding and unfolding of other proteins. Their expression is increased when cells are exposed to elevated temperatures or other stressors (Wu, 1995). Upregulation of the HSPs is a key part of any normal organism's response to stress produced by hyperthermia and perhaps other conditions, such as ischemia, as well.

HSPs are named according to their molecular weight. For example, Hsp60, Hsp70, and Hsp90 (the most widely studied HSPs) refer to families of HSPs on the order of 60, 70, and 90 kDa in size, respectively (Wu,1995). The presence of HSP is not specific for any one disease, but these proteins appear to interact with the cardiovascular system at many points.

In experimental animals, even low-dose methamphetamine causes damage to cardiomyocytes (mainly the endoplasmic reticulum), with measurable leakage from the cell of Hsp32, 60, 70, and 90, especially Hsp70 (Tomita et al., 2011). Other studies have shown Hsp70 elevation in animals treated with phencyclidine (Hashimoto et al., 1996). It would be a leap to say that cocaine-induced ExDS is "caused" by Hsp70 elevation, but it is interesting to note that use of two other psychosis-inducing drugs is also associated with Hsp70 elevation.

Serum Hsp70 concentrations can be measured by using a commercially available ELISA kit and Hsp70 quantified by interpolating the absorbance readings from a standard curve generated from a series of calibrated Hsp70 protein standards. This technology is not particularly complex, making Hsp70 both a desirable and relatively easy biomarker to measure (Sandstrom et al., 2008).

The relationship between Hsp70 and death in ExDS has been questioned by Johnson et al. (2012). The objections were based on one experimental paper where the temperature was omitted most of the time and the criteria were to establish the diagnosis of ExDS were not specified. The case series had only 18 cocaine-related deaths (and 17 controls), but the data showed that HSP70 was associated with any cocaine-related death. Until further experimental work is brought forward, the original observations would seem to be the most reliable of the two studies. The compound should be measured, even if interpretation of its significance is a matter of some dispute (Mash, 2013).

1.15.9 Cause of Death Determination in ExDS

The cause of death in excited delirium remains a matter of contentious dispute, especially in court. Nonetheless, we do, in fact, know a great deal more about ExDS today than when it was first described by Bell (1849).

There is no question that chronic cocaine use leads to myocardial remodeling, global fibrosis, and endothelial hypertrophy of intramyocardial vessels. Individually and collectively, these alterations favor the occurrence of sudden cardiac death. Nor is there any question that chronic cocaine abuse leads to chronically elevated plasma concentrations of catecholamines (chiefly NE) or that elevated catecholamines promote arrhythmias and make defibrillation harder to accomplish.

Thus, it would appear that the cause of death in virtually all cases of positional asphyxia is unrecognized heart disease. However, such a conclusion may represent an oversimplification. The cardiac rhythm in every ExDS death ever witnessed and documented was primary asystole, and none of the factors enumerated earlier causes primary asystole (interestingly, all alleged Taser-related deaths have also been asystolic at presentation).

An intriguing, but yet to be investigated possibility is that both respiratory and cardiac arrests are centrally mediated, perhaps because of some receptor imbalances in the tractus solitarius or the red nucleus. Cocaine users often experience seizures, and patients with seizures occasionally develop ictal asystole. There is no reason why the same mechanism could not explain ExDS deaths, but there has been no research, and the idea remains only an untested hypothesis (Zhu et al., 2007).

In some cities in the United States, medical examiners have taken the sensible approach of contacting the deceased's family and asking them to retain their own pathologist to witness the autopsy. In the United Kingdom, this is standard practice. Even the presence of an independent observer, however, may not be enough to prevent litigation or to prevent individuals from confusing temporal proximity of an action, such as "hog tying," with causality. Aristotle identified this type of logical error more than 2000 years ago. One would hope that, in the interim, pathologists would have learned to avoid this mistake and base their decisions on factual analysis, not flawed reasoning.

1.16 Cocaine-Associated Pulmonary Disease

Chronic coca leaf chewers may sometimes develop stomatitis, glossitis, and buccal mucosal leukoderma (Hammner and Villegas, 1969; Grattendick et al., 2000), although this effect seems to be quite uncommon and not a subject of research interest.

The practice of "snorting" probably did not begin until shortly before 1903, the year when the first cases of septal perforation were reported (Maier, 1926). In the ensuing years, septal perforation has become a well-known complication of coca leaf chewing and numerous case reports and cases series have been published (Newton et al., 2003; Neumann et al., 2011).

Many of these cases present as midline granulomas that look, for all the world, like Wegener's granulomatosis, but histologic examination will disclose necrosis and atrophy of the inferior and middle nasal turbinates bilaterally, along with prominent naso- and oropharyngeal ulcers (Trimarchi et al., 2006; Scheenstra et al., 2007). Biopsies can be expected to reveal focal areas of chronic inflammation and necrosis (Figure 1.67). Until the recent introduction of levamisole as a contaminant, no evidence of vasculitis or granuloma formation had ever been observed (Becker and Hill, 1988; Deutsch and Millard, 1989; Daggett et al., 1990; Allbery et al., 1995; Sevinsky et al., 1995; Heller et al., 2005; Teymoortash and Werner, 2009).

When the histologic appearance of the septal mucosa in 20 chronic nasal cocaine inhalers and 15 controls was compared, chronic inflammatory disease was seen in the cocaine users. The glandular elements were in total disarray, and mononuclear cells, particularly lymphocytes, were seen surrounding arterioles and glands (Chow et al., 1990). At autopsy, even if sections of the septum are not obtained, the mucosa should be swabbed with saline; cocaine may be recovered via this route for some time, possibly days, after the last episode of use.

Recently, the differential diagnosis has become more complicated. Antineutrophil cytoplasmic antibody (ANCA)-positive vasculitis from levamisole-tainted cocaine, with concomitant cocaine-induced midline destructive lesion (MDLs) of the palate and nasal septum, has been reported (Zwang et al., 2011). The detection of antineutrophil cytoplasmic antibodies reacting with human neutrophil elastase (HNE) has been suggested as one way to distinguish the cocaine-related syndrome from a true autoimmune vasculitis. One recent case report described two women, aged 39 and 49 years, with cocaine-related retiform purpura, mainly affecting the legs. The initial clinical and serologic profile in Case 1 led to a suspicion of antiphospholipid syndrome. However, in one of the cases, Wegener's granulomatosis was in fact present along with an unexplained associated neutropenia.

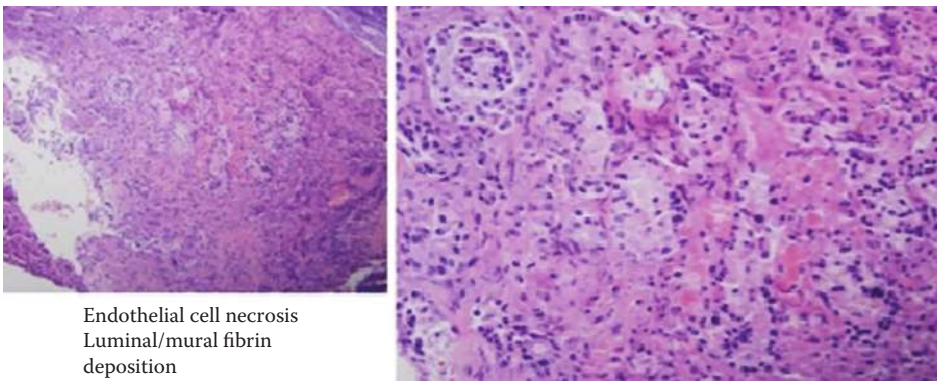


Figure 1.67 Midline granuloma in a cocaine abuser. In cocaine-associated midline facial destruction, the necrosis can be relatively paucicellular reflecting an ischemic sequela. Higher-power magnification of the mucosal vessels will reveal paucicellular fibrin deposition and endothelial cell injury, likely reflective of the direct toxic effect of the cocaine and/or catecholamines on the mucosa. (Reproduced from *Head Neck Pathol.*, 2(2), 116, June 2008. From the author's collection.)

Skin biopsies revealed a mixed pattern of leukocytoclastic vasculitis and microvascular thrombosis in Case 1 and pure microvascular thrombosis in Case 2. Identification of anti-HNE antibodies in both patients links their disease to cocaine. The mixed vasculopathic pattern in Case 1 and the associated neutropenia in Case 2 are both known adverse effects of levamisole (Walsh et al., 2010; Chung et al., 2011).

In the living, computed tomography remains the preferred method for evaluating calcified nasal masses, or masses that originate from bone or cartilage. Pott's puffy tumor, an old name for subperiosteal abscess of the frontal bone associated with underlying frontal osteomyelitis, has rarely been reported in stimulant abusers but it is a known complication of chronic frontal sinus infection. When it does occur in association with cocaine use, the underlying mechanism presumably is recurrent vasospasm and ischemia, favoring the spread of infection (Noskin and Kalish, 1991).

Black sputum is reported by nearly half of all cocaine smokers and is thought to be secondary to the practice by some of smoking the tarry residue that coats the "crack" pipe (Tashkin et al., 1992; Greenebaum et al., 1993; Laposata and Mayo, 1993). Interestingly, cocaine smoking appears to lead to fewer significant bronchial mucosal alterations than marijuana or tobacco when smoked alone and does not add to the changes associated with marijuana. When smoked together with tobacco, however, cocaine appears to augment the bronchial injury caused by tobacco smoking (Fligiel et al., 1997).

Inhaled cocaine particulate can be seen as black particles within collections of macrophages. Some individuals may even sustain upper airway burns, caused by the inhalation of hot particulate matter from inadequate filters used in their "crack" pipes, which are often composed of steel wool (Bezmalinovic et al., 1988; Snyderman et al., 1991; Reino and Lawson, 1993). Others will experience severe stridor, but some may present with uvular edema unaccompanied by any apparent respiratory distress. No predictable pattern of symptoms is diagnostic for upper airway injury occurring as a consequence of cocaine use (McQueen et al., 1995).

1.16.1 Barotrauma

The general classification of barotrauma includes disorders where increased intra-alveolar pressure or decreased interstitial pressure leads to rupture of alveoli with leakage of air. Whether pneumothorax or pneumomediastinum results will depend on the location of the alveolus. Increased intra-alveolar pressure is usually the result of coughing or performing the Valsalva maneuver, which "crack" smokers routinely do. There are, however, other possibilities. Pulmonary inflammation could weaken the alveolar wall and lead to leakage of air. Alternatively, vasoconstriction in the vessels adjacent to an alveolus could cause decreased interstitial pressure, leading to alveolar rupture without any great increase in intra-alveolar pressure (Maklin and Macklin, 1944; Seaman, 1990; Uva, 1997; Dorfmuller et al., 2003; Cabanas et al., 2009; de Almeida et al., 2014).

Another possibility that cannot be dismissed is idiopathic pulmonary hypertension (IPH) (Figure 1.68). It has been shown that humans and animals both convert levamisole to aminorex (Bertol et al., 2011), and aminorex causes pulmonary hypertension. Repeated exposure over a long period is required before the disease becomes evident, and changes that could weaken the alveoli walls precede the development of classic plexiogenic arteriopathy (Dorfmuller et al., 2003). Patients with IPH and high concentrations of hair levamisole have been described (Karch et al., 2014).

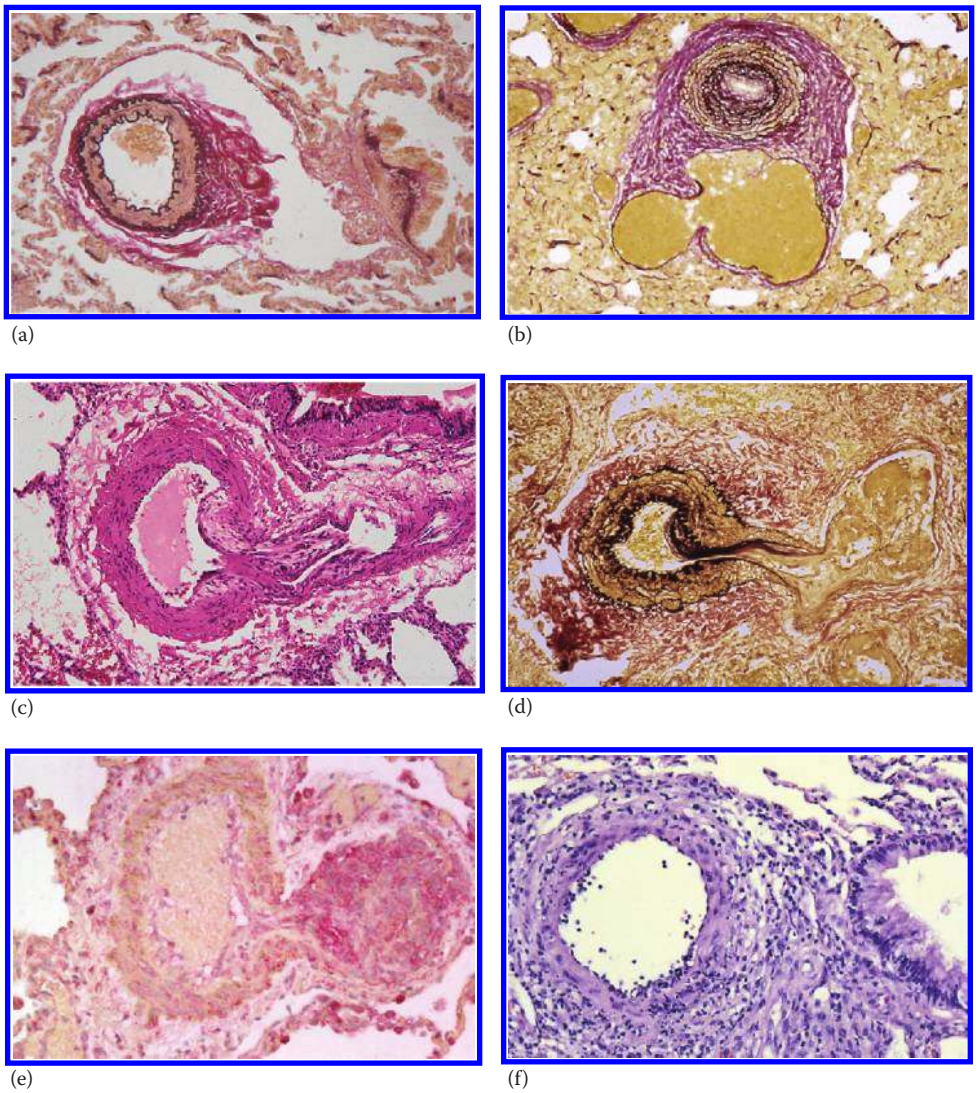


Figure 1.68 Plexogenic pulmonary arteriopathy (WHO group 1.1–1.3). (a) Medial hypertrophy and some adventitial thickening of muscular pulmonary artery. In the early (and presumably reversible) phase of the disease process, these nondiagnostic alterations are the only findings. (b) Advanced plexogenic pulmonary arteriopathy; a dilatation lesion, presumably representing greatly widened and very thin-walled supernumerary arterial vessels, lies next to parent artery showing marked medial hypertrophy. (c and d) Plexiform lesion. Axial muscular pulmonary artery (left) giving rise to supernumerary artery showing intimal fibrosis at the orifice and proximal part, with associated increased cellularity, irregular shape of lumen, some mural thrombus formation (d), and marked widening of lumen of the most distal part of the lesion (right). (e) Vascular endothelial growth factor immunostain highlighting a plexiform lesion (red). (f) Focal pulmonary arteritis in a case of advanced plexogenic pulmonary arteriopathy. (a, b, d, and f) Hematoxylin and eosin (HE) stain, (c) elastic van Gieson (EvG) stain, and (e) immunostain (alkaline phosphatase). Whether or not these individuals were smoking aminorex-laced cocaine is not known. (Reproduced from Mooi, W.J. and Grunberg, K., *Curr. Diagn. Pathol.*, 12(6), 429, December 2006. With permission; Also see Pietra, G.G. et al., *Circulation*, 80(5), 1198, 1989.)

Another possible cause for pneumo- and hemothorax is injection into central veins, although this practice is much more common in heroin abusers; heroin tends to be adulterated with material that is not water soluble, making the injection process much more difficult. Repeated injections of adulterated heroin can lead to sclerosis of peripheral veins. When that happens, the users are forced to inject central veins. The two most popular central sites are the great vessels in the neck ("pocket shot") (Lewis et al., 1980) and the vessels of the femoral triangle ("groin shot") (Pace et al., 1984). Injections are also made into the general area of the supraclavicular fossa (Figure 1.69), either by the addict himself or by a hired "street doc." Because the lung apex is directly contiguous with the area, pneumothorax commonly results (Kurtzman, 1970; Feldman et al., 1976; Merhar et al., 1981; Douglass and Levison, 1986; Patel et al., 1987; Welch et al., 1990; Cheng et al., 1992; Naqi et al., 2006). This practice is much more common than many suspect. In the Vancouver Drug Injectors study, published in 2008, an alarming 28% reported having injected the jugular (Hoda et al., 2008).

Cocaine-associated pneumomediastinum occurs with some frequency (Viswanathan and Navaneethan, 2007). Pneumomediastinum is a generally benign condition; no fatal cocaine-related cases have been reported, and no autopsy studies have been performed.

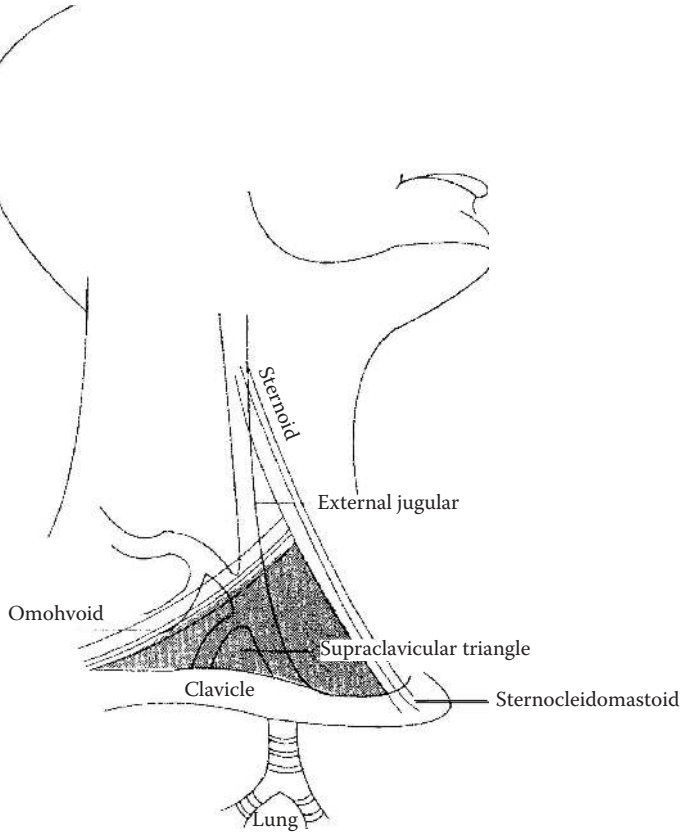


Figure 1.69 The supraclavicular fossa. As peripheral veins become sclerosed, chronic abusers resort to injecting themselves in the supraclavicular fossa and in the femoral triangle. The supraclavicular fossa overlies the great vessels and the apex of the lung. Pneumothorax and hemothorax are the predictable results.

Presumably, the mechanism has to do with the performance of a Valsalva maneuver by a deeply inhaling smoker. No cases have been reported after intravenous or intranasal use. This is another entity that almost disappeared over the last decade but which seems to have reemerged in this decade. Perhaps its occurrence is some sort of marker for population-wide cocaine abuse.

Surprisingly, barotrauma has not been proposed as the mechanism for some cocaine-related strokes, but it might offer just as good an explanation as cocaine-induced vasospasm. Air from ruptured alveoli may diffuse into pulmonary capillaries and veins, then pass through the left side of the heart and embolize via the systemic arteries to the brain. This possibility seems especially likely, now that the role of atrial septal defect in stroke is increasingly understood (Berthet et al., 2000). The diagnosis would be particularly difficult to make because, if the patient survives for more than a few hours, the air bubbles will have dissolved and typical morphologic changes (multiple small, well-circumscribed foci of cortical necrosis, sometimes associated with laminar necrosis) will not have had time to develop (Wolf et al., 1990).

1.16.2 Parenchymal Disease

Crack smokers have carbonaceous sputum and emphysematous changes in their lungs. The pattern is readily apparent in microscopic sections, even before they are placed under the microscope; the appearance of these slides is highly reminiscent of the pattern seen in "coal miner's lung." Sputum from these individuals is usually turbid, gray or even black, and considerably darker than sputum seen in heavy tobacco smokers dwelling in the same urban environment. Microscopic examination of sputum smears from "crack" smokers will disclose excessive carbonaceous material in the cytoplasm of pulmonary alveolar macrophages and also in the extracellular compartment (Figure 1.70) (Klinger et al., 1992; Greenebaum et al., 1993; Barsky et al., 1998; Janjua et al., 2001; Ali et al., 2002; Hirche et al., 2002; Restrepo et al., 2007).

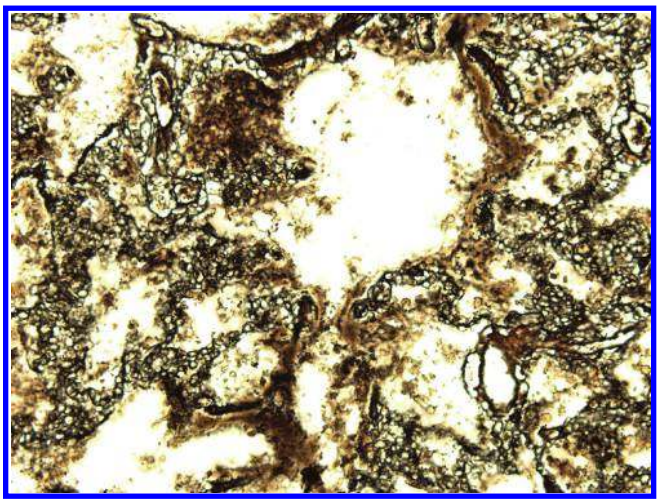


Figure 1.70 Pulmonary anthracosis. The lungs of "crack" smokers often are emphysematous and contain dense carbonaceous deposits. The changes can be apparent to the naked eye. Hemosiderin-laden macrophages are often present, probably reflecting recurrent bouts of focal hemorrhage. (Courtesy of Dr. Vittorio Finnechi, Sienna.)

In animal studies, smoked cocaine causes several forms of injury to the respiratory tract, including asthma exacerbations, lung edema and hemorrhage, and nasal mucosal alterations. There have been far fewer studies of respiratory tract pathology in habitual crack cocaine users. However, histologic alterations in the respiratory tract of mice caused by chronic inhalation of crack cocaine have been studied. Twenty 2-month-old BALB/c mice were exposed to the smoke of 5 g crack cocaine in an inhalation chamber once a day for 2 months and compared to controls ($n = 10$). The serum cocaine level was 212.5 ng/mL after a single inhalation. The mucus content of the nasal epithelium increased in crack-exposed animals, and the nasal and bronchial epithelium thickness decreased significantly. The alveolar hemosiderin content and the alveolar and bronchiolar macrophage cell density increased. The vasoconstriction index increased in the pulmonary arteries of the exposed group, suggesting that cocaine itself, and not just cocaine adulterated with levamisole, can cause IPH (Herculiani et al., 2009).

Levels of ET-1 are significantly increased in bronchoalveolar fluid recovered from "crack" smokers, and the percentage of hemosiderin-positive alveolar macrophages tends to correlate with ET-1 concentrations. This suggests that clinically silent alveolar hemorrhage occurs frequently in otherwise healthy "crack" cocaine smokers (Figure 1.71). These hemorrhages must, in some way, be associated with elevated levels of ET-1, further suggesting that a cocaine-induced pulmonary microvascular injury has occurred (Baldwin et al., 2002; Terra Filho et al., 2004).

Whether or not past autopsy studies of cocaine users remain relevant in the era of levamisole-adulterated cocaine is impossible to say; prudence suggests that observations made decades ago may no longer provide an accurate picture of the histologic changes that are being observed today. Earlier reports recorded the presence of hemorrhages and hemosiderin-laden macrophages in 27%–58% of patients (Murray et al., 1988; Bailey et al., 1994). Bailey et al. (1994) found evidence of acute and/or chronic hemorrhage in 71% of cases examined, even in the absence of any clinical history of hemoptysis. Bailey concluded that placing too much reliance on the presence or absence of hemoptysis would lead to serious underestimation of how frequent alveolar hemorrhage actually was in "crack" smokers. But whether this was a result of cocaine itself or one of the cocaine adulterants is hard to say. Diffuse alveolar hemorrhage has been described, and clinical reports suggest

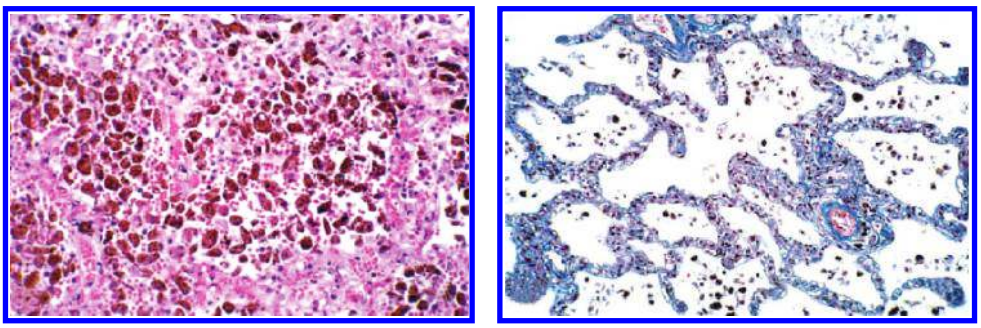


Figure 1.71 The micrograph on the left illustrates acute alveolar hemorrhage, frequently seen in crack smokers even as an incidental finding. The trichrome-stained section on the left illustrates mild alveolar septal fibrosis and intra-alveolar hemosiderophages, which may also be an incidental finding. (Reproduced from Tomashefski, J.F. and Felo, J.A., *Curr Diagn Pathol*, 10, 413, 2004. With permission.)

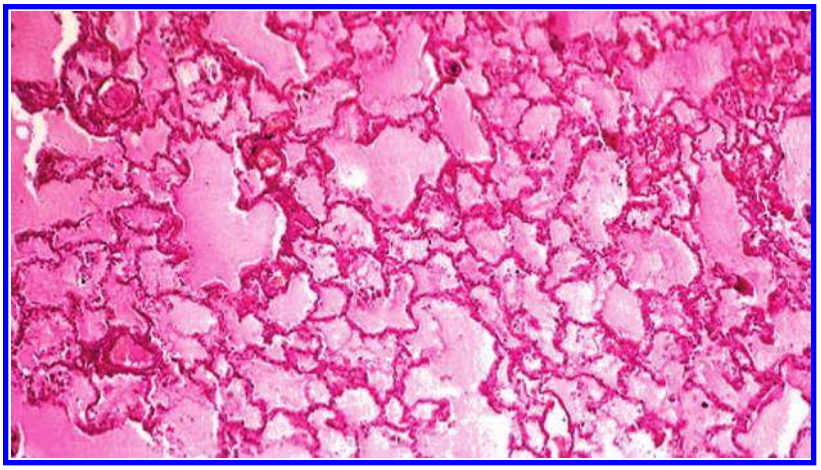


Figure 1.72 Pulmonary edema.

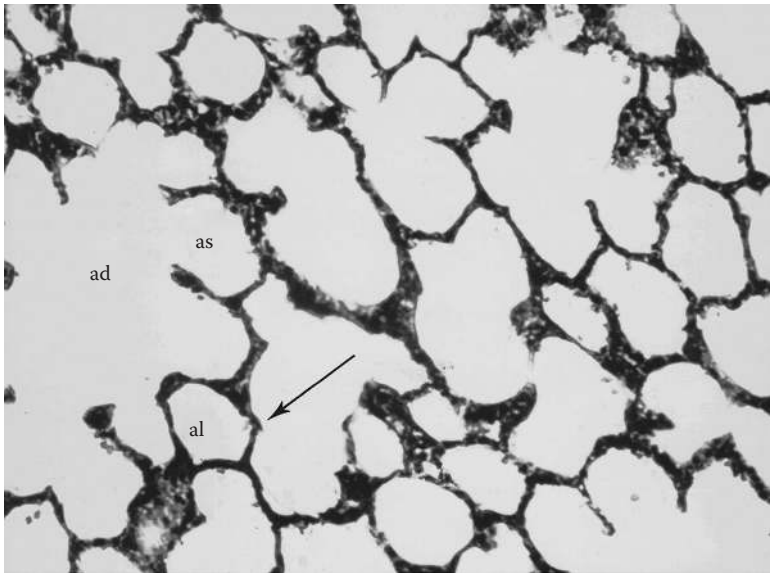
that spontaneously resolving hemoptysis is not that uncommon among cocaine smokers (Perez-Reyes et al., 1982; Forrester, 1990; Bouchi et al., 1992; Garcia-Rostan y Perez et al., 1997; Gallouj et al., 1999; Baldwin et al., 2002).

Another aspect of parenchymal disease in cocaine users is pulmonary congestion (Figure 1.72). The presence of congestion in cocaine-related deaths was first recognized at the turn of the twentieth century; interestingly, it can even occur in nonfatal cases (Purdie, 1982; Cucco et al., 1987; Efferen et al., 1989; Hoffman and Goodman, 1989; Bakht et al., 1990; Kline and Hirasuna, 1990; Batlle and Wilcox, 1993; Raijmakers et al., 1994; Kuczkowski, 2005; Diskin et al., 2006; Ksienski et al., 2007). The etiology of cocaine-associated pulmonary edema is obscure; however, the relatively low protein content of the edema fluid (it does not froth like the edema fluid seen with heroin overdose) suggests that it is of cardiogenic, not neurogenic origin (Robin et al., 1989). Alternatively, pulmonary edema in cocaine users could just be another manifestation of catecholamine toxicity affecting the lungs, the heart, or both (Kurachek and Rockoff, 1985; Karch, 1989; Arfi et al., 2005; Rassler, 2007).

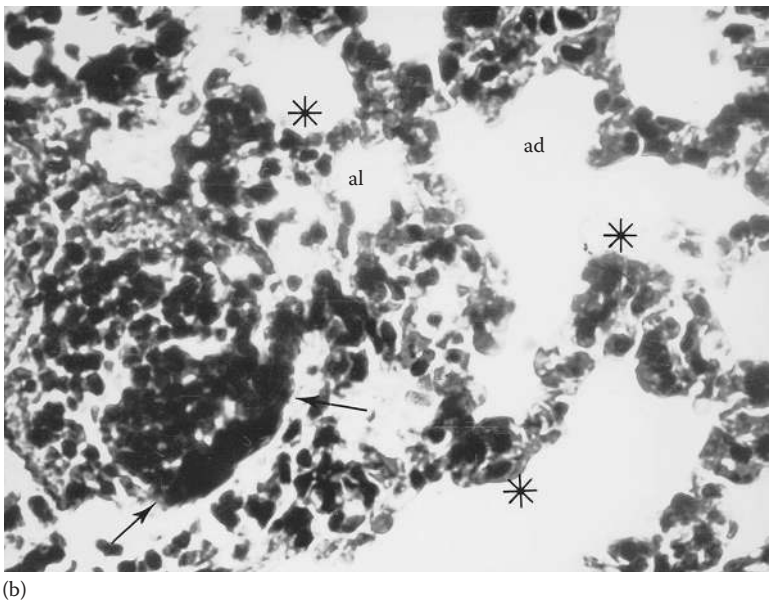
Cocaine itself, and the catecholamine excess that occurs in association with cocaine use, both decrease myocardial contractility (Perreault et al., 1993). If contractility is depressed enough to lower cardiac output, heart failure and pulmonary edema will develop. A direct effect of cocaine on the lungs may also be possible. It has been suggested that the local anesthetic action of cocaine impairs the movement of sodium and fluid across the alveolar epithelium (Raijmakers et al., 1994), though the suggestion remains unproven.

The lungs of chronic cocaine smokers usually display thickening in some of the interalveolar septa, along with interstitial hemorrhages, progressive thrombosis, and transformation of reticular and elastic fibers into diffusely fibrotic tissue. These findings are indicative of severe cocaine-induced microvascular disease and are consistent with the findings of Baldwin and others. They have been reproduced in animals chronically treated with cocaine (Barroso-Moguel et al., 1999). The typical alveolar changes seen in this animal model are seen in Figure 1.73.

Prior to the advent of "crack" cocaine, mentions of cocaine-related pulmonary disease were rare. As smoking cocaine became more popular, reports of patients with inflammatory infiltrates, sometimes associated with fever, hypoxia, hemoptysis, and



(a)



(b)

Figure 1.73 Typical alveolar changes. (a) Control rat in which the alveolar ducts (ad), sacs (as), and alveoli (al) are all normal; the arrows point to an alveolar wall of normal thickness. (b) Alveolar hemorrhage (arrows) in a rat after 30 days of treatment with 30 mg/kg/day of intraperitoneal cocaine; the lumen of the ducts and alveoli are narrowed by fibrous wall thickening. Both sections were stained with Masson's trichrome, 100×. (From Barroso-Moguel, R. et al., *Toxicol. Lett.*, 110(1–2), 113, 1999. With permission.)

even respiratory failure, began to appear (Forrester et al., 1990). Specimens from some of these patients have demonstrated diffuse alveolar damage with hyaline membrane formation and type II cell hyperplasia, as well as intra-alveolar and interstitial inflammatory infiltrates with eosinophilia. Some, with x-ray demonstrated inflammatory infiltrates, have had peripheral eosinophilia (Mayron et al., 1972; Murray et al., 1988), while others did not (Cucco et al., 1987; Kissner et al., 1987; Patel et al., 1987; Forrester et al., 1990; Talebzadeh et al., 1990; Oh and Balter, 1992; Nadeem et al., 1994; Opalka et al., 2002; Strong et al., 2003).

1.16.3 Vascular Adaptations

Interpretation of histologic changes in the lungs of cocaine users is now complicated by the fact that most cocaine sold in the United States, and much of the cocaine sold in Europe, is adulterated with levamisole. Once ingested, levamisole is converted in humans into aminorex, and aminorex unequivocally causes pulmonary hypertension. Anecdotal reports from U.S. crime laboratories suggest that most (i.e., >80%) samples of street cocaine are found to contain at least 10% levamisole. If all of the levamisole were to be converted to aminorex, the amount present would be equal to or greater than the amount that caused an epidemic of deaths in Europe during the early 1970s (Kay et al., 1971).

In January of 1971, a brief report in the *British Medical Journal* described a sudden increase in the incidence of a particularly virulent form of pulmonary hypertension of undetermined cause in 40 young Swiss women. The only feature shared by all of these patients was the prolonged use of a new anorectic drug called aminorex fumarate, sold in Switzerland, Austria, and Germany beginning in 1965. The first cases of IPH in aminorex users were not reported until 6–12 months after the drug came to market (sold by McNeil Laboratories as Menocil™ or Apiquel™) (Gurtner, 1979). The average dose per day ingested by the affected women ranged from 14 to 42 mg/day; the average patient had been taking the drug for more than 1 year (Follath et al., 1971). A Swiss researcher reported that in 1968 alone, the number of patients presenting with pulmonary hypertension had increased 10- to 20-fold (Rivier, 1970). It is now understood that aminorex is a substrate for 5-HT (serotonin) transporters (SERT) that influence the structure of pulmonary vessels. The predominant histopathologic features in these patients were pulmonary arteriopathy with plexiform lesions characterized by a combination of concentric laminar intimal fibrosis and eccentric intimal fibrosis. In some, recanalized thrombi were also present. The question that now must be addressed by clinicians is whether humans can convert enough levamisole to aminorex to cause IPH. The answer is not yet known, and is not likely to be forthcoming quickly. Several studies have shown that cocaine itself (uncontaminated with levamisole) can cause IPH (Abenhaim et al., 1996).

Hypertrophy of the pulmonary artery smooth muscle is also seen in the lungs of heroin and cocaine abusers. It results from the intravascular deposition of foreign materials that have been injected along with the heroin (see Chapter 5, Section 5.12.3.3). Their deposition causes foreign body granulomas to form and sets in motion a series of events that eventually leads to pulmonary hypertension, even in the absence of aminorex. The specific particles causing the granulomas are easily seen with polarizing microscopy, but they can also be differentiated by their staining properties (Tomashefski and Hirsch 1980; Tomashefski et al., 1981). These alterations are much more common in the subpopulation of abusers who inject crushed pills (Ritalin, oxycodone, hydrocodone, etc.). In autopsy studies of heroin

and polydrug abusers, the incidence of medial hypertrophy of small- and midsized pulmonary arteries has ranged from 8% (Hopkins, 1972) to as high as 40% (Rajs et al., 1984).

It seems likely that other mechanisms cause injury to the blood vessels of cocaine users: excessive stimulation of α_1 receptors causes smooth muscle to contract and also induces the proliferation of smooth muscle growth in vessel walls. Proliferation could also be the result of 5-HT2 overstimulation. Cocaine prevents platelet reuptake of 5-HT (Patkar et al., 2003), and platelet reuptake is one of the mechanisms by which the body normally maintains 5-HT concentrations within physiologic ranges. Many abused drugs and selective serotonin reuptake inhibitor antidepressants influence circulating 5-HT concentrations. Although the effects of cocaine have not been specifically studied, amphetamine analogs (which also block 5-HT reuptake) can induce large dose-dependent increases in plasma 5-HT.

Without additional information, there is no way to determine the cause of medial hypertrophy within lung vessels. However, granulomatous changes can also be seen in the lungs of individuals who only sniff cocaine, which implies that the method of ingestion may not be all that important. Cellulose granulomas were identified in a patient who denied intravenous drug use and who had no occupational exposure to cellulose products (Cooper et al., 1983). Talc granulomas have been identified in two other patients (Buchanan et al., 1981; Oubeid et al., 1990). Mycotic aneurysm formation, local cellulitis, and abscess formation have all been described, usually in conjunction with injection of the neck or groin, although the incidence is much higher in heroin than in cocaine users (Roszler et al., 1989; Henriksen et al., 1995; Raso et al., 2000; Gotway et al., 2002; Maliphant and Scott, 2005; Chan and Burnand, 2006; Coughlin and Mavor, 2006; Yegane et al., 2006).

1.17 Neurologic Disorders: Introduction

1.17.1 Molecular Basis of Cocaine Neurotoxicity

Within minutes of cocaine administration, expression of several immediate early genes increases: *c-fos*, the transcriptional regulator, is one of the first genes to be upregulated (Graybiel et al., 1990), followed by increased production of mRNA coding for tyrosine hydroxylase and tryptophan hydroxylase. These two enzymes catalyze the rate-limiting steps in the production of dopamine and 5-HT, the neurotransmitters most obviously involved in cocaine's ability to cause euphoria and seizures (Ritz and George, 1993).

Postmortem studies have shown that the numbers of both D_1 and D_2 dopamine receptors are altered in cocaine abusers (Graybiel et al., 1990; Seeman and Van Tol, 1994). The number of dopamine transporters is critical in determining the concentration of extracellular dopamine and overall dopaminergic tone and function (Zahniser and Sorkin, 2004). When cocaine blocks the dopamine transporter protein, presynaptic dopamine that has been released into the synapse persists in the extracellular space and prolongs dopamine receptor stimulation. The result is behavioral activation.

Chronic cocaine abuse also leads to a compensatory upregulation of the number of dopamine transporters (Staley et al., 1994; Riddle et al., 2002). In an increasingly common disorder, known variously as excited delirium, agitated delirium, or ExDS, there is a failure of dopamine transporter upregulation (Staley et al., 1994; Chen et al., 1999). The use of any psychostimulant (cocaine, methamphetamine, and MDMA) always results in elevated synaptic concentrations of dopamine (Riddle et al., 2005). Failure to upregulate dopamine

transporters is thought to explain the association of chronic psychostimulant abuse with the occurrence of excited delirium (Mash et al., 2009).

Hyperthermia is also considered strong (but not absolutely necessary) evidence for the diagnosis of ExDS. Even if the death investigation is incomplete, the presence or absence of hyperthermia at the time of death can be established with the aid of postmortem neurochemical studies. Such studies will inevitably show a marked induction of the HSP A1B (HspA1B). The production of HSP is an immediate, though transient, response to elevated temperature, and the amount produced most closely correlates with the magnitude of the thermal stress (Sun and MacRae, 2005).

1.17.2 Psychiatric Syndromes

Clinical studies suggest that a high percentage of regular cocaine abusers exhibit symptoms of paranoia and hallucinations (Brady et al., 1991). Heavy users often believe they are being watched and/or followed. This complaint is not confined to cocaine users; it is also frequently observed in methamphetamine abusers (Brecht et al., 2004; Urbina and Jones, 2004). Cocaine psychosis is more likely to develop in men, and its occurrence appears to be dose related (Brady et al., 1991). Intravenous abusers are more prone to develop paranoia and hallucinations than nonintravenous abusers (Kaye and Darke, 2004).

Cocaine abusers with psychosis become sensitized—their disease becomes more and more severe the more drug they consume (Brady et al., 1991). While the relationship between chronic cocaine abuse and psychosis is well established, the underlying etiology for this disorder remains speculative. The latest genomic studies suggest that there is an association between catechol-*O*-methyltransferase (COMT) polymorphisms and psychosis. COMT is one of the major enzymes involved in catecholamine metabolism, and COMT polymorphism is associated with cocaine-induced paranoia (Ittiwut et al., 2011), presumably because it leads to pathologically elevated concentrations of dopamine.

Soon after cocaine became widely available (in the late 1860s), neurologists recognized that abusers were subject to paranoid psychosis. Maier, Magnan, and Lewin (Magnan and Saury, 1889; Maier, 1926; Lewin, 1931) all wrote on the subject and took pains to distinguish cocaine psychosis from symptoms induced by alcohol and other drugs. More recent studies have tended to confirm the earlier observations (Siegel, 1978; Gawin and Kleber, 1986). Transient or "binge" paranoia is common among heavy users; the incidence in one study was nearly 70% (Satel et al., 1990). What distinguishes cocaine-associated psychosis from the schizophrenic symptoms induced by amphetamines is that the paranoia occurs only for a very brief period in amphetamine abusers (Janowsky and Risch, 1979). The development of paranoia among cocaine abusers is unpredictable and its occurrence is clearly not dose related; some individuals just appear to be more vulnerable than others.

When Magnan first described cocaine psychosis, over 130 years ago, he published clinical histories of three patients, all of whom thought they had "bugs" crawling under the skin. All three individuals were covered with self-inflicted cuts and scratch marks made in vain attempts to remove the imagined parasites. This particular paranoid manifestation came to be known as Magnan's syndrome, and for a time, it was thought to be diagnostic for cocaine abuse. The syndrome is now referred to as "delusional parasitosis" or "formication" and clearly is not confined to cocaine abusers. Magnan's syndrome may occur in association with a number of different psychotic disorders, such as schizophrenia and organic mental disorders, or even in dementia with behavioral and psychological

symptoms. Evidence for the efficacy of treatment is weak and little is known about the results of treatment with specific antipsychotics (Huber et al., 2007; Cubala et al., 2011).

Cerebral glucose metabolism, as accessed by (^{18}F)-fluorodeoxyglucose PET scanning of cocaine abusers in an early state of cocaine withdrawal, shows increased rates of glucose uptake. This increase in glucose metabolism involves all areas of the brain but is particularly noticeable in the basal ganglia and orbitofrontal cortex. The increase in these two areas correlates with clinical measures of cocaine craving and is consistent with the notion that the changes in glucose uptake are due to changes in brain dopamine activity (Volkow et al., 1990, 1991).

Cocaine-induced changes in cerebral perfusion and glucose utilization appear to be gender related. Brain scans of cocaine-dependent women do not disclose the same set of abnormalities seen in men (Levin et al., 1994). Longer-lasting episodes of psychosis due to chronic cocaine abuse can occur. When they do, it is likely that the victim will be misdiagnosed as a schizophrenic. Because drug takers, schizophrenic or not, often deny drug use, routine drug screening of such patients is prudent (Shaner et al., 1993). Hair testing may be the preferred matrix for drug screening (Vearrier et al., 2010), especially when the urine-screening test is negative. Another important diagnosis to consider in cocaine users with psychiatric symptoms is stroke. Cocaine-induced ischemic infarcts have occasionally been mistaken for acute-onset psychosis (Reeves et al., 1995).

Results of the most recently published research suggest that in contrast to a D_2 deficiency, truly cocaine-dependent individuals may have elevated D_3 receptor levels. D_3 upregulation is emerging as a biomarker in preclinical models of addiction, and human PET studies of this receptor system can help guide novel pharmacologic strategies for treatment. Of little value to the pathologist, PET scanning may yet become a common tool during the initial evaluation of the addicted (Payer et al., 2014).

1.17.3 Cognitive Impairment

Cocaine-related impairment is frequently an issue in court proceedings, but causation is difficult to establish. Regular cocaine users rapidly become tolerant to cocaine's stimulant effects. In fact, there is experimental evidence suggesting that tolerance begins to emerge with the first dose (Mendelson et al., 1998). Whether or not this tolerance extends to performance and impairment is not known, though it is certainly possible, and the notion has some support (Verdejo-Garcia et al., 2007; Fernandez-Serrano et al., 2011; Chu et al., 2012); however these findings are far from universal and do not apply in all areas. For example, in Switzerland, cannabinoids were found in almost half of the impaired drivers tested, but cocaine in only 25% and methamphetamine in 10% (Senna et al., 2010). On the other hand, epidemiologic surveys of individuals actually arrested for impaired driving found quite the opposite result. Spanish researchers who studied the effect of cannabis and cocaine use on nonfatal traffic injuries suffered by 17,484 car or motorcycle drivers, surveyed in 2005, disclosed that cocaine use for one or more days a week and cannabis use for more than 4 days a week were both associated with an increased risk of traffic injury (Pulido et al., 2011).

Against the epidemiologic findings is the undisputed ability of psychostimulants, including cocaine, to increase the ability to sustain attention over prolonged periods of time, especially during the performance of monotonous tasks. Both amphetamine and cocaine have also been shown to improve performance on auditory and visual reaction time tests, on other tests of psychomotor skills and attention, and on tests of selective and

divided attention. Taken together, these findings suggest that moderate cocaine use should enhance driving performance, but that hypothesis has never been tested directly (Higgins et al., 1990; Farre et al., 1993; Stillman et al., 1993). Modest performance improvement on a number of cognitive performance measures has also been observed after intravenous dosing with cocaine (0.325 or 0.650 mg/kg) (Johnson et al.,1998).

1.17.4 Ischemic Stroke

A 100-year hiatus occurred between the first reports of cocaine-associated stroke in the 1880s, and Brust's report on the same subject in 1977 (Brust and Richter, 1977). Now, new case reports appear every year, some involving areas of the brain where ischemic infarct is uncommon. There have been reports of mesencephalic infarcts (Rowley et al., 1989), lateral medullary syndrome and anterior spinal syndrome (Mody et al., 1988), embolization from a left atrial thrombus (Petty et al., 1990), central retinal infarction (Devenyi et al., 1988; Zeiter et al., 1992; Libman et al., 1993; Sleiman et al., 1994), and massive cerebellar infarcts (Aggarwal and Byrne, 1991). The strange distribution of these lesions seems to be explained by cocaine's ability to alter endothelial function, leading to a prothrombotic environment (Figure 1.74). These abnormalities may persist even after short-term drug withdrawal (Sáez et al., 2011).

With the arrival of the great cocaine pandemic of the 1980s, cocaine-related stroke emerged as a significant medical problem, but its incidence now seems to be decreasing. Most cases of ischemic stroke in young adults (15–45 years) are of undetermined etiology. In a recent retrospective study reviewing the records of patients 15–45 years old who were experiencing their first ever stroke, the etiologic diagnoses were undetermined in 36%, large artery atherosclerosis in 21%, cardioembolism in 17%, nonatherosclerotic vasculopathy in 17%, and other specific etiologies in 9% (Varona et al., 2007). These results are in

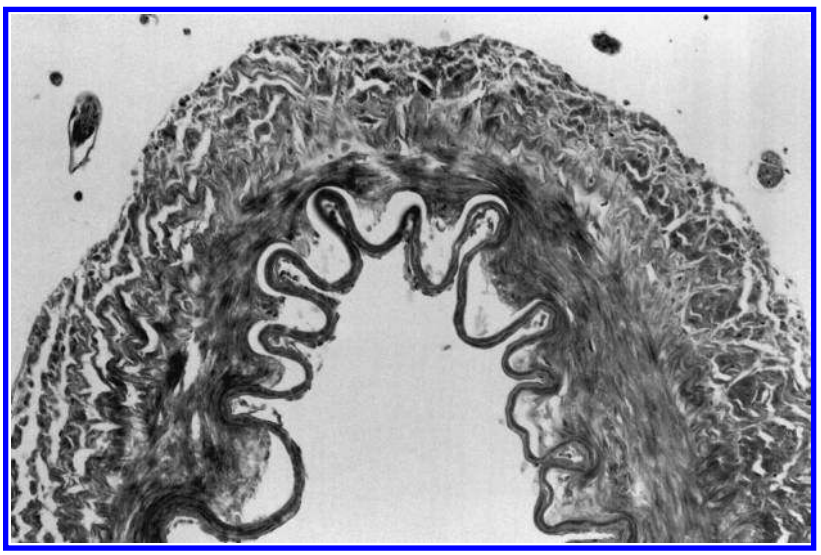


Figure 1.74 Markedly infolded, irregular internal elastic lamina in the anterior cerebral artery of a cocaine user with ischemic stroke. The irregularity of the elastic lamina may be a marker for cocaine-induced vasospasm. (From Konzen, J. P. et al., *Stroke,* 26, 1114, 1995. With permission.)

conformity with an older retrospective study from Hong Kong that failed to find evidence that crack use (historical or acute) had any significant association with stroke or cerebral infarction (Qureshi et al., 1997).

A study performed in 2010 compared stroke risk factors, comorbidities, complications, laboratory findings, medications, and outcomes in patients with cocaine-related (n = 41) and non-cocaine-related (n = 221) ischemic stroke (n = 147). The patients with cocaine-related stroke were younger (mean age, 51.9 vs. 59.1 years; $p = 0.0008$) and more likely to be smokers (95% vs. 62.9%; $p < 0.004$). The prevalence of arrhythmias was significantly higher in the patients with cocaine-related stroke and that of diabetes was significantly higher in those with non-cocaine-related strokes. The prevalence of hypertension and patient lipid profiles were similar in the two groups; however, those with cocaine-related stroke were less likely to have been receiving statins. The same percentages of both groups received antiplatelet therapy. Survivors of both groups had similar modified Rankin scores (the modified Rankin scale is commonly used for measuring the degree of disability or dependence in the daily activities of people who have suffered a stroke) and similar lengths of hospital stay. Of course, in the older urban population, smoking and cocaine use may coexist with cerebrovascular disease; within this subgroup, smoking and cocaine-related strokes have similar morbidities and mortality as non-cocaine-related strokes (Bhattacharya et al., 2011).

MRI studies in human volunteers clearly show that cocaine administration causes dose-related vasoconstriction (Figure 1.75), even when low doses of cocaine are given and even in the absence of other risk factors (Kaufman et al., 1998b). The situation has recently become more complicated because most of the world's cocaine supply is now adulterated with levamisole (Centers for Disease Control, 2009). The ability of levamisole to cause generalized vasculitis is well known (Macfarlane and Bacon, 1978), although no specific case of levamisole-induced cerebral vasculitis has been reported. Thus, vasospasm in cocaine users could be the result of some direct action exerted by cocaine on cerebral blood vessels or be secondary to catecholamine elevation (Brust and Richter, 1977; Levine et al., 1987; Covert et al., 1994) or a consequence of levamisole contamination (Laux-End et al., 1996; Bagga and Hari, 2000).

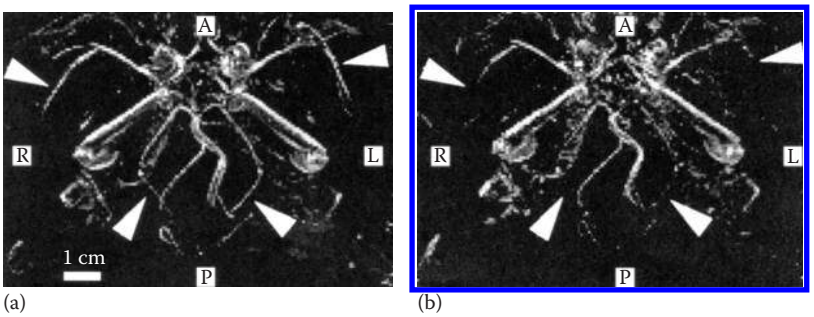


Figure 1.75 Cocaine-induced vasoconstriction. Axial maximum intensity projection images at baseline (a) and 20 min following intravenous cocaine (0.4 mg/kg) administration (b). Cocaine induced a signal loss at distal segments of the middle cerebral arteries (upper arrowheads) and in the posterior cerebral arteries (lower arrowheads), indicative of vasoconstriction. A indicates anterior; P, posterior; L, left; R, right. Scale bar = 1 cm. (From Kaufman, M. J. et al., *JAMA*, 279(5), 379, 1998. With permission. See also Sheikh, H.U. and Mathew, P.G., *Curr. Pain Headache Rep.*, 18(5), 414, 2014.)

Cocaine users may be subject to decreased cerebral flow, even in the face of a normal cardiac output, and there is no question that cocaine users are subject to accelerated atherogenesis (Dressler et al., 1990; Kolodgie et al., 1991; Karch et al., 1995). If cardiac output is reduced, blood pressure fluctuations could lead to cerebral infarction, especially in the presence of preexisting CNS atherosclerotic lesions. Cocaine-associated cardiomyopathy (Duke, 1986; Lathers et al., 1988; Williams, 1990) and arrhythmias are both recognized consequence of cocaine abuse, and either could result in sudden blood pressure fluctuations. A sudden drop in blood pressure combined with asymptomatic stenotic CNS lesions could lead to infarction. The situation is somewhat analogous to cocaine-associated myocardial infarction. The presence of preexisting lesions may exacerbate transient flow decreases, which otherwise would have been tolerated without consequence.

1.17.5 Cerebral Vasculitis

The frequency of cerebral vasculitis (Figures 1.76 and 1.77) in cocaine users is very low, but since the introduction of levamisole into the cocaine supply, the situation may be subject to change. Episodes of biopsy-proven vasculitis are rarely reported today (Krendel et al., 1990; Fredericks et al., 1991; Scully et al., 1997; Kumar and Smith, 2000; Spittell and Spittell, 2001; Gertner and Hamlar, 2002), and even though authors continue to cite cocaine-induced vasculitis as a cause of stroke, in most instances, it almost certainly is not. In virtually all of the published case reports, there is no microscopic evidence of vasculitis and/or multiple drug ingestion (Mockel et al., 1999). Vasculitis has been notably absent in the few autopsy reports of cocaine-related stroke (Klonoff et al., 1989; Nolte and Gelman, 1989; Konzen et al., 1995).

If cocaine does, in fact, cause cerebral vasculitis, it probably has nothing to do with catecholamine toxicity. Patients with pheochromocytoma and animals treated with exogenous catecholamines generally do not show signs of CNS inflammation. Small, perivascular hemorrhages can be induced in animals by giving massive amounts of epinephrine, but

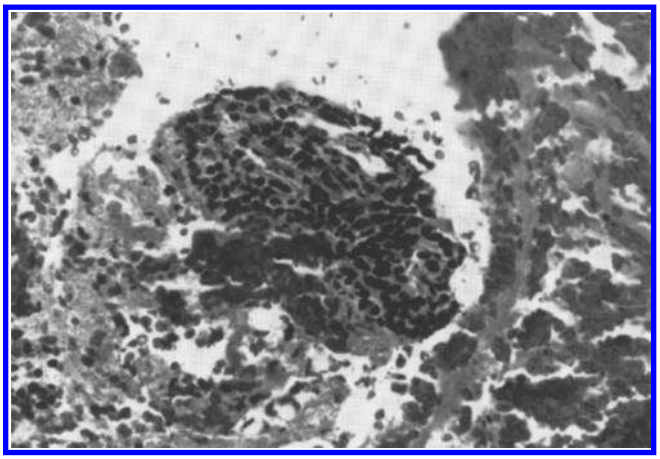


Figure 1.76 Cerebral vasculitis in a cocaine user. Biopsy specimen from a patient surviving an episode of vasculitis. Transmural infiltration of a small cortical vessel. Both acute and chronic inflammatory cells are present. (Original magnification 800×.) (Courtesy of Dr. David A. Krendel, Section of Neurology, The Emory Clinic, Atlanta, GA.)

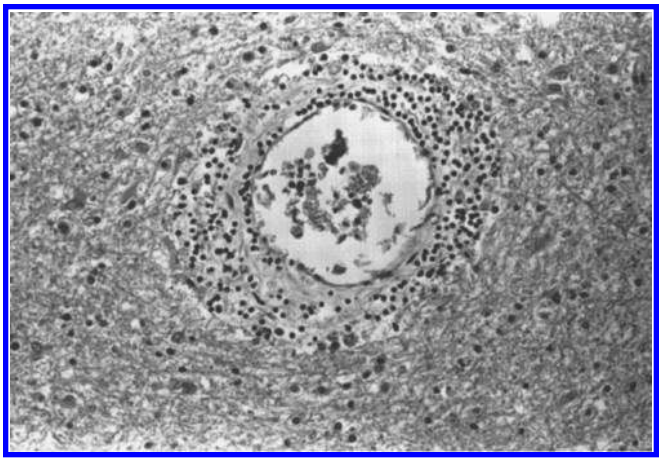


Figure 1.77 Cerebral vasculitis in a cocaine user. Autopsy specimen from another patient with cerebral vasculitis. Illustration shows a lymphocytic infiltrate around a small cerebral vessel. (Original magnification 800×.) (Courtesy of Dr. David A. Krendel, Section of Neurology, The Emory Clinic, Atlanta, GA.)

in this model, the cerebral vessel wall remains normal, with no necrosis and no infiltrates (Stief and Tokay, 1935).

When cocaine-associated CNS vasculitis is encountered, the most plausible explanation is likely to be levamisole adulteration, some other disease totally unrelated to cocaine abuse, or designer amphetamines (Kahn et al., 2014). Even though there is no question about levamisole's ability to cause vasculitis, the process appears to be confined to the skin. Instances of levamisole-related CNS vasculitis have yet to be reported.

Necrotizing angiitis is a form of periarteritis nodosa associated with the abuse of amphetamine and other stimulant drugs (Citron et al., 1970). A few anecdotal reports suggest it may be seen in cocaine users, but only insofar as they used cocaine along with intravenous amphetamines and heroin, and/or the user suffered from hepatitis B infection (Guo et al., 2001), or the decedent was taking levamisole-contaminated cocaine. Cases of documented ANCA-positive necrotizing vasculitis and thrombotic vasculopathy have been documented in the latter (Jenkins et al., 2011). Necrotizing vasculitis in stimulant abusers was first described in the early 1970s, but its incidence seems to have steadily declined over the last 30 years, and no new cases had been reported until the introduction of levamisole. The fact that this disorder has essentially disappeared (save for levamisole-related cases), while intravenous abuse of cocaine and amphetamine has not, suggests that the originally reported cases may have been due to a contaminant introduced into the amphetamine during the course of manufacture and/or distribution. The current practice of referring to cocaine "pseudovasculitis" (Friedman and Wolfsthal, 2005; Bhinder and Majithia, 2007) to describe an ill patient with symptoms and/or laboratory findings that mimic true vasculitis, but with a negative biopsy, is not particularly helpful.

1.17.6 Subarachnoid and Intraventricular Hemorrhage

Intracerebral hemorrhage (ICH) (Figure 1.78) is a devastating event, carrying a very high morbidity and mortality rate. Hypertension and age-related amyloid angiopathy are the strongest risk factors for ICH, but smoking, anticoagulation with warfarin, excessive alcohol

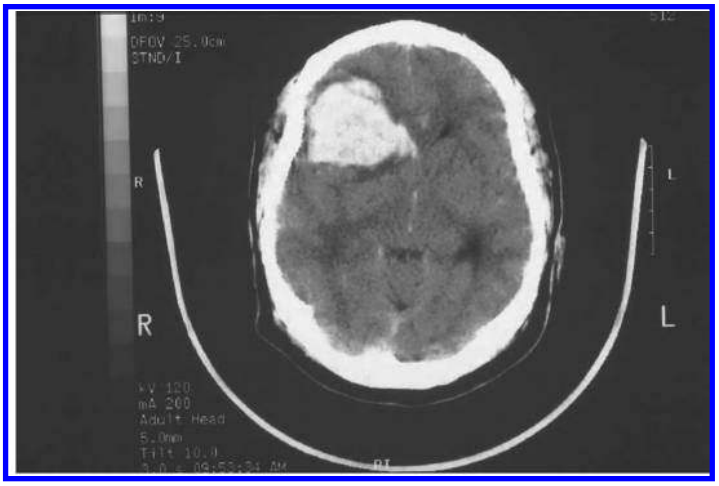


Figure 1.78 Intracerebral hemorrhage in a young "crack" cocaine smoker. Stimulant abuse is becoming an increasingly common cause of stroke in young people. Scan shows massive hemorrhage in the temporal–frontal region. (Courtesy of Dr. Kari Blaho, University of Tennessee, Memphis, TN.)

intake, and cocaine also increase risk (Rymer, 2011). The relationship between cocaine and cerebral hemorrhage is much clearer than that between cocaine and ischemic stroke. Large retrospective studies have shown that cocaine ingestion is associated with hemorrhages that occur more frequently in subcortical locations, that their occurrence is associated with a higher risk of intraventricular hemorrhage, and that the prognosis is worse in cocaine-related hemorrhage than in hemorrhage due to other causes (Martin-Schild et al., 2010).

In the most conclusive study to date, Nolte et al. (1996) performed a prospective autopsy study of all cases of fatal nontraumatic intracranial hemorrhage seen over a 1-year period in a large metropolitan medical examiner's office (Nolte et al., 1996). Ten of 17 (59%) nontraumatic intracranial hemorrhages were found to be associated with the presence of cocaine. Seven (70%) individuals had sustained parenchymal hemorrhages, and the remaining three (30%) had subarachnoid hemorrhages from ruptured berry aneurysms. Neither vasculitis nor any other form of vasculopathy was identified in any of the cases. Nolte et al. concluded that the relationship between severe cocaine-induced hypertension and the development of subarachnoid or ICH "seems fairly clear and is apparently related to sudden transient increases of blood pressure related to cocaine use." Nothing published in the last 15 years would seem to contradict this conclusion (Toossi et al., 2010). This connection has been noted by others (Kibayashi et al., 1995).

Other, more recent studies have confirmed similar findings (Nanda et al., 2006; Chan et al., 2013). Subarachnoid hemorrhage is more common than ICH by a ratio of 4:3. As is the case with cocaine-related cerebral infarction, individuals are in their early 30s. Most (80%) subarachnoid hemorrhages are the result of saccular aneurysms involving the anterior communicating artery, with the posterior communicating system being the next most frequent site. Other retrospective studies have shown that aneurysms are significantly smaller and rupture at a younger age among cocaine users compared with nonusers (Vannemreddy et al., 2008).

Intracerebral hemorrhages occur as a consequence of structural changes in the small perforating vessels of the basal nuclei. They produce deeply situated hemorrhages in the

cerebral hemispheres (basal nuclei and thalamus) and brain stem (Kase, 1995). White matter hemorrhage has been reported in some cocaine users, but it is usually the result of amyloid angiopathy, not cocaine, and not hypertension (Shvartsbeyn et al., 2010). The most common site for hypertensive hemorrhage is the basal ganglia, outnumbering the second most common site, the cerebral white matter, by a ratio of 7:1 (Adams et al., 1984).

In a 2010 study of 45 patients with cocaine-associated ICH and 105 patients with cocaine-negative ICH, gender and age were identical; there was a significantly higher incidence of Black patients in the cocaine-positive group. Cocaine-associated ICH patients had higher admission blood pressures, significantly more subcortical hemorrhages, and higher rates of intraventricular hemorrhage compared to patients with cocaine-negative ICH. Patients with cocaine-associated ICH were nearly three times more likely to die during their acute hospitalization when compared to cocaine-negative patients (Martin-Schild et al., 2010).

Long-term cocaine use may be necessary before hypertensive cardiovascular disease and ICH occur, but even occasional use can lead to transient blood pressure elevations sufficient to cause the rupture of preexisting malformations or bleeding into a tumor (Yapor and Gutierrez, 1992). Cocaine potentiates the increases in blood pressure and cerebral blood flow produced by the administration of NE (Muir and Ellis, 1993). Since cocaine users already have elevated circulating levels of NE, use of the drug would potentiate the normal response of blood vessels to catecholamines. This potentiation may account for many of the reported hemorrhages. Hemorrhage in individuals without underlying lesions, as in a reported case of spinal epidural hematoma (Huff, 1994), remains unexplained.

1.17.7 Seizures

Grand mal seizures in cocaine users are an uncommon occurrence, and very little is known about them. Published case series have placed the incidence of this complication at somewhere between 2% and 10% (Lowenstein et al., 1987; Derlet and Albertson, 1989). In one series of 43 cocaine users with seizures, ages ranged from 17 to 54, with a mean age of 31 years; 53% were male. Forty-two of the forty-three experienced a single tonic-clonic seizure and one developed status epilepticus. Almost all the seizures were self-limiting, and it would appear that status epilepticus occurs only rarely (Majlesi et al., 2010). In experimental animals, dopamine D_3/D_2 receptor agonists seem to protect against the convulsions and the other lethal effects of cocaine after intravenous administration (Witkin et al., 2008), but whether these observations apply to humans is not known. Like all local anesthetics, cocaine is a sodium channel blocker and all local anesthetics can cause seizures (Di Gregorio et al., 2010). Another possibility is that other neurotransmitters, especially γ-aminobutyric acid (GABA), may be involved (Ye et al., 1997; Ye and Ren, 2006). Alternatively, seizures may be a consequence of stroke, intracerebral hemorrhage, or even of massive overdose. There is also the possibility that cocaine use may simply exacerbate a preexisting seizure disorder. This possibility should be considered when seizure activity is prolonged, since, as mentioned previously, most cocaine-associated seizures are self-limited (Ye and Ren, 2006; Majlesi et al., 2010).

"Kindling" is a term used to describe the development of generalized convulsions in response to repeated subconvulsive brain stimuli; cocaine kindling can be induced in animals. Whether this process also occurs in humans as a consequence of cocaine, or any other kind of drug use, has been debated for some time, but the issue is far from

resolved. Kindling appears to be associated with increased *N*-methyl-D-aspartate receptor binding activity in the striatum, amygdala, and hippocampus (Itzhak, 1994; Chihara et al., 2011).

1.17.8 Movement Disorders

Movement disorders, including choreoathetosis, akathisia, and parkinsonism with tremor, have all been described in cocaine users (Daras et al., 1994; Catalano et al., 1997; Riley et al., 2001; Supervia et al., 2006; Kamath and Bajaj, 2007). This phenomenon has become so common that it has even been given the slang name of "crack dancing." Symptoms are generally self-limiting and do not bring the victims to medical attention. One recent controlled study, using MRI imaging, demonstrated an increased incidence of basal ganglia abnormalities that were not seen in controls (Bartzokis et al., 1999); however, there has been little additional research.

Dystonic reactions are extrapyramidal motor abnormalities that paradoxically occur where there is an insufficient supply of nigrostriatal dopamine, even though the accepted mechanism of cocaine intoxication is dopamine excess. The main symptom is spasm within isolated muscle groups. Neuroleptic drugs are a known cause of dystonia and are the most frequently encountered trigger, but the same symptoms can be caused by cocaine (Hegarty et al., 1991; Cardoso and Jankovic, 1993; Fines et al., 1997; Van Harten et al., 1998). Similar reasons may explain why cocaine users are also prone to multifocal tics, which cocaine precipitates (Pascual-Leone and Dhuna, 1990; Linazasoro and Van Blercom, 2007).

Interestingly, movement disorders have been reported among users of "bath salts" (3,4-methylenedioxypyrovalerone) that, like cocaine, inhibit reuptake of NE and 5-HT. One case report describes a sympathomimetic syndrome including high blood pressure, tachycardia, tachypnea, and hyperhidrosis with choreoathetotic movements and renal failure (Sutamtewagul et al., 2014). It is too early in this epidemic to determine the extent of the problem.

1.17.9 Blood–Brain Barrier Alterations

Cocaine alters the permeability of the BBB. This effect is mediated via the actions of platelet-derived growth factor (PDGF) (Yao et al., 2011), which, in turn, downregulates tight junction proteins (proteins that bind membranes together forming a virtually impermeable barrier to fluid) (Kanmogne et al., 2005; Toborek et al., 2005). Blood microvascular endothelial cells structurally and functionally control the BBB. When cocaine binds to σ receptors, it sets off a signaling cascade, leading to the induction of the PDGF-B chain, a key mediator of increased endothelial permeability. Activation of mitogen-activated protein kinase and transcription factor (Egr1) pathways leads to increased expression of PDGF-B. The Egr1 pathway is involved in neuronal plasticity and there is some evidence it may be involved in regulating apoptosis via the phosphatase and tensin homolog pathway (Knapska and Kaczmarek, 2004). HIV virus exerts much the same effect, and a synergistic interaction between cocaine and HIV-1 may well explain why the BBB in HIV-infected patients is "leaky." It may also explain why, despite combined antiretroviral therapy, HIV-associated neurocognitive disorders (HANDs) continue to afflict approximately 60% of HIV-infected individuals, most likely because of the partial or complete inability of antiretroviral drugs to cross the BBB (Kousik and Shih, 2012).

1.18 Renal Disease

1.18.1 Cocaine-Related Kidney Disease

The pathophysiologic basis of cocaine-related renal injury is multifactorial (Figure 1.79), which is why it is associated with a very nonspecific set of pathologic alterations. At a minimum, changes in renal hemodynamics and in glomerular matrix synthesis, cell degradation oxidative stress, the promotion of renal atherogenesis (Nzerue et al., 2000), and the density of ET-1 receptors within the renal vascular smooth muscle of resistance vessels all play a part (Kohan, 1997). In addition, increased ET-1 production causes decreased renal blood flow and glomerular filtration rate (Kon et al., 1989).

The results of large epidemiologic studies suggest that cocaine is an uncommon cause of chronic kidney disease (Akkina et al., 2012). In the most recent study of cocaine users with nephropathy, individuals were mostly white (99.2%), mostly male (82.2%) and mostly intravenous drug users (IVDUs) (81.4%). Median age at death was 39 years and duration of drug abuse was 17 years. The majority (79.1%) continued with their use of illicit drugs during treatment. Despite the relatively young age of these individuals, a large number of comorbid conditions were present, especially cardiovascular disease, liver cirrhosis, and infections. Evaluation of the kidneys demonstrated a broad spectrum of pathologic alterations dominated by arteriosclerotic and ischemic damage, mild interstitial inflammation, calcification of renal parenchyma, interstitial fibrosis, tubular atrophy, and hypertensive-ischemic nephropathy. Interstitial inflammation (OR, 16.59; 95% CI, 3.91–70.39) and renal calcification (OR, 2.43; 95% CI, 1.03–5.75) were associated with severe IVDU, whereas hypertensive and ischemic damage were associated with cocaine abuse (OR, 6.00; 95% CI, 1.27–28.44). Somewhat surprisingly, neither specific glomerular damage indicative for heroin- and hepatitis C virus-related disease, nor signs of analgesic nephropathy were found (Buttner et al., 2014).

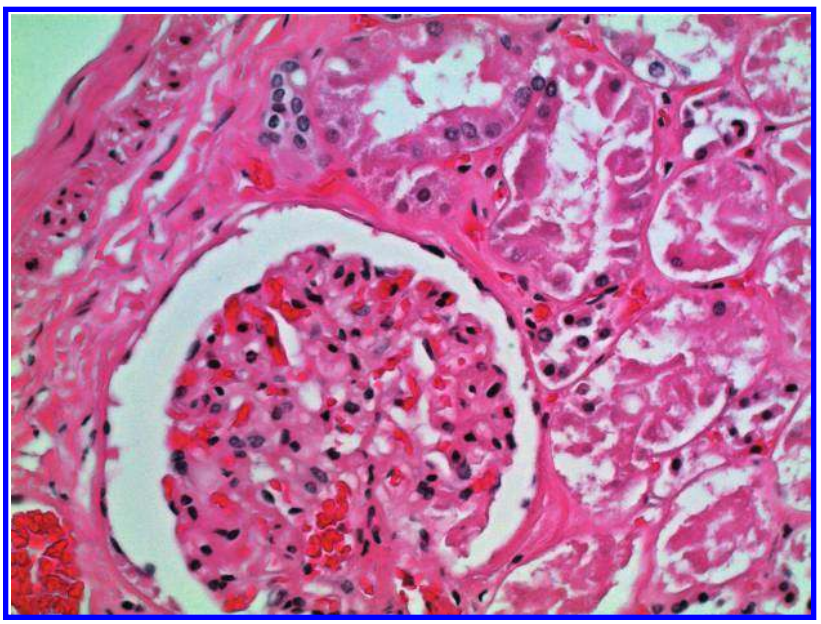


Figure 1.79 Marked glomerulosclerosis in a chronic cocaine abuser. This is a nonspecific finding, but kidney disease is highly prevalent in stimulant abusers.

Nonetheless, cocaine use has been linked to accelerated atherogenesis in both animal experiments and human studies and autopsy findings (Karch and Billingham, 1988; Kolodgie et al., 1991; Karch, 2005). Cocaine increases renal cellular oxidative stress and decreases intracellular glutathione in cocaine-exposed cultured renal epithelial cells (Togna et al., 1985), as well as increasing thromboxane production and platelet aggregation in in vitro platelet cultures (Togna et al., 1985).

Increased platelet activity, measured by platelet p-selectin expression, occurs in human and canine models (Kugelmass et al., 1993). Any of these actions could favor the development of kidney disease and no doubt does. The problem is the lack of systematic studies to assess the magnitude of the problem. Attempts have been hindered by several factors, perhaps the most important of which is that lesions seen in experimental animals treated with cocaine bear no resemblance to those seen in humans.

In vitro studies of human proximal tubular endothelial cells have shown quite dramatically that the kidneys, not just the liver, metabolize cocaine, that they produce substantial amounts of norcocaine (not the case in the liver), and that the concentration of norcocaine achieved can cause apoptosis. It has also been shown that inhibition of CYP3A2 prevents the norcocaine-associated tissue injury (Valente et al., 2011).

Cocaine-induced renal infarction is not frequently reported (Hoefsloot et al., 2009; Fabbian et al., 2012). Suggested pathophysiologic mechanisms include direct cocaine-induced platelet activation combined with vasoconstriction and secondary endothelial damage (Hoefsloot et al., 2009), or simple vasoconstriction, for whatever cause (Furaz et al., 2008; Madhrira et al., 2009). None of these mechanisms has ever been proven and, unfortunately, there is no proven therapy for this complication.

The first case of rhabdomyolysis directly related to cocaine was described in 1987 (Merigian and Roberts, 1987), and hundreds of case reports have appeared since (Gitman and Singhal, 2004; Vets et al., 2006). In a large percentage of these cases, rhabdomyolysis is not the primary disorder but it is often a component of the ExDS where very high core temperatures and extreme physical activity combine to cause muscle breakdown.

Except for cases of ExDS, the underlying process by which stimulant drugs produce rhabdomyolysis is poorly understood. In some cocaine-related cases, the relationship to prolonged seizure activity is clear; however, seizures are rarely reported in recreational users. In other settings, pressure-related injury seems to be the most likely explanation (Singhal and Faulkner, 1988; Singhal et al., 1989; McCann et al., 2002; Gitman and Singhal, 2004; Vets et al., 2006). Cocaine-induced vasospasm leading to myocyte necrosis has also been proposed as a mechanism (Roth et al., 1988), but this mechanism has never been reproduced in the laboratory or even described in other case reports. Accelerated renal artery arteriosclerosis, with histologic changes reminiscent of those that are occasionally observed in the coronary arteries of cocaine users, has also been reported (Bacharach et al., 1992; van der Woude and Waldherr, 1999) yet, here too there have been no similar reports in the last 15 years. This raises the possibility that a cocaine adulterant, then in common use, may have been responsible for the anatomic changes.

A common thread in many, but not all, cases of rhabdomyolysis is hyperthermia (Rosenberg et al., 1986; Campbell, 1988; Menashe and Gottlieb, 1988; Pogue and Nurse, 1989; Lomax and Daniel, 1990; Nolte, 1991). In humans, heritable malignant hyperthermia is inherited as an autosomal dominant caused by a defect in the ryanodine receptor. Over 90 mutations have been identified in the *RYR1* gene located on chromosome 19q13.1, and at least 25 are causal for malignant hyperthermia (MH). The pathophysiologic changes of

MH have been extensively characterized in animal models and are the result of an uncontrolled rise of cytosolic calcium that, in turn, activates biochemical processes related to muscle activation. As ATP is depleted, muscle membrane integrity is compromised, ultimately leading to hyperkalemia and rhabdomyolysis (Rosenberg et al., 2007). This mechanism very likely applies to humans as well. The latest animal studies show that exposure to cocaine (10 μM) significantly increases RYR 1 and 2 proteins and their production of mRNA, but not expression of RYR. One can only infer that cocaine-induced D+ receptors play a regulatory role in RYR expression and that cocaine is part of the regulatory process (Kurokawa et al., 2011).

Of the 90 mutations identified in the *RYR1* gene, at least 25 can cause for MH. Diagnostic testing relies on assessing the in vitro contracture response of biopsied muscle to halothane, caffeine, and other drugs. Elucidation of the genetic changes has led to the introduction, so far on a limited basis, of genetic testing for susceptibility to MH. This practice will no doubt become much more widespread with the advent of second-generation DNA sequencers. As the sensitivity of genetic testing increases, and the price for testing decreases, molecular genetics will be used to identify those at risk.

Dantrolene sodium is a specific antagonist of the pathophysiologic changes in MH and is the recommended drug of choice (Probert and Macnair, 2008). Thanks to the dramatic progress in understanding the clinical manifestations and pathophysiology of the syndrome, the mortality from MH has dropped from over 80% 30 years ago to less than 5% today. From the pathologist's perspective, the most interesting feature of this disorder is that it produces anatomic alterations that are grossly visible at autopsy. These include not only orange discoloration of the subcutaneous fat but also marked pallor of individual muscle groups. The nature of the underlying process can be confirmed with routine microscopy (Byard et al., 2011).

Repeated exposure to CEW devices has also been proposed as a possible etiology in cocaine-associated rhabdomyolysis. Repeated CEW applications could, in theory, result in repetitive, sustained muscle contraction, with little or no muscle recovery period, especially if exposures are longer than 5 ms. Other studies have demonstrated that, in humans, duration of CEW application has no predictable relationship to elevation of creatinine kinase (Dawes et al., 2011). However, CEW application has only been demonstrated in experimental animals under extreme conditions (exposure periods of up to 3 min), and even then the changes observed were transitory (Jauchem et al., 2006).

A systemic autoimmune disease, thought to be caused by levamisole-contaminated cocaine, has been described. It appears to be a consequence of ANCA-induced vasculitis, no different than the vasculitis now so commonly reported in the face and ears (McGrath et al., 2011).

Cocaine-associated tubular necrosis (Figure 1.80) is a multifactorial disorder. Hypovolemia, renal arterial vasoconstriction, and myoglobinuria all combine to produce the syndrome. Except for one case report (Turbat-Herrera, 1994), morphologic alterations in cocaine users have not been described. In the one case where a renal biopsy was performed, no abnormal antibody deposition or myoglobin was identified in the tubules, and the picture was otherwise typical for acute tubular necrosis, with vacuolation, fragmentation, and desquamation of the proximal lining tubular epithelial cells and pigmented casts in some distal nephrons (Turbat-Herrera, 1994). In theory, catecholamine toxicity and other well-known sequelae of cocaine use could lead to hemolytic

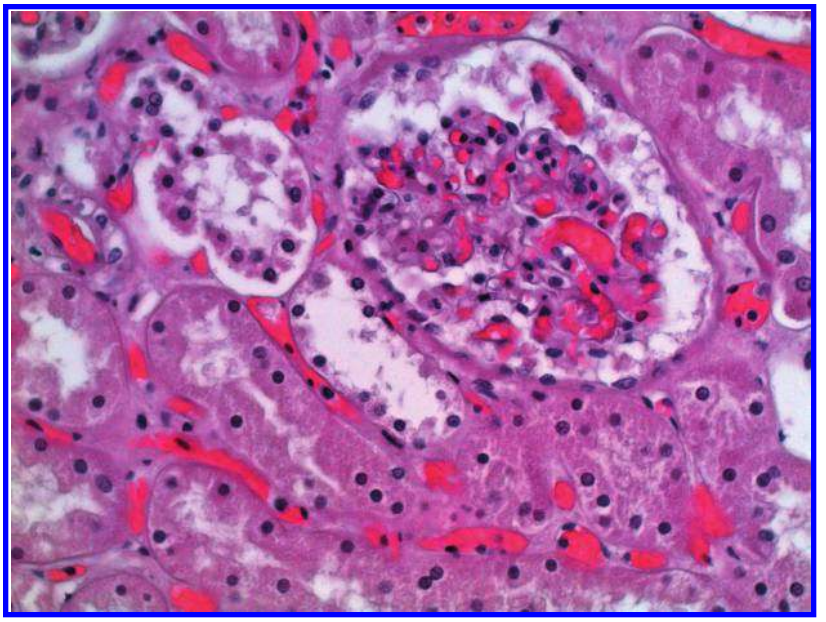


Figure 1.80 Kidney of a chronic cocaine abuser. Note the shrunken glomeruli, some still adherent to Bowman's capsule. The endothelial cells of the tubules are disintegrating and fragments can be seen free in the tubules.

uremic syndrome or microangiopathy. After an initial endothelial injury, intravascular coagulation and all the other elements of hemolytic uremic syndrome (HUS) can result. This possibility is supported by the observation that not all cocaine users with hemolysis and thrombocytopenia have demonstrable histologic lesions. The focal type of glomerulosclerosis associated with heroin-related nephrotic syndrome has not been reported in cocaine or stimulant abusers, but similar lesions have been produced in rats (Barroso-Moguel et al., 1995).

The presence of glomerulosclerosis in cocaine abusers should also raise the suspicion of HIV infection. Glomerulosclerosis is the most common renal lesion seen in AIDS patients (Sanders and Marshall, 1989). Sometimes the picture becomes very confusing because cocaine users, who may be HIV positive, can present with refractory hypertension and renal failure but only modest proteinuria and no evidence of either renal shrinkage or cardiomegaly. Because renal biopsies are no longer routinely performed in patients with end-stage renal disease and evidence of accelerated hypertension, the etiology in such cases may never be determined.

Cocaine has been implicated as a potential teratogen since the early 1990s when a few case reports describing congenital abnormalities of the genitourinary tract were published (Chavez et al., 1989). However, a prospective study using ultrasound to evaluate 100 consecutive infants exposed to cocaine in utero failed to find any consistent teratogenic effect (Rosenstein et al., 1990). A similar study repeated in 1995 yielded almost identical results. Renal ultrasound scans performed on 79 infants born to cocaine-using mothers demonstrated abnormalities in 11 of the babies, including 1 case each of horseshoe kidney, unilateral abnormal small kidney, duplex kidney, and hypospadias. Renal tract dilation was seen in several of the children. The authors of the study concluded that the risk of urogenital

malformation might be slightly increased, but a much larger study would be needed to reach any conclusion (Battin et al., 1995). Cocaine-induced teratogenesis has never been demonstrated in a controlled human trial, even though there is abundant experimental evidenced suggesting that oxidative stress produced by cocaine is responsible (Omoya et al., 2001).

Finally, even though cocaine use might seem a contraindication to organ donation, but it is not. One-year survival rates for individuals receiving kidneys from cocaine abusers appear to be no different than rates for drug-free donors (Leikin et al., 1994). Donor hearts from cocaine abusers are similarly unaffected, with survival rates comparable to those for drug-free donors (Freimark et al., 1994; Caballero et al., 2003).

1.19 Hematologic Abnormalities

1.19.1 Thrombocytopenic Purpura

Thrombocytopenic purpura (TTP) seems to have, at best, a weak association with cocaine use. One study (Koury, 1990) described seven HIV-seronegative intravenous cocaine abusers, all with extensive cutaneous petechiae, ecchymoses, and heme-positive stools. The patients all had normal bone marrow aspirates or, at most, increased numbers of megakaryocytes. Platelet counts in each of the individuals all improved promptly after steroid administration. No other etiology for their condition could be identified. These cases seem to have actually been caused by cocaine consumption, but since cocaine is almost never available in a pure form (more than 80% of street cocaine in the United States contains levamisole), it is impossible to be sure.

A handful of other similar cases have been reported (Savona et al., 1985; Burke and Ravi, 1990; Gallup et al., 1991; Klein et al., 1991; McDonough and Nolte, 1991; Freimark et al., 1994; Valenza et al., 1995; Keung et al., 1996; Takkenberg et al., 1997; Volcy et al., 2000; Alcazar-Guijo et al., 2005). However, reports have gradually decreased, again suggesting that some adulterant then present was responsible for these disorders in the first place.

This disorder was first recognized as a potentially fatal disease in 1924. It appears to be the result of ADAMTS-13 (a metalloprotease) deficiency. The enzyme is supposed to act as an essential regulator of the extremely adhesive and unusually large vWF that is secreted by endothelial cells. Hereditary TTP is caused by homozygous or double heterozygous mutations of *ADAMTS13*, many of which have been identified. Acquired TTP is often but not always associated with severe, autoantibody-mediated ADAMTS-13 deficiency. The pathogenesis of cases without severe deficiency of the von Willebrand factor (vWF)-cleaving protease remains unknown and it is not clear why cocaine should have anything to do with the process. Survivors of acute TTP, especially those with autoantibody-induced ADAMTS-13 deficiency, are at a high risk for relapse, as are patients with hereditary TTP. Patients with thrombotic microangiopathies (TMAs) associated with hematopoietic stem cell transplantation, neoplasia, and several drugs (cocaine) usually have normal or only moderately reduced ADAMTS-13 activity, with the exception of ticlopidine-induced TMA. Constitutional TTP can be treated by infusions of fresh frozen plasma. The infusions rapidly reverse acute disease and, if given prophylactically every 2–3 weeks, will prevent relapse (Lammle et al., 2005).

1.19.2 Thrombosis

Taking into account recent developments in the illegal cocaine trade, namely, the appearance of levamisole as a cocaine adulterant, cocaine-induced thrombosis is likely to become an increasingly common entity, and it appears that other organs may be involved as well—there are already reports of renal infarction (see Section 1.18.1) and even stent thrombosis (Singh et al., 2007; Ibrahim et al., 2013). A recent case report described five patients with an ANCA-associated cutaneous vasculopathy that was secondary to the use of cocaine adulterated with levamisole. In all five cases, the presenting sign was retiform purpura, with ear involvement being the most characteristic finding. High-titer polyspecific ANCA and positive antiphospholipid antibody tests were also evident; thrombosis and/or leukocytoclastic vasculitis were seen on all the skin biopsies.

Episodes of vasculitis and thrombosis occur in organs besides the skin (Zoghby et al., 2007). But new case reports of skin lesions are appearing at a rapid rate (Bonaci-Nikolic et al., 2005; Walsh et al., 2010; Chung et al., 2011; Gross et al., 2011).

In vitro, cocaine increases thromboxane generation; however, the consequences of this are not really known (Togna et al., 1985, 1996). When placental segments are incubated with cocaine-containing solution, thromboxane production increases and concentrations of prostacyclin decrease (Monga et al., 1994), but what these changes mean to the clinical status of the user are not known.

Studies of platelet and clotting mechanisms in cocaine users have yielded what can only be described as conflicting results, but the results of some recent pharmacologic studies are more suggestive. It only logical to suspect that, since monoamines such as serotonin and epinephrine are involved in platelet activation and aggregation, serotonin and dopamine should be as well. In vitro studies of human tissue show that dopamine is an ADP-dependent platelet agonist that acts via D_2-like but not D_1-like receptors or adrenergic receptors. The observation is of some concern because many psychiatric drugs are directed at D_2-like receptors, as is cocaine; platelet dysfunction in patients being treated with such drugs may be the culprit in some of these cases (Schedel et al., 2008, 2011).

1.19.3 Erythrocytosis

In a 1999 study, changes in hemoglobin concentration, hematocrit, and red blood cell counts were measured in a group of chronic cocaine users, both before and after cocaine administration. Hemostatic parameters, including vWF, fibrinolytic activity, fibrinogen, plasminogen activator inhibitor antigen, and tissue-type plasminogen activator antigen, were also measured. Hemoglobin levels, hematocrit, and red blood cell counts increased significantly 30 min or less after the cocaine had been given. At the same time, there was no change in white blood cell or platelet counts, but concentrations of vWF increased by 40% over baseline levels. Thus, it is apparent that cocaine induces a transient erythrocytosis that may increase blood viscosity and concentrations of vWF (Siegel et al., 1999). In a separate study of 79 consecutive chest pain patients presenting at an emergency room for treatment, hemoglobin and hematocrit levels were significantly elevated in the cocaine-using subjects compared with controls, but there was no corresponding elevation in reticulocyte count. Multivariate logistic regression revealed that male chest pain patients were significantly more likely to be exposed to cocaine than females ($p = 0.001$) and that all of the relative increases in hemoglobin

concentration in the cocaine-exposed group were attributable to gender. Cocaine exposure was not significantly associated with reticulocyte count (Weber et al., 2003).

An epidemiologic survey of patients with myocardial infarction (Determinants of Myocardial Infarction Onset Study) showed that, among the subset of patients who were cocaine users, the risk for onset of myocardial infarction was elevated 23.7 times over baseline (95% CI, 8.5–66.3) 60 min after cocaine ingestion, but within the first hour the risk rapidly returned to that of the general population (Mittleman et al., 1999). Because the observed hematologic changes seem to match precisely the timing of the transient increases in vWF, platelet aggregability, and the risk for acute myocardial infarct, it is tempting to suppose that all of these events are related.

1.19.4 Methemoglobinemia

Another hematologic abnormality associated with cocaine use, at least indirectly, is methemoglobinemia. Street-level cocaine is occasionally diluted with benzocaine or other related local anesthetics, and oxidation of ferrous (Fe_2) hemoglobin to the ferric (Fe_3) state is a well-recognized complication of benzocaine administration. One case report described a 27-year-old man with a massive overdose who developed classic methemoglobinemia. Blood cocaine levels were not measured; however, urine cocaine levels were 106 mg/L, while benzocaine levels were 3.8 mg/L (Tada et al., 1987; McKinney et al., 1992). Cocaine itself has never been implicated as a cause of this disorder. These results are confirmed by the reported findings in a 2009 case series where 242 episodes (40.1% published in year 2000 or after) were accumulated. A discrepancy between the pulse dosimeter saturation (≤90%) and the arterial oxygen partial pressure (≥70 mm Hg) was present in 91.8% of the episodes. The difference between oxygen saturation measured by pulse oximetry and co-oximetry varied from ≥6.2% to 44.7%. Plain prilocaine may induce clinically symptomatic methemoglobinemia in children older than 6 months when given in doses exceeding 2.5 mg/kg. In adults, doses of prilocaine less than 5.0 mg/kg (3.2 mg/kg) in the presence of renal insufficiency, and to 1.3 mg/kg if other oxidizing drugs are used concurrently are, apparently, safe.

A single spray of benzocaine may induce methemoglobinemia. Clinical symptoms may be observed at relatively low methemoglobin values, including coma at 32.2% and 29.1% in children and adults, respectively (Guay, 2009).

1.20 Hormonal Alterations

1.20.1 Overview

Opiates suppress the hypothalamic–pituitary–adrenal (HPA) axis, but cocaine use leads to HPA axis activation. HPA axis activation also occurs during withdrawal from either opiates or cocaine. It is also speculated that reactivation of the HPA axis may reinforce relapse behavior (Brown and Kiyatkin, 2006). Cocaine and amphetamine transport (CART) is a neuropeptide but it also acts as a neurotransmitter. It is a potent anorexigen and can be found in most parts of the body. It is also expressed in pituitary endocrine cells, adrenomedullary cells, islet somatostatin cells, and rat antral gastrin cells. CART is regulated by leptin. CART is an object of interest to addiction professionals because fasting animals show a pronounced

decrease in CART mRNA expression within the arcuate nucleus, the same brain nucleus that is deeply involved in the process of cocaine addiction (Fekete and Lechan, 2006).

In the past, it had been thought that CART played a role in regulating the rewarding and reinforcing effects of many abused drugs; CART given directly into the ventricular cistern deters previously addicted experimental animals from drinking again; however, the site(s) of action remains unclear. The paraventricular thalamus seems to be the target for CART signaling because of its direct contact with the hypothalamus and also because it connects with the hypothalamus and the arcuate nucleus, the single brain nucleus thought to be most deeply implicated in the addiction process.

Men and women respond differently to cocaine. Even though cocaine is classically thought of as a reuptake inhibitor exerting its effects on tissue and organs that respond to NE and 5-HT, it also acts on the endocrine system. In many ways, cocaine's effects resemble those exerted by monoamine oxidase inhibitors. In particular, acute cocaine administration stimulates the release of gonadotropins, adrenocorticotropic hormone (ACTH), and cortisol or corticosterone, while at the same time it suppresses prolactin concentrations (Mello and Mendelson, 1997). Taken together, these actions may account for the apparent inability of cocaine users to fight infections (Avila et al., 2003). Other studies have shown that, in addicted rats, abrupt cessation of cocaine or morphine leads to increased concentrations of corticosterone, a sign that stress system-related pathways have been activated (Avila et al., 2003). It has recently been demonstrated that in patients with multiple sclerosis, cerebrospinal fluid contains elevated levels of orexin (hypocretin-1), which is thought to account partly for weakness associated with that disease. Orexin levels are increased by the infusion of CART—still another link with the hypothalamus (Constantinescu et al., 2011).

1.20.2 Prolactin

Prolactin is episodically secreted by the hypothalamus. During sleep, the amplitude of the secretory pulses increases. Unlike other pituitary hormones, hypothalamic secretion of prolactin is under tonic inhibition by dopamine and possibly by GABA. Release of prolactin is favored by increasing concentrations of thyroid-releasing hormone, vasoactive intestinal polypeptide, and 5-HT (Molitch, 1992). Initially, the results of animal studies suggested that acute administration of cocaine caused prolactin levels first to decrease and then, later, to rebound causing rebound hyperprolactinemia (Mello et al., 1990). Other studies have found that, in humans, intranasal cocaine increases plasma cortisol but not prolactin, and there is no evidence that tolerance changes the situation at all. In studies with human volunteers given high doses of cocaine, 24 h after the last dose prolactin concentrations were immediately and significantly reduced, but no sustained alterations were observed during abstinence. The observation suggests that exposure to controlled high doses of cocaine produces modest symptoms consistent with cocaine withdrawal within hours of cessation of dosing but provides no evidence of symptoms persisting beyond 24 h (Walsh et al., 2009). The findings suggest that, even if changes in prolactin concentration do occur, they may not be of very great clinical significance.

1.20.3 Sex Hormones

Effects on other human hormonal systems have been difficult to demonstrate, mainly because cocaine users also use alcohol and other drugs that alter hormone production. Studies comparing male and female addicts have shown sex differences in all aspects of

drug abuse history, including age of first drug abuse, progression to dependence, and propensity to relapse following drug abstinence (Becker and Hu, 2008). Because of the number of possible variables, studying the situation in humans is difficult, if not impossible.

Most studies agree that males and females respond differently to drugs of abuse. In females, estradiol enhances the behavioral response to cocaine. It has also been demonstrated that, in males, testosterone not only modulates the behavioral response to a single and to repeated cocaine injections, but that it is also essential for male rats to become sensitized to cocaine (Menendez-Delmestre and Segarra, 2011). This observation simply confirms what has been strongly suspected for years: sex hormones modulate the response to cocaine (Caine et al., 2004; Festa and Quinones-Jenab, 2004).

Drug abuse and HIV infection alter the production of female sex hormones. Luteinizing hormone levels are significantly higher in women with low CD4 cell counts, and current methadone use is associated with lower levels of total testosterone ($p = 0.03$) and higher levels of prolactin ($p = 0.002$). Mean estradiol levels are 43% lower in women using intravenous drugs than in those who do not ($p < 0.001$). There is, at present, no explanation why female alcoholics and "crack" smokers seem to have normal concentrations of sex hormones, but the HIV infected do (Cofrancesco et al., 2006). For unexplained reasons HIV-infected individuals are less likely to be receiving highly active antiretroviral therapy than are those who have discontinued the practice, which could alter hormonal concentrations (Cofrancesco et al., 2008).

The explanation why there are so many more men than women drug abusers with cardiovascular disease may also have its basis in hormonal differences. Female sex hormones, even when present in normal concentrations, are said to cause decreased ET-1 release, whereas testosterone increases endothelin production, although the data are conflicting. In the most recent study with cultured human aortic endothelial cells, estradiol did not change the number of cells secreting ET-1 during 4 h incubation under basal conditions, but did decrease the number of secreting cells stimulated with angiotensin II. Testosterone, on the other hand, induced an increase in the number of cells secreting ET-1. It appears that angiotensin II, testosterone, and estradiol all have parallel effects on the production of ET-1 (Pearson et al., 2008). That would almost guarantee that, in the presence of cocaine, there will be changes in ET-1 production and release. In addition to classic steroid hormone receptors, cocaine receptors also mediate the effects of female sex hormones on ET-1 release (Wilbert-Lampen et al., 2005).

Testosterone levels in chronic cocaine abusers have not been systematically characterized, and laboratory studies on gonadal uptake have produced conflicting results. However, there is real concern that combining cocaine with supplemental testosterone (as body builders may do) places these individuals at increased risk for thrombosis—greater than the risk conferred by the single use of either agent (Togna et al., 2003); however, clinical evidence for this link has never been established.

1.21 Immune System Abnormalities

Drug users are susceptible to pulmonary, endovascular, skin, soft tissue, bone and joint disease, and also to sexually transmitted infections, caused by a wide range of bacterial, viral, fungal, and protozoal pathogens. In addition, injection drug users are at increased risk for parenterally acquired infections such as HIV, hepatitis B virus, hepatitis C virus,

tetanus, and malaria. Factors related to drug use, such as unsterile injection practices, contaminated drug paraphernalia, and drug adulterants, all increase exposure to microbial pathogens. Illicit drugs also affect several components of the complex immune system and thus modify the host's normal immune response. Drug takers are also at risk because of lifestyle practices such as multiple sexual partners, overcrowded housing arrangements, and malnutrition.

Drug abuse–mediated immune dysfunction increases host susceptibility to microbial pathogens. This action has assumed greater importance since the onset of the AIDS pandemic (Siegel, 1986; Siegel et al., 1986). Cocaine affects several different components of the immune system by enhancing or suppressing the function of immune response cells and factors, such as chemokines and cytokines. Effects on the immune system may be mediated directly through activation of receptors located on immune cells or indirectly through drug interactions in the CNS. Th1 (cellular)/Th2 (antibody-mediated) responses often lead to inhibition of Th1-associated cytokine conduction, including IL-12 and gamma interferon (IFN-γ). Alternatively, there may be elevation of Th2-associated (IL-4) cytokines (Cabral and Staab, 2005). Increased host susceptibility would explain unusual infections such as that seen in Figure 1.81.

Cocaine exerts mainly indirect effects on the immune system. It does so via the sigma-1 (σ_1) receptor located in the brain and peripheral nervous system (Friedman et al., 2003). In the pathogenesis of HIV, cocaine has been shown to increase viral replication in peripheral blood mononuclear cells, increase viral load, and decrease the CD4+/CD8+ ratio (Matsumoto et al., 2002). Notwithstanding experimental limitations and challenges in extrapolation of in vitro data, illicit drugs act at least as cofactors that can increase the severity of microbial infections by altering host resistance (Kaushik et al., 2011).

1.22 Gastrointestinal Disorders

1.22.1 Introduction

Most of the gastrointestinal disorders associated with cocaine are due to cocaine's catecholamine-mediated effects on blood vessels. However, cocaine metabolites, and possibly even cocaine itself, may be directly toxic to the liver. Norcocaine is hepatotoxic in experimental animals and so is cocaethylene; both compounds are synthesized by the human liver in the presence of ethanol. The overall toxicity of cocaethylene seems to be quite similar to that of cocaine itself. In spite of convincing animal studies, there is no evidence that cocaine use causes any significant damage to the human liver, and when liver disease is observed, it can be presumed to be the result of lifestyle disease (hepatitis, HIV, or polydrug abuse), especially hepatitis C. The other disorder seen with some frequency in the gastrointestinal tracts of cocaine users is an unintended side effect of the smuggling process: smugglers with intestines full of cocaine or other illicit drugs (Karch et al., 1999b).

1.22.2 "Body Packing"

The practice of "body packing" has increased drastically in the last 20 years and complications are ever more common. Most of these cases are managed with laxatives or bowel irrigation but surgery is occasionally required because of either drug toxicity or small bowel obstruction. If clinically indicated, drug-containing packets can be retrieved using a combination of

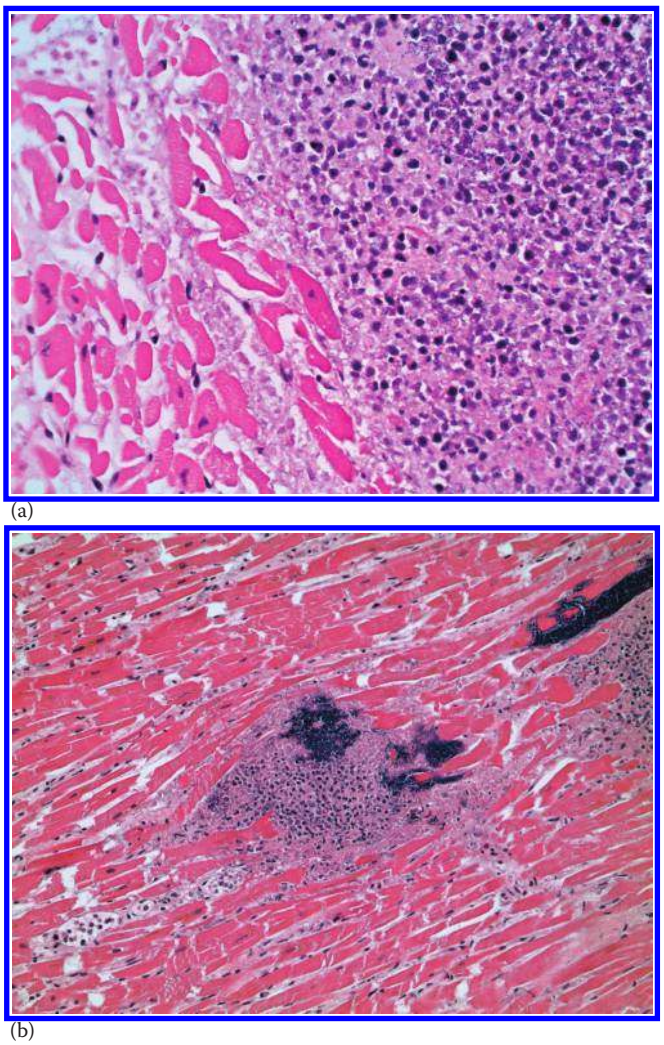


Figure 1.81 (a and b) The patient was a young cocaine user who developed septicemia of undetermined origin. Multiple abscesses were seen, mainly in the myocardium, even though there was no evidence of endocarditis.

milking and multiple enterotomies, but this generally leads to a high rate (40%) of postoperative wound infection (Silverberg et al., 2006; Wilcher, 2011). Should a packet rupture, blood concentrations can reach levels approaching 2 mg/L (de Prost et al., 2010).

1.22.3 Ischemic Bowel and Stomach Injuries

Ischemic colitis due to cocaine abuse is a well-recognized entity (Fishel et al., 1985). It was first described in 1985 (Nalbandian et al., 1985) and reports have been published regularly ever since (Ruiz-Tovar et al., 2010; Edgecombe and Milroy, 2012). The disease process tends to involve the proximal colon (Ellis and McAlexander, 2005). Cocaine-associated enterocolitis usually presents within three days of cocaine use. The majority of patients will recover with no operative therapy; however, those who do develop peritonitis and undergo

laparotomy have a 50% mortality (Ellis and McAlexander, 2005). Microscopic examination of ischemic bowel removed from cocaine users will demonstrate findings consistent with pseudomembranous colitis, with some areas of the bowel resembling acute ischemic colitis more closely than others. Otherwise, there are no distinctive features.

In the largest series published to date, which was comprised of seven patients, endoscopy revealed lesions restricted to the left colon, including hemorrhagic edema of the mucosa, pseudopolyps, and ulcerations. Rectal involvement, which is not generally considered a feature of ischemic colitis, was seen in five patients. The histologic changes were classified as acute/subacute in two of the five patients and as subacute/chronic in the other three (Niazi et al., 1997). Given that genital cocaine application is not uncommon, it could be that the rectal involvement noted in two of the cases had as much to do with sex practices as with drug-induced ischemia.

Perforation from any cause is said to occur in 7 to 10 patients per 100,000 of the general population annually and is thought to be a complication in 5%–10% of peptic ulcers. "Crack"-related gastroduodenal perforations are typically prepyloric and usually presumed to be ischemic, though motility disorders, increased air swallowing, platelet-related thrombosis, and increased ACTH and corticosterone secretion could all play a role (Yahchouchy et al., 2002).

During the beginning of the "crack" pandemic, it was thought that maternal cocaine use might, in some way, be related to necrotizing enterocolitis in the neonates, but no evidence has ever been brought forward to substantiate the claim.

When bowel obstruction and ischemia occur in cocaine users, the pathologic findings are similar to those seen in patients with pheochromocytoma (Bravo and Gifford, 1993). In fact, catecholamine-mediated gastrointestinal lesions have been recognized since the 1930s, when treatment of asthmatics with nebulized epinephrine came into fashion. Treatment was occasionally complicated by tracheal hemorrhages and ulceration of the gastrointestinal mucosa (Galgaini et al., 1939).

Szakacs et al. systematically studied the effects of chronic catecholamine administration in experimental animals and humans (Szakacs et al., 1959). They observed that fibrinoid degeneration and necrosis could be seen in the arteriolar walls of vessels, both in the heart and the gastrointestinal tract. Prolonged NE infusion induced endothelial proliferation, occasionally sufficient to cause complete obstruction of small arteries of the gastrointestinal tract, leading to infarction and perforation of the bowel (Szakacs et al., 1959). Similar lesions are observed in experimental animals and in patients with pheochromocytoma.

More than 40 years after Szakacs first presented his observations, exactly the same lesion has been identified in cocaine users (Garfia et al., 1990).Thrombotic lesions have also been described, presumably caused by the same sequence of events that leads to thrombosis in the heart and other blood vessels (Ottolini and Foster, 1994). The bowel is not the only part of the gastrointestinal tract subject to ischemic injury. There is at least one report of spontaneous hepatic rupture in a pregnant cocaine user, presumably a result of the same mechanism responsible for ischemic gut injury (Moen et al., 1993).

1.22.4 Hepatic Disease

Cocaine hepatotoxicity has been repeatedly demonstrated in man and animals (Marks and Chapple, 1967; Wanless et al., 1990), and clinical experience suggests that the incidence of hepatic microsteatosis is increased with cocaine use (Karch et al., 1998; Rajab

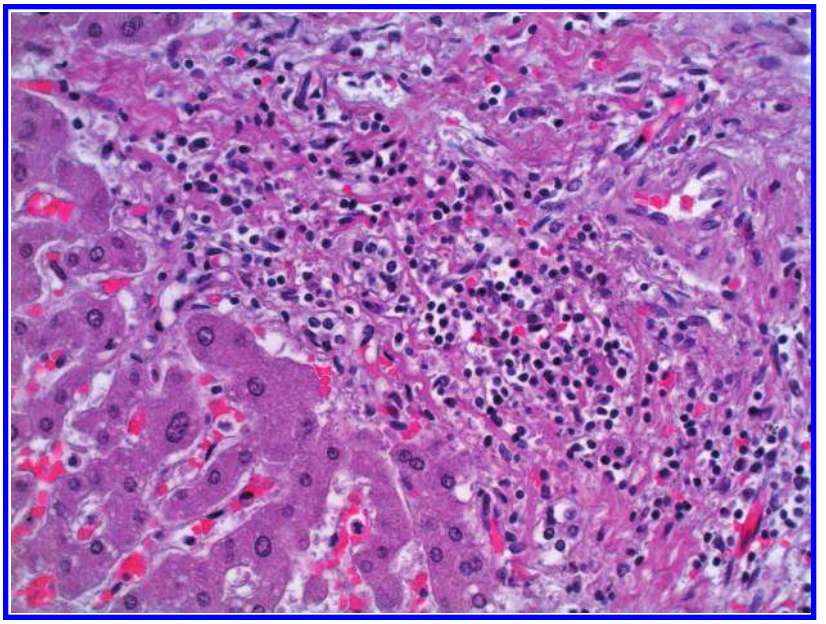


Figure 1.82 Cellular infiltrates are often seen within the portal triads. They may represent a reaction to drug adulterants or, more likely, hepatitis C, which was probably not diagnosed in life. Microsteatosis is present even more frequently, but the mechanism is not known, nor is the significance. Neither finding is diagnostic for cocaine abuse, but their presence should suggest that possibility.

et al., 2008), though whether by cocaine or another drug abused simultaneously is impossible to say (Figure 1.82). A 2013 report described a chronic cocaine abuser with chronic hepatitis leading to marked liver fibrosis (Payance et al., 2013), but such reports are extremely rare when compared to reports of the same lesions secondary to designer amphetamine abuse.

Several different mechanisms of toxicity are possible and quite probably dose dependent. Humans metabolize cocaine via a butyrylcholinesterase (mice and other experimental models possess both the butyrylcholinesterase and carboxylesterase). In 2008, a "knockout" mouse lacking butyrylcholinesterase was produced. After 7 days of intraperitoneal cocaine injections, a distinct combination of hepatocellular changes was observed: the cells were enlarged and showed considerable cytoplasmic granulation and vacuolization. The changes were confined mainly to centrilobular locations. In addition, nuclear pyknosis and regeneration with mitotic figures were present, and lymphocytic infiltrates were consistently seen adjacent to the central veins (Duysen et al., 2008).

Hepatotoxicity may also be caused by the generation of free oxygen species. These are generated by cytochrome P450 and flavin adenine dinucleotide containing monooxygenase (Boelsterli et al.,1993). *N*-demethylation to norcocaine (which, in the absence of alcohol, is only formed in small amounts by humans) leads to the formation of *N*-hydroxynorcocaine and norcocaine nitroxide (Bornheim, 1998). And then in turn to both cocaine itself and norcocaine. While normally metabolized by CYP3A4, three forms of polymorphic butyrylcholinesterase can also perform the same action, and do so with more efficiency, especially with norcocaine. This new observation might have some

significance in cases of massive cocaine overdose, or when a patient urgently needs to go to surgery (Zhan et al., 2014). It has also been proposed, though hardly proven, that redox cycling between the oxidation of *N*-hydroxynorcocaine to norcocaine nitroxide ultimately leads to membrane damage and cell death (Bornheim, 1998). Cells are protected from the damaging effects of free radical oxygen by superoxide dismutase conversion to peroxide.

Oxidative cocaine metabolism plays a very minor role in humans, and in most controlled human studies, the amount of norcocaine formed has been found to be quite small. In a recently published study of 18 healthy volunteers given a 150 mg/70 kg dose of subcutaneous cocaine, concentrations of cocaine and its major metabolites were in the hundreds of ng/mL, while that of norcocaine (and all of the other minor metabolites) was less than 18 ng/mL (Kolbrich et al., 2006). This observation cast some doubt on the role that ROS actually play in human, cocaine-induced, liver disease. When hepatic toxicity does occur in humans, it is usually manifested only as enzyme elevation and, even then, elevations in liver enzymes tend not to be impressive (Cantilena et al., 2007).

When fatal cases occur, they usually involve polydrug abusers, so it is not surprising that the pattern of damage observed seems to vary from report to report. Because most drug abusers use multiple drugs that can induce, or block, the P450 system (Bornheim, 1998), different patterns of injury may be the result. There are still no case reports or controlled clinical series suggesting that liver disease is a regular occurrence among cocaine abusers.

It has also been suggested that hepatic injury could be secondary to cocaethylene production. This compound is produced in the liver by transesterification of cocaine, but only in the presence of large amounts of ethanol (Kanel et al., 1990). In animal experiments, cocaethylene is nearly as toxic as cocaine itself and, when experimental animals are simultaneously treated with ethanol and cocaine, tissue necrosis, presumably secondary to lipid peroxidation, is much worse than when the animals are treated with cocaine alone (Figure 1.83) (Odeleye et al., 1993). Cocaethylene given to mice produces dose-dependent hepatic zone 2 (midlobular) necrosis. Pretreatment with P450 inducers makes the necrosis worse and shifts the zone of necrosis to zone 1 in the periphery. Treatment with inhibitors, such as cimetidine, reduces toxicity. This is essentially the same pattern seen in mice given cocaine, suggesting that both cocaine and cocaethylene share common mechanisms of toxicity (Roberts et al., 1992). Nonetheless, cocaethylene liver damage in humans has never been reported, and it is hard to say how it could be identified, given that cocaine and alcohol would also be present. Nonetheless, since cocaethylene was discovered, most papers on the subject ritually conclude that cocaethylene and norcocaine are neurotoxic (Farooq et al., 2009). That may well be the case in animal models but it has yet to be a significant clinical issue in humans.

There is some evidence that cocaine may interact with ketamine to cause illness, at least in experimental animals. Animal studies have shown that pretreatment of mice with ketamine (100 mg/kg) produced a 76-fold increase in serum alanine aminotransferase activity level and a 260-fold rise in those mice pretreated with 80 mg/kg ketamine for 4 days. This strongly suggests that ketamine can induce multiple forms of P450 in rat liver microsomes, thereby increasing the liver toxicity of both CCl_4 and cocaine, increasing liver damage. Other studies have shown that ketamine in some way prevents tissue uptake, which might explain the observed higher plasma concentrations (Roberts et al., 1992). In spite of these theoretical considerations, and in spite of the fact that both drugs are used together with some regularity, there have been no reported deaths.

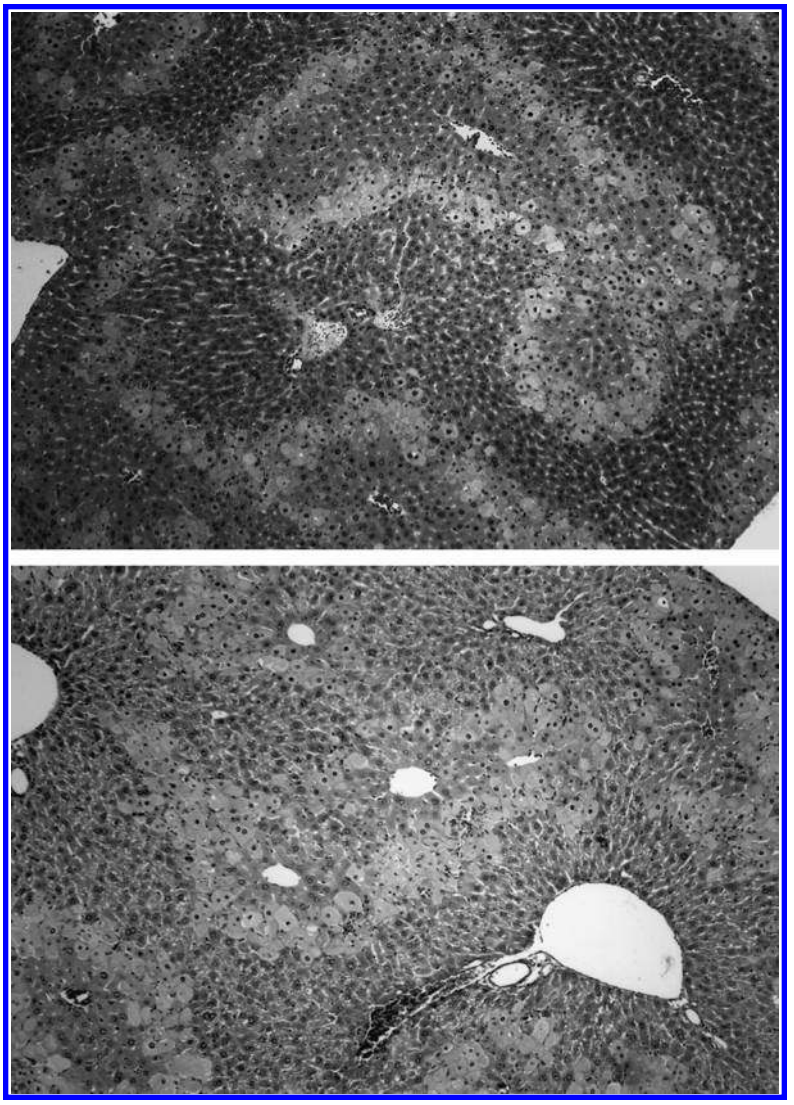


Figure 1.83 Effects of cocaine and cocaethylene on the rat liver. The upper microphotograph is from a rat treated with cocaine; the lower one is from a rat treated with cocaethylene. The patterns of injury are the same. (Courtesy of Stephen M. Roberts, Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL.)

1.23 Analytic and Forensic Toxicology

1.23.1 Bioanalytic Considerations

Cocaine hydrochloride is relatively stable as a pure substance; however, once cocaine has been dissolved in solution or is taken up by biological material, loss of cocaine is fairly rapid (McCurdy et al., 1989; Klingmann et al., 2001). The use of collection tubes with sodium fluoride substantially reduces loss of cocaine when stored at ambient temperature, although after 2 days, a loss of 10% is still likely (Brogan et al., 1992). Complete loss of cocaine occurs when blood containing 100 mg sodium fluoride and 20 mg potassium oxalate cocaine is stored at 4°C for 1 year (Baselt et al., 1993).

Cocaine is converted to BZE in living persons, but in the deceased, much of the drop in cocaine concentrations occurs because cocaine is converted to EME, even after death (Isenschmid et al., 1992). BZE can even be detected in serum samples stored at room temperature (Hilal et al., 2009).

Cocaine and BZE leach readily into formalin solutions used to fix tissues. In buffered formalin (pH 7.4), cocaine is hydrolyzed to BZE with a pseudo-first-order reaction rate and a half-life of approximately 7 days, whereas in unbuffered formalin (pH approximately 3.5), it is relatively stable over a period of 30 days (Viel et al., 2009).

BZE is stable in frozen specimens but not those stored refrigerated or at higher temperatures; hence, the amount of BZE and EME measured in urine will vary depending on how the specimen is stored. It is also a function of the preservative used (Toennes and Kauert, 2001).

EME is stable for as long as 3 years in urine samples with pH ranges of 3–5, but at pH 9, 100% will have disappeared in 30 days (Vasiliades, 1993). Under alkaline conditions, EME will hydrolyze to form ecgonine and thus will not be detected at all. Most postmortem urine samples contain more BZE than EME (Clark and Hajar, 1987), but changes in sample acidity can also lead to artificially high ratios of EME to BZE in postmortem blood samples.

In life, circulating EME is rapidly converted to ecgonine, and EME levels generally remain quite low. After death, however, anaerobic metabolism continues and conditions are not suitable for spontaneous hydrolysis, so the conversion of cocaine to BZE and EME to ecgonine ceases. At the same time, enzyme-mediated hydrolysis continues, albeit at a slower rate, and EME continues to accumulate. The resultant high EME levels and relatively low BZE levels will thus provide a misleading picture of the situation before death (Logan and Peterson, 1994).

Methylecgonine (MEG) or AEME is a marker of crack cocaine use but is less stable and requires storage of plasma at −80°C (Fandino et al., 2002). Similarly, unless it is collected with NaF preservative, it undergoes breakdown to cocaine (Fandino et al., 2002).

Cocaethylene is not produced postmortem even in the presence of ethanol and appears to be more stable than cocaine itself (Moriya and Hashimoto, 1996).

It is recommended that collections occur in tubes containing at least 1% fluoride/oxalate and that samples are frozen as soon as possible. Analysis should occur as soon as practicable.

1.23.2 Preferred Analytic Methods

The detection of cocaine in biological fluids is generally not difficult for analysts since concentrations are generally relatively high; however, it is unstable and has a complicated metabolism with numerous metabolites and breakdown products.

1.23.2.1 Screening Methods

A host of commercial immunoassay kits are available. These are generally quite good at detecting the presence of cocaine and its metabolites since there is little cross-reactivity with other substances. In urine, typical screening cutoffs applied are 300 ng/mL for BZE. ELISA-based testing in blood has also used a screening cutoff of 300 ng/mL (Moore et al., 1999). In oral fluid screening, cutoffs are less developed since it is still not entirely clear what the best cutoff should be; these have varied from 3 to at least 50 ng/mL (Drummer, 2007;

Crooks and Brown, 2010). In hair, screening cutoffs are far lower since concentrations are much lower. The Society of Hair Testing recommends a cutoff of 0.5 ng/mg (Barroso et al., 2010).

In most of these cases, applications are geared toward workplace testing or testing drivers suspected of using drugs and are directed largely at the detection of the BZE metabolite. In other forensic cases, such as some criminal cases, lower threshold limits will be useful to prove exposure. In these situations immunoassays may not be sufficiently sensitive to detect much lower concentrations unless prior extraction has occurred and/or a chromatographic method has been used.

Recent applications of time-of-flight mass spectrometry (TOF-MS) and LC–MS methods have been published to screen biological samples for cocaine and metabolites (Pesce et al., 2010). These are sensitive and are much more specific screening methods. They have the real advantage of being able to screen for a large number of substances and relevant metabolites in one assay.

1.23.2.2 Confirmatory Methods

Presumptive immunoassay results or other screening tests must be confirmed. Mass spectrometry is the gold standard—either GC–MS or LC–MS in their various configurations. Numerous methods have been published. In the main, the methods work satisfactorily with variations in the type of extraction method used, the specimen used, the range of metabolites that can also be detected, and of course the limits of detection and quantification (Barroso et al., 2009). GC–MS methods require derivatization since BZE does not chromatograph easily underivatized (Paul et al., 2005); however, AEME can be produced at the injection port if the temperature is too high (see Section 1.5.4).

As with other drugs of abuse, LC–MS/MS is beginning to dominate laboratory instrumentation since it can detect most, if not all, of the metabolites without derivatization and also confirm other drugs present in the sample (Barroso et al., 2009; Concheiro et al., 2009; Shakleya et al., 2010). These include cocaethylene, BZE, EME, and the marker of smoking cocaine, AEME.

1.23.3 Tissue Concentrations, Analytical Considerations

Finally, it should be noted that segmental hair analysis may be used to demonstrate external contamination in postmortem cases; however, standard decontamination procedures do not completely remove external contamination in postmortem material. The use of a single hair for analysis is to be discouraged, although homogenous segmental analyses can be probably be used to discriminate between external contamination and long-term exposure, but with considerable caution, because metabolites can also be present in putrefactive material (Kintz, 2012b).

1.23.3.1 Heart

This finding suggests that toxic levels of NE may persist in the heart for some time after the cocaine has been cleared. It may also explain the typical patterns of catecholamine-induced necrosis seen in the hearts of some abusers. The relatively high uptake by the heart, in spite of the rapid rate at which cocaine is cleared, makes it possible that cocaine could be detected in myocardium at autopsy, especially in chronic users or those who have ingested large amounts of drug.

1.23.3.2 Kidneys

In recent human PET studies, both ^{11}C-*d*- and *l*-methamphetamines showed rapid uptake and clearance from the heart, lungs, and spleen, with ^{11}C being retained slightly longer in the spleen than in the heart and lungs. In contrast, both enantiomers showed high uptake and relatively slow clearance of ^{11}C from the kidneys and the liver. The rank-order half-time for clearance from peak uptake for both enantiomers was lung ≫ heart > spleen ≫ kidneys ≫ liver (Fowler et al., 2007).

1.24 Pregnancy Interactions

In the early 1990s, studies based on either meconium or hair testing were first published. The results of these studies indicated that prevalence rates for maternal cocaine use at inner-city hospitals fell in the 12%–20% range (Forman et al., 1992). Results of more recent studies suggest that the real prevalence of maternal cocaine abuse is much lower. In a pilot study to determine the local prevalence of maternal drug misuse, cocaine was detected in the meconium of 2.75% of 400 infants tested (Williamson et al., 2006). In a similar, but much larger study from Spain, the rate was only 1.8% (Pichini et al., 2005). Before a fetal effect can be attributed to cocaine use, other possible etiologies must be excluded. The results of a recent study from a large Canadian pediatric center strongly suggest that even attempting to exclude other causes may not be worth the effort. The researchers analyzed nearly 1400 meconium samples and found that a positive meconium test result for opioids was associated with positive results for cocaine, oxycodone, methadone, benzodiazepines, and fatty acid ethyl esters (alcohol) (Moller et al., 2010).

Newborns exposed to drugs in utero can suffer from a varying degree of transient, usually benign, withdrawal symptoms a few days after birth. Cocaine and other stimulants seem to present little problem, but mothers addicted to opiates often deliver children who undergo full-blown withdrawal syndrome (Blaser et al., 2008) and the mothers are at increased risk for placental abruption (Ortigosa Gomez et al., 2011).

Prospective studies have compared cocaine-exposed with non-cocaine-exposed infants and toddlers with respect to anthropometric growth, infant neurobehavior, visual and auditory function, cognition, motor development, and language development. Now that scientists have collected a substantial body of controlled scientific evidence relating to the use of cocaine in pregnancy, it is clear that the much anticipated epidemic of "crack babies" never materialized. Evidence now accumulating from well-designed prospective investigations has shown that in the majority of prenatally exposed infants, the sequelae have been far less significant than anticipated. Prenatal cocaine exposure appears to be associated with statistically significant but extremely subtle decrements in neurobehavioral, cognitive, and language function (Bandstra et al., 2010). Even if such decrements can be demonstrated, establishing causality is nearly impossible. Such was always the case, but now that nearly the entire U.S. cocaine supply is contaminated with levamisole/aminorex, given the lack of pathognomonic lesions, indeed, the complete lack of knowledge about either drug's effect on the fetus, it may be impossible to attribute causality, especially when viewed in the context of other exposures and the caregiving environment, not to mention social status, education, and finances.

According to data that are now more than 20 years old, which may or may not be relevant to the situation today, women who use cocaine during pregnancy are likely to be

older (Richardson and Day, 1994), less likely to have sought prenatal care (Cherukuri et al., 1988), more likely to be malnourished (Knight et al., 1994), and more likely to be suffering from HIV infection, syphilis, and hepatitis (Ellis et al., 1993). They are also more likely to be cigarette smokers, the apparent explanation for the lower birth weight of children born to cocaine-using mothers (Shiono et al., 1995). Multicenter trials have confirmed that low birth weight, preterm birth, and intrauterine growth restriction were all more common among children whose mothers were cocaine users but that the effects exerted by tobacco were greater than those exerted by cocaine (Bada et al., 2005).

Cocaine also stimulates human myometrial contraction, both in vitro and in vivo. Strips of uterus obtained at the time of Cesarean section contract much more forcefully when they are exposed to modest concentrations of cocaine (Monga et al., 1993). This increased force of contraction is mediated both by α-adrenergic stimulation and other factors that have yet to be identified (Hurd et al., 1998). Cocaine use during pregnancy is also associated with downregulation of myometrial β-adrenergic receptors, a change that could inhibit uterine relaxation and speed labor (Smith et al., 1995; Wang et al., 1996).

Cocaine does interact with the placenta (Prasad et al., 1994; Ganapathy et al., 1999). Since cocaine inhibits the serotonin transporter, NE transporter, and dopamine transporter, inhibition can occur in the pregnant mother as well as in the fetus. It had been thought that the placenta did not play any direct role in the in cocaine exposure except that it allowed the passage of these drugs from maternal circulation into fetal circulation. It is now known that the placenta expresses both the serotonin transporter and the NE transporter on the maternal-facing side of the syncytiotrophoblast, exposing it to the inhibitory actions of cocaine and amphetamines. Inhibition of these transporters in the placenta could lead to elevation of serotonin and NE in the intervillous space that may cause uterine contraction and vasoconstriction, resulting in premature delivery, decreased placental blood flow, and intrauterine growth retardation. Since the placental serotonin transporter and NE transporter are also inhibited by many antidepressants, therapeutic use of these drugs in pregnant women may have similar detrimental effects on placental function and fetal growth and development (Ganapathy, 2011).

Histopathologic studies of either the cocaine-exposed fetus or placenta are rare. Detailed examination of placentas from 13 pregnancies with cocaine-related complications failed to demonstrate any specific changes (Gilbert et al., 1990). Vascular changes have, however, been documented in the human fetus by noninvasive means. Doppler studies have shown renal artery vasoconstriction and a simultaneous decrease in urine output (Mitra et al., 2000). Neonatal myocardial infarction and reversible myocardial calcification have both been described (Bulbul et al., 1994; Yap et al., 1994). All of these abnormalities could be explained by exposure of the fetal heart to high circulating levels of catecholamines in utero, but it is difficult to draw any firm conclusion based solely on isolated clinical and anecdotal reports, especially in the virtual absence of histopathologic studies.

There are several other possibilities that might account for the observed changes. Death might be due to cocaine-induced neurologic dysfunction, which is well documented in animal and in vitro studies (Azmitia, 2001). Heart rate variability, an indicator of autonomic stability and predictor for sudden death, is decreased in infants exposed to cocaine (Mehta et al., 2001). Another possible explanation might be hereditary channelopathy. It is believed that approximately 10% of all SIDS cases may stem from potentially lethal cardiac

channelopathies, with approximately half of channelopathy SIDS involving the Na(V)1.5 cardiac sodium channel. Recently, Na(V) beta subunits have been implicated in various cardiac arrhythmias. Thus, the four genes encoding Na(V) beta subunits within the normal channel represent plausible candidate genes for SIDS (Wang et al., 2007; Tan et al., 2010), and this estimate fails to account for the fact that cocaine binds to the hERG potassium channel as well (Karle and Kiehn, 2002; Berul and Perry, 2007; Karch, 2007; Risgaard et al., 2014).

1.25 When Is Cocaine the Cause of Death?

It is distressing to see how often deaths caused by cocaine are misclassified. Misconceptions about cocaine-related deaths persist, partly because death certification practices are not standardized and partly because there is no reproducible method by which to make the decision. Open any pathology or toxicology text at random and read the section on drug death determination; they all say the same thing, namely, that the process involves three separate elements that the pathologist (not the toxicologist, and not the death investigator) must integrate in order to reach an equitable, defensible, decision as to cause of death (Figure 1.84).

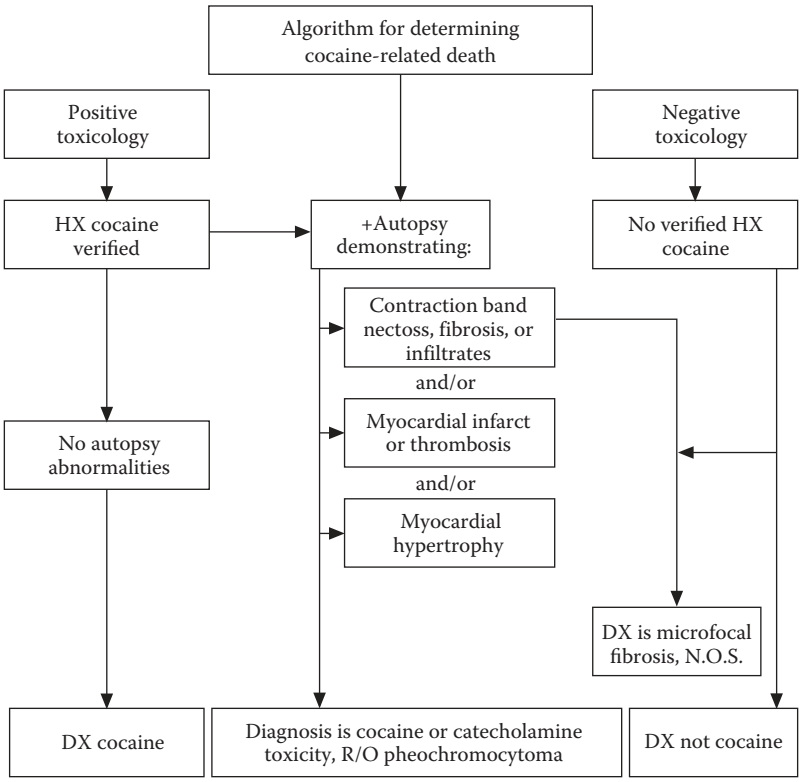


Figure 1.84 This is a simplified schematic outlining the convergent pathways that can lead to sudden death in cocaine abusers. Except in the case of massive overdose, cocaine-related deaths are always multifactorial.

The three elements are (1) history and scene investigation, (2) complete autopsy findings, and (3) toxicology findings. Similar statements are repeated by virtually every text in print. Where the books fail is that they offer absolutely no guidance on how to integrate the three elements. The system itself fails because death scene investigation (including perusal of all the medical records) is rarely, if ever, complete. It also fails because the autopsy is always incomplete, and so is the toxicologic investigation. Even if all three elements were performed to a high standard, the pathologist would still be at a loss as to how to weigh and integrate the findings of the three components.

In many large medical examiners' offices, it is now common practice to collect and store tissue, but not examine it microscopically. If limited toxicologic testing reveals no obvious cause of death, and only then, the microscopic portion of the autopsy will be completed. The results can be inaccurate if not catastrophic. Consider the example of a young cocaine abuser who dies from smoldering myocarditis that has been present for many weeks. If microscopic examination is not performed, or, even worse, if no autopsy was performed except to draw blood for toxicology testing with "a blind stick," even if the level of cocaine present is trivial, there is a very good chance that death will be attributed to the drug.

Even if the pathologist does a very thorough autopsy, including microscopic examination of many tissues, the autopsy is not complete. What if the decedent carries a genetic mutation that made death more likely to occur? Then the cause of death may very well be missed. In a 2014 study of 245 U.S. college athletes who had died suddenly, with no abnormalities detected at autopsy, nearly a third of the individuals had fully normal autopsies. At about the same time, researchers in Denmark reviewed nearly 439 cases where the decedent was aged 1–49 years (Bjarke et al., 201). As might be expected, nearly 70% of the deaths were the result of coronary artery disease. But in 135 of the U.S. cases, complete autopsy disclosed no anatomic abnormality, very strongly suggesting an underlying mutation (Maron et al., 2014).

The pathologist will never know the key mutation is present because, except for extraordinary circumstances, DNA resequencing is still not routinely performed at medical examiner offices. Fortunately, this is a rapidly changing situation. Advances in DNA technology (most notably "NextGen" sequencing) mean that, in the not very distant future, medical examiner offices will be able to screen for a vast array of genes, including those sometimes responsible for sudden death. These discoveries create another problem that should be extremely obvious. Suppose a person with a heritable cardiac channelopathy is found dead and a modest amount of BZE is found in autopsy blood. For many, the temptation to classify the death as drug related may be too strong to resist if they are unaware that a potentially lethal mutation is present.

Similar problems apply to the toxicologic component of death investigation. Often, there simply is no money to enable measurement of drug metabolites, and without knowing how much of which metabolites are present, there is no way to interpret the amount of cocaine measured. Is it high because the sample came from the wrong part of the body, or is it falsely elevated because the body is decomposed? The fewer data generated, the fewer data there are to interpret and the more likely misdiagnosis is to occur.

The mere presence of cocaine does not prove it was the cause of death or even the cause of toxicity. Drug use is pervasive in most societies and postmortem testing frequently reveals the presence of low levels of cocaine and their metabolites. The existence of these compounds only proves environmental exposure or, at most, use of cocaine within the last week or so of life. There is convincing evidence of prolonged cocaine excretion in the

urine of chronic users (Weiss and Gawin, 1988; Cone and Weddington, 1989; Burke et al., 1990; Preston et al., 2002). Cocaine is stored in reservoirs such as skin or fat (Levisky et al., 2000). Very low concentrations of cocaine or cocaine metabolites measured in postmortem samples are almost certainly the result of drug released from these reservoirs. The greater the steady-state volume of distribution (V_{ss}) of a given drug, and the V_{ss} for cocaine is fairly high, the more likely it is to be present in fat and other deep tissue stores (Flanagan, 1998; Drummer et al., 2004; Ferner, 2008).

Nor does the situation work very well in reverse. A very high cocaine concentration may have nothing to do with death. Howell and Ezell described the case of a man, looking and behaving normally, who had nonetheless been using cocaine all day. He died from a gunshot wound to the head. Blood obtained at autopsy had a cocaine concentration of 35,000 ng/mL (Howell and Ezell, 1990).

In the absence of any confirmatory histopathologic changes, cocaine levels of less than 50 ng/mL should not be deemed the cause of death. However, if extensive myocardial remodeling is obvious (hypertrophy, microfocal fibrosis, and microvascular disease), then cocaine may well be the cause of death in a cocaine abuser, even when blood cocaine is not measurable. To ensure the correct diagnosis, physical and laboratory findings must be integrated with information from detailed case histories and meticulous scene investigations, but we have already seen the limitations of that approach.

The unpleasant truth is that there is no reliable methodology by which an isolated postmortem measurement can be relied upon to predict antemortem cocaine concentrations. The half-life of cocaine varies greatly from individual to individual, while the V_{ss} has been measured for only one metabolite (BZE; roughly 1.0 L/kg—less than one-half that of cocaine) (Ambre et al., 1991). Because the V_{ss} of cocaine is so much greater than its metabolite, it is not legitimate to argue that, just because cocaine concentrations are high and metabolite concentrations are low in postmortem blood, ingestion occurred in close proximity to the time of death. However, such conclusions are warranted if brain is the testing matrix. BZE does not cross the BBB but cocaine crosses freely; any BZE detected in the brain was produced in the brain. It follows that very high cocaine/BZE ratios suggest remote use, while low ratios suggest just the opposite (Spiehler and Reed, 1985; Bertol et al., 2008).

The rapid emergence of tolerance explains why cocaine-related deaths are not dose related. Blood levels of over 5000 ng/mL may be present as an incidental finding. No upper concentration limit can be guaranteed fatal and, in chronic users with abnormal hearts, no lower concentration limit can be guaranteed safe. Anatomic alterations resulting from chronic cocaine use, in both heart and brain, may persist indefinitely, even after drug use is discontinued. These changes may be the cause of death, even when no cocaine or metabolite is detectable.

In some cases, postmortem blood concentrations can reasonably be used to identify those deaths resulting from acute toxicity and those due to chronic toxicity. The very first dose of cocaine might produce myocardial infarction from coronary spasm, particularly if significant underlying coronary artery disease is present. Cocaine-induced rises in blood pressure may lead to the rupture of a preexisting berry aneurysm or arteriovenous malformation, which explains why most strokes in cocaine users are hemorrhagic (see Section 1.17.6). Cardiac standstill can be another manifestation of acute toxicity but usually only at the very high blood concentrations (>20 mg/L) seen in drug smugglers with massive amounts of cocaine sequestered in their bowels. All local anesthetics have toxic effects on

the myocardium and can cause marked depression of cardiac output (Rhee et al., 1990), leading to infarction or asystolic arrest secondary to ion channel blockade (Nademanee 1992; Guo et al., 2006; Ma et al., 2006).

Chronic cocaine abuse initiates a type of myocardial remodeling that favors sudden cardiac death. Cocaine activates calcium/CMKII and causes cardiomyocyte hypertrophy, as well as elevating intracytosolic calcium—two actions that favor arrhythmic sudden death (Swaminathan and Anderson, 2011). Part of the remodeling process, whether due to cocaine or hypertension, involves alterations in sodium and potassium conductance channels that also favor sudden death (Guo et al., 2006; Ma et al., 2006).

The degree of myocardial hypertrophy seen in cocaine users, while highly significant, is nonetheless modest (less than 10% above predicted weight). Because the increase is small, it is likely to go unrecognized. The only way to make the diagnosis is by comparing the heart weight of the deceased to a standard nomogram (Kitzman et al., 1988) (see Section 1.11.2.1). Myocardial hypertrophy, taken in isolation, need not increase the chances of sudden death. However, concentric enlarged ventricular myocardium is relatively underperfused. Eccentric hypertrophy, of the variety seen in athletes, is not ischemic, and hypertrophy in athletes does not constitute an increased risk for sudden death (Maron and Pelliccia, 2006). The situation is far different from that seen in hypertensives and cocaine users (concentric hypertrophy), and the two situations are easily distinguishable. Obviously, if the heart is not examined microscopically, the pathologist will remain unaware of the underlying abnormalities.

In summary, the existence of a strong history of cocaine abuse in the presence of typical myocardial pathology strongly suggests that cocaine is the cause of death, even in the face of negative toxicology. Presuming that appropriate measures have been taken to rule out pheochromocytoma, there simply is no other diagnosis except, perhaps, chronic methamphetamine abuse. If typical pathologic findings are present, but toxicology and history are both negative, the diagnosis must be microfocal fibrosis or microvascular disease (perhaps syndrome X) (Picano, 1999), etiology not otherwise specified. In the event that additional information becomes available at a later date (e.g., exhumation with hair testing), the diagnosis can be revised. However, the mere presence of isolated myocardial alterations is not sufficient for diagnosis. Many states (California is an important exception) simply list the cause of death as "drug related." The designation covers all deaths not considered to be suicide, but rather unexpected complications of chronic drug usage. It is unnecessary, therefore, to attempt to make the artificial separation between toxicity and poisoning.

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Natural Stimulants

2

The plant-derived alkaloids in absinthe, caffeine, khat, and ephedra are capable of producing amphetamine-like effects.

Europeans stopped drinking absinthe at the turn of the nineteenth to twentieth century, but it is now making a comeback. The cessation of absinthe abuse in the early 1900s occurred almost entirely in step with the decline in the popularity of cocaine. Until 2007, the U.S. law prohibited the importation and sale of absinthe, but it can now be purchased in stores and over the Internet. The U.S. government rescinded its prohibition after nearly a century when it was discovered that the drink contained smaller amounts of potentially toxic thujones than had previously been supposed, although it is not entirely clear that the Drug Enforcement Agency agrees with this position. Absinthe is sold over the counter in European Union (EU) liquor stores. Alternatively, the key herbal ingredients contained in absinthe can be purchased as "health food supplements" and then added to vodka or pure ethanol.

Khat (qat) has been used in the Sub-Saharan Africa for more than 1000 years. Its use is now common in many parts of Africa and, until quite recently, legal in the United Kingdom, although there is an increasing movement to ban the plant. Its use is increasingly prohibited in Europe, not so much because chewing khat is associated with toxicity, but because distribution has fallen into the hands of organized crime. Khat is illegal in the United States because it contains restricted drugs. Khat usage has grown clandestinely in many areas of the United States, although the amounts consumed are relatively insignificant. The clandestine synthesis of derivatives of the cathinone found in khat has become a real problem in recent times. See Section 3.6 for further discussion of cathinone and khat.

Xanthine derivatives, especially caffeine, are the world's most widely consumed natural stimulants. Massively caffeinated drinks are very popular, both in the United States and Europe, particularly among the young.

The other important naturally occurring stimulant is ephedrine. A ruling by the U.S. Food and Drug Administration (FDA) caused it to be withdrawn from the herbal supplement market in 2004 (Thompson, 2004). The ruling was appealed and overturned, but now the FDA has won its appeal, and ephedra-containing products can no longer be sold (although ephedrine, its active ingredient, is still a legal drug used daily, on a large scale, by hospital anesthesiologists). Pharmaceutical grade ephedrine still remains, arguably (Lee et al., 2004), one of the preferred drugs for the treatment of anesthetic-related hypotension, especially in cardiac and obstetric surgery, where its use seems rarely to cause complications.

Outside of the hospital setting, ephedrine can be an abused drug: when smoked or injected intravenously, it is a potent stimulant. Ephedrine was widely abused in Asia during World War II (it was injected into kamikaze pilots) and was considered a major threat to public health in Japan during the 1950s (Karch, 2005). Sales of pseudoephedrine remain legal, but they are strictly controlled for fear that the drug will be used to

make methamphetamine. Nonetheless, pseudoephedrine continues to be used routinely as a cold medicine and decongestant. Methylephedrine, which is not sold in the United States, is a potent cough suppressant widely abused in Japan, and small amounts are occasionally found in ephedra plants. Except for caffeine and ephedrine, little is known about the human pharmacology, toxicology, and kinetics of these drugs, and knowledge of the pathologic changes associated with the chronic abuse of any of these agents remains very limited.

2.1 Absinthe

2.1.1 Incidence and Epidemiology

Absinthe is not even mentioned in any of the various iterations of Drug Abuse Warning Network (DAWN) reports (Table 2.1). The lack of DAWN mentions does not necessarily mean that no episodes of toxicity have occurred. It just means that standard urine toxicology screens, either in the emergency room or at autopsy, detect none of the ingredients contained in absinthe except for alcohol, which is present in high concentrations. The true incidence of toxicity, if any, remains unknown. Absinthe contains the same toxic monoterpene ketones, called thujones, present in the essential oils of eucalyptus, fennel, hyssop, pennyroyal, rosemary, sage, savin, tansy, thuja, and other popular herbal remedies. Published case reports have described seizures and even status epilepticus following the use of some of these oils (Burkhard et al., 1999). In the late 1990s, the EU voted to allow absinthe back on the market (Directive 88/388/EEC), provided that the total content of α- and β-thujone did not exceed a concentration of 35 mg/kg. Hundreds of different brands of absinthe, easily recognized by their pale green color (Figure 2.1), are now available in EU liquor stores and pubs. Analysis shows that the thujone content of all the commercial products remains below the mandated 35 mg/kg standard. More or less, the same restrictions apply in the United States. The total sales volume is not known, so even if cases of alleged toxicity were reported, there would be no way to determine the incidence of the problem.

Table 2.1 Physiochemical Properties and Pharmacokinetics of Thujone

(−)-α-Thujone	
Chemical name	(1S-(1-,4-,5-α))4-methyl-1-propan-2-yl-bicyclo[3.1.0] hexan-3-one bicyclo[3.1.0]hexan-3-one
Structure of beta form	CAS 471-15-8 MW 152.23
Synonyms and common names	Main monoterpene ketone in absinthe, found in *Artemisia absinthium* and *Artemisia pontica,* Roman wormwood
Metabolism	7- and 4-hydroxy-thujone, minor 2-hydroxy-thujone, and carvacrol, plus cysteine and glutathione conjugates
Key papers	Lachenmeier et al. (2006a,b); Abass et al. (2011)

Figure 2.1 Photograph of a glass of absinthe. (Retrieved from Wikipedia Commons November 18, 2014. Photo made available and published under the Creative Commons Attribution-ShareAlike 2.5 Generic license by user and author Eric Litton, original upload date March 18, 2006. License can be found here and no changes were made to the image: http://creativecommons.org/licenses/by-sa/2.5/legalcode.)

2.1.2 History

Absinthe is a French word for wormwood (*Artemisia absinthium* and *Artemisia pontica*), a perennial herb related to sage (*Salvia officinalis*). The Egyptians used wormwood for medical purposes. Pliny, in the first century A.D., recommended it as a vermifuge, and wormwood is mentioned in several of Shakespeare's plays (Karch, 2005). In *Romeo and Juliet*, Act I, Scene 3, the nurse discourses:

> For I had then laid wormwood to my dug,
> Sitting in the sun under the dove-house wall;
> My lord and you were then at Mantua:—
> Nay, I do bear a brain:— but, as I said,
> When it did taste the wormwood on the nipple
> Of my dug, and felt it bitter, pretty fool….

Late in the 1700s, techniques for the mass production of grain alcohol were introduced, and shortly afterward herb-based liqueurs appeared on the market. A French general practitioner named Courvet, working in Switzerland, is credited with first devising the formula

Figure 2.2 Advertisement for the Pernod brand of absinthe. The inventor of absinthe sold the formula to Henri-Louis Pernod in 1797.

(Lachine, 1967). In early 1797, he sold the formula to Henri-Louis Pernod, who ushered in the era of absinthe abuse when he opened his factory in Pontarlier. Pernod's liquor became immensely popular in France and throughout Europe (Figure 2.2) (Lachenmeier et al., 2006a). By the time of the Parisian Exposition Universelle in 1867, most boulevard cafes in Paris had their equivalent of a "happy hour," except that it was called the *heure verte* ("green hour") because of absinthe's green color.

Absinthe drinking became popular only a few years before Angelo Mariani started selling his coca-fortified wines, and the popularity of both coca and absinthe rose almost in parallel. French impressionist painters left an enduring record of just how popular absinthe was (Figures 2.3 and 2.4). During the 1860s and 1870s, Degas and Manet immortalized images of absinthe drinking. Toulouse-Lautrec painted van Gogh with a glass of absinthe (Morrant, 1993). Some even speculate that van Gogh's mental illness was related to his abuse of absinthe, either as a consequence of its direct toxicity (Hughes, 2005) or because it exacerbated an undiagnosed (and at the time not even recognized) case of acute intermittent porphyria (Bonkovsky et al., 1992).

Toulouse-Lautrec's painting of van Gogh was completed just 3 years after Freud published his infamous paper *Über Coca*. Baudelaire used both cocaine and absinthe, though he only wrote about the latter. Valentine Magnan studied the medical complications of both cocaine and absinthe (Magnan, 1874) and sounded warnings about the potential toxicity of both drugs. Just as American and European manufacturers of cocaine-containing patent medicines minimized the medical problems associated with cocaine use, so did the manufacturers of absinthe cordials.

Figure 2.3 Absinthe drinkers. These gentlemen were obviously intoxicated, but whether from the terpenes or the alcohol in their drinks is not entirely clear. Early evidence suggested that the active ingredients in this drink may have been very similar to those in marijuana, but this suggestion has been disproven. (From *Harper's Magazine*, April 1889.)

Figure 2.4 Absinthe abuse was common in the late 1800s. Individuals who were intoxicated with it were said to be in possession of the "green faire" illustrated here.

Public relations campaigns mounted by the absinthe makers and the coca wine industry were very similar. Manufacturers minimized the risks, and sales boomed. From 1875 to 1913, annual consumption of absinthe per French citizen increased by 1500% (Arnold, 1989). However, the success of the advertising campaigns was short lived, and, just 2 years before the Harrison Narcotic Act banned cocaine from patent medications in the United States, the French government passed legislation limiting the alcohol and absinthe content of commercial products. In 1915, the sale and manufacture of absinthe was banned entirely. Since the breakup of the former Soviet Union, absinthe production has resumed in several Eastern European countries, particularly the Czech Republic, although the EU

and now the United States have followed suit. Regulatory agencies in both Europe and the United States limit the β-thujone content. The results of periodic government monitoring suggest that the limits are being followed.

The thujone content of absinthe produced in the 1930s and product manufactured today has been measured and compared. The thujone content of the older samples was relatively low (mean 1.3 ± 1.6 mg/L, range 0–4.3 mg/L), but it is impossible to say whether this is the result of deterioration or the original manufacturing process (Lachenmeier et al., 2006b).

2.1.3 Manufacture

Absinthe is prepared either as a distillate of aromatic herbs or as a solution made by steeping the herbs in alcohol, so the physical characteristics of any particular sample will depend on the production techniques used by the manufacturer. It will also vary from batch to batch, particularly if the absinthe is home brewed or if the liqueur is made with components purchased from a "head shop." A fairly high concentration of alcohol is required to keep the various essential oils in solution. According to the older classifications, absinthe ordinaire contained 47.6% alcohol, absinthe demi-fine contained 68% alcohol, and premium grade, also known as absinthe Suisse, contained 80.66% ethanol (Vogt and Montagne, 1982). Some of the components, such as thujone, are found in other plants (sage, in particular) (Ishida et al., 1989; Loza-Tavera, 1999). It has been suggested that thujone concentrations might have been as high as 260 mg/L in the nineteenth century versions, but testing of product from that era has not shown levels even approaching that magnitude (Lachenmeier and Nathan-Maister, 2008). Either thujone deteriorates with time or, more likely, the colorimetric methods used were not that accurate.

The typical formula used in the 1890s to produce a premium-grade absinthe is given as follows (Fritsch, 1891). Kits for home production usually also contain star anise and use caramel and food dyes for coloring (Lachenmeier et al., 2006a).

Classic absinthe production formula is as follows:

1. Combine 2.5 kg wormwood + 5 kg, anise 5 kg, and 5 kg fennel with 91 L of 85% ethanol.
2. Allow mixture to sit for 12 h (the process is called maceration).
3. Add 45 L of water.
4. Distill to 95 L of colorless distillate.
5. Add coloration consisting of 1 kg Roman wormwood + 1 kg hyssop + 500 gm lemon balm.
6. Add water to a final volume of 100 L, yielding 74 proof absinthe.

2.1.4 Routes of Administration

Absinthe is only taken orally. Resultant blood concentrations of the individual herbal components are not known. The ethanol content can be exceedingly high, but whether or not the herbal components have any effects that would alter the rate of absorption or excretion has not been determined. The sale of absinthe in the United States is now legal, but even when it was not, human exposure still occurred because thujone diastereomers of the active ingredient were contained in 20 different approved food flavorings, as well as perfumes and fragrances, all allowed for sale within the United States. The best known of these products is Vicks VapoRub® (Hold et al., 2001).

2.1.5 General Pharmacology

Three different types of terpenoids are found in absinthe: thujones (α and β) (Figure 2.5), camphor, and pinenes. Thujone is a monoterpene composed of two isoprene units with the molecular formula of $C_{10}H_{16}$. Thujone has two stereoisomeric forms: (+)-3-thujone or α-thujone and (–)-3-thujone or β-thujone. Thujone is the principal terpene extracted from wormwood, and its structure was first published in 1900. Both the α and β forms of thujone are noncompetitive blockers of the γ-aminobutyric acid (GABA)-gated chloride channel, which explains their proconvulsant activity (Hold et al., 2000). The α form is also more likely to trigger episodes of porphyria in those with a genetic susceptibility (Bonkovsky et al., 1992).

There are no clinical studies, but metabolic studies in human liver microsomes show two major hydroxylated metabolites (7- and 4-hydroxy-thujone) and two minor (2-hydroxy-thujone and carvacrol) metabolites. Glutathione and cysteine conjugates were also produced (Abass et al., 2011). In animals, enzymatic reduction (possibly by a cytosolic ketone reductase) of thujone to thujol and neothujol has been demonstrated in rabbit, but not mouse, liver. Mouse liver cytochrome P450 (CYP) 3A4 rapidly converts thujone to 7-hydroxythujone, to smaller amounts of the 4-hydroxythujone diastereomer, and other hydroxythujones. Dehydrothujone is also formed. It is presumed that the various hydroxythujones then undergo conjugation and excretion. The CYP enzymes are largely controlled by CYP2A6 with a minor component catalyzed by 3A4 and 2B6. In animal toxicity studies, brain concentrations of 7-hydroxythujone metabolite are several times greater than those of thujone, suggesting that the metabolite may also be toxic (Hold et al., 2000).

In the absence of more definitive studies, it would be a mistake to dismiss the potential toxicity of all the other absinthe components. In addition to wormwood, absinthe also contains the essential oils of angelica, anise, marjoram, and calamus. Some of these other plants contain pharmacologically active compounds. Angelica (*Angelica archangelica* L., Umbelliferae) contains ferulic acid, which acts both as a cyclooxygenase and as a thromboxane A_2 synthetase inhibitor (Kuenzig et al., 1984; Lanhers et al., 1992).

Calamus (*Acorus calamus* L. var. *americanus* Wulff or *A. calamus* L. var. *vulgaris* L., Araceae) is a hallucinogen and a potential carcinogen (Vohora et al., 1990). The β-asarone in calamus causes cancer in experimental animals but is found only in calamus grown in Europe and Asia; α-asarone, also found in calamus, is similar in structure to reserpine. The structures of both α- and β-asarone bear a strong resemblance to that of ecstasy (3,4-methylenedioxymethamphetamine) (Vohora et al., 1990). Asarones tend to decompose over

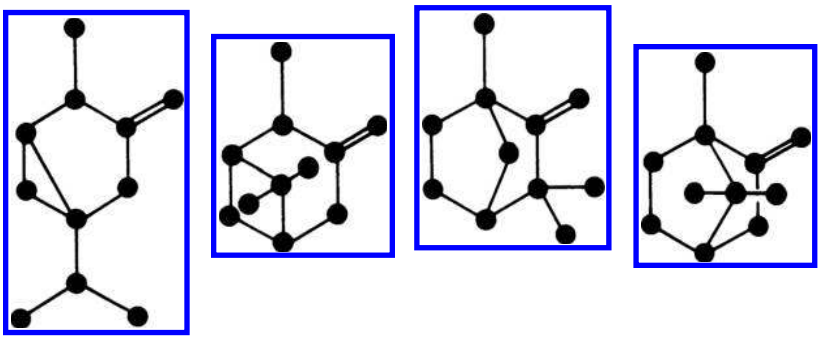


Figure 2.5 Terpenes. Absinthe contains many different compounds; thujone is the principal agent.

time, losing their psychoactive properties within a few months of harvesting. Once asarones have been dissolved in ethanol, their psychoactivity may be longer lasting. Calamus is still used as a "recreational" hallucinogen in the United States, although its popularity is somewhat limited by nausea that accompanies its use (Karch, 1999).

2.1.6 Tissue Concentrations

Tissue concentrations of thujone have never been measured, nor have blood and tissue concentrations for camphor-associated deaths ever been reported in humans.

2.1.7 Toxicity by Organ System

Relatively, little is known about the toxicity of the concoction, let alone the individual active components, thujone, camphor, pinene, or asarone and their likely effects when consumed. It is even unknown what other herbs and secret ingredients have been added; hence, it is difficult to say what, besides ethanol, absinthe drinkers are actually consuming and whether that amount is sufficient to cause toxicity. It seems likely that the amount of thujone actually required would be far less than the 30 mg/kg suggested as the fatal dose in the older literature (Amory, 1898). It is most likely that any toxicity is largely, if not exclusively, due to the ethanol in the absinthe itself.

A recent article suggests that the consumption of as much as 1 L of an alcoholic beverage containing thujone would result in an intake well below the "no observed effects level" of 5 mg/kg body weight (Lachenmeier and Nathan-Maister, 2008).

Only one-tenth of a liter of a preparation containing 50% ethanol would raise the blood alcohol content by about 0.1%, which is sufficient for alcohol to exert a significant central nervous system (CNS) depressant effect. At this concentration, ethanol causes cognitive and psychomotor dysfunctions, and long-term abuse leads to numerals of abnormalities involving the brain, liver, heart, metabolic and gastrointestinal systems that are much more likely to be a consequence of absinthe drinking.

The molecular structures of thujone and tetrahydrocannabinol are in some respects very similar, and in the past, it had been suggested that the psychological effects ("high") experienced by absinthe drinkers were similar to marijuana intoxication (del Castillo et al., 1975). More recent studies have shown that not to be the case. Thujone is the only component of wormwood oil that has any affinity at all for the CB_1 cannabinoid receptor and then only in massive doses far exceeding those that could conceivably be encountered in any absinthe drinker (Meschler and Howlett, 1999). A more plausible explanation for the intoxicant effects of absinthe may have to do with the recent finding that thujones are noncompetitive blockers of the GABA-gated chloride channels (Hold et al., 2000). On the other hand, the ethanol content of these beverages is so high that the neurologic symptoms observed are likely to be ethanol related. Drugs in this category are known to cause seizures by disrupting calcium homeostasis within neurons (Weiergraber et al., 2006).

Questions about absinthe-related impairment are not uncommon within the E.U., where there have actually been controlled studies assessing the impact of absinthe on attention, performance, and mood. The administration of ethanol containing a high concentration of thujone has a negative effect on attention performance. In controlled studies, subjects tended to direct their attention to signals in the central field of attention and to neglect peripheral signals and their reaction times increased significantly. However, when

ethanol was combined with low doses of absinthe, performance was actually better than when ethanol was consumed alone (Dettling et al., 2004).

Whether absinthe has any effect on the cardiovascular system is not known. One case report describes a 29-year-old absinthe-drinking man with no risk factors with a plasma ethanol concentration of 198 mg/dL, but otherwise negative toxicology, who developed Mobitz type I atrioventricular block that converted to a rapid junctional rhythm (Benezet-Mazuecos and de la Fuente, 2006). No similar cases have been reported. Stomach upset was a common complaint of absinthe drinkers in Paris during the late 1800s. Vincent van Gogh wrote several letters to his brother Theo complaining of stomach upset after bouts of absinthe drinking (Morrant, 1993). This appears to be a rare complaint among van Gogh's modern cousins.

2.2 Caffeine

2.2.1 Incidence

Caffeine is present in many different plants and goes by many different names depending on the plant source. It is sometimes called guaranine when extracted from the guarana plant, mateine when found in yerba mate, and theine when found in tea. The main sources of caffeine in consumed beverages are the beans of the coffee plant and the leaves of the tea and yerba plants. Because of their high caffeine content, guarana berries are the main sources of caffeine in commercial food products. Only small quantities of caffeine are found in cocoa, the kola nut, and the yaupon holly. Smaller amounts of caffeine can be detected in the beans, leaves, and fruit of over 60 different plants, where its primary purpose appears to be to act as a natural pesticide. No matter the name used, caffeine remains the most widely used stimulant in the world. Citizens of the United States, with an estimated consumption of more than 100,000 tons in 2002, are its largest consumers. According to testimony before the U.S. Senate, the coffee market is grossly oversupplied. Total global supply in 2002–2003 was forecast to reach 143.6 million bags, up to nearly 6% from the previous year, largely as a result of increasing production in Brazil. Coffee supplies have not reached this level since 1991–1992. This oversupply is not likely to lessen anytime soon (Lee, 2002).

The United Nations states that world tea production continues to increase at rates parallel to those of coffee. Since 2004, tea production has grown by 2% a year to reach an estimated 3.6 million tons. In China, tea output for 2004 approached 800,000 tons, as policy initiatives to promote production, passed years earlier, finally began to have an impact (Anon, 2005).

Estimates from many different sources suggest that formidable amounts of caffeine are also consumed in soft drinks, cold medications, and pain-relief formulas. In spite of caffeine's widespread use, neither the medical examiner's component nor the emergency room component of the most recent DAWN survey makes any mention of caffeine, suggesting that episodes of serious toxicity are very uncommon (Ball and Duchama, 2005).

Guarana, officially known as *Paullinia cupana*, is the primary source of caffeine added to commercial products, both in the United States and Europe. Unlike other sources of caffeine, guarana comes only from the Amazon, with Manaus as the exporting center. In Brazil, it is used mainly as a soft drink and has some traditional uses in folk medicine, where it is used to treat fever and diarrhea. The seeds contain 4%–5% caffeine as well as theophylline and theobromine (Carlson and Thompson, 1998), making it the richest source of caffeine.

Guarana production figures are sketchy, but, thanks to modern growing techniques, the average production is thought to be on the order of 1200 tons/year (Hernándo Bermejo and León, 1994). Yerba mate (Rioplatense Spanish) and erva mate (Portuguese) (*Ilex paraguariensis*) both belong to a species of holly (family Aquifoliaceae) that is native to subtropical South America, particularly northern Argentina, Paraguay, Uruguay, southern Brazil, and Bolivia. Steeping the dried leaves in hot water, rather than boiling water as with tea or coffee, prepares an infusion called mate. It is slightly less potent than coffee and said to be much gentler on the stomach. The flavor is grassy, but much stronger than green tea, to which it is generally compared (Dickel et al., 2007).

2.2.2 Epidemiology

Table 2.2 shows the caffeine content of some commonly consumed beverages and medications. The average American adult consumes 2.4 mg/kg/day of caffeine. Intake for children between the ages of 5 and 18 is thought to be half that amount. European consumption is said to be even higher: 3.5 mg/kg/day (Scientific Committee for Food, 1983). An average cup of coffee contains 40–100 mg of caffeine. The average American drinks two cups a day, the average European three. The caffeine content of cola drinks is lower, ranging from 30 to 65 mg/8 oz serving, but the caffeine intake of some cola drinkers is substantially higher than that of coffee or tea drinkers.

2.2.3 History

The origins of coffee drinking are a mystery. According to legend, the prior of a Muslim convent observed that goats eating beans from certain trees tended to stay up all night. He assumed that the beans were responsible and concluded that using the beans might help him and his followers stay awake during their long prayer vigils in the mosque. The prior brewed a beverage called *kahweh* and was said to have been quite pleased with the results. The first convincing evidence of coffee's widespread popularity is from the sixteenth century. In 1511, when a new Egyptian governor arrived in Mecca, he noticed people sitting around the mosques drinking coffee. He asked what they were doing, and he was told that they were drinking coffee to give them the energy they needed to pray all night.

The governor had his doubts about the propriety of the practice and convened a meeting of clerics and elders to discuss the subject of coffee drinking. The governor feared that coffee might be some sort of intoxicating agent and therefore, its use would be prohibited by the Koran. The assembly concluded that coffee was indeed an intoxicant and therefore, should be banned. Sales of coffee were prohibited and stocks were burned. Had the new governor bothered to check with his superiors, he would have found that the sultan of Cairo was an avid coffee drinker; the sultan promptly overruled the governor's decision, and coffee drinking in Mecca has been legal ever since.

Venetian traders introduced coffee to Europe. In London, the first coffee shop opened in 1652 and was located in St. Michael's Alley, Cornhill. Its owner, Pasqua Rosee, advertised extensively, making mostly medicinal claims for the drink. According to Rosee, coffee was "a very good help to the digestion…and makes you fit for business" (Thompson, 1928). In spite of Rosee's claims, coffee drinking was at first considered highly suspect by Europeans. Coffee drinkers were said to have a haggard appearance and to be "subject to fits of agitation and depression." Coffee drinking had been introduced into France 9 years

Table 2.2 Caffeine Content (in mg) of Some Common Beverages and Medications

Carbonated Beverages (12 oz Can)	
A&W Root Beer®	0
AMP, 8.4 ounces	74
Barq's Root Beer®	23.0
Coca-Cola®	64.7
Coca-Cola Classic®	34.0
Diet Coke®	45.6
Diet Mountain Dew®	55.0
Diet Sunkist Orange®	41.0
Dr. Pepper®	42–44
Diet Dr. Pepper®	54.2
Dunkin Donuts, 16 oz	143–206
Enviga, 12 ounces	100
Full Throttle, 16 oz	144
Generic instant coffee	27–173
Jolt®	71.2
Lipton Brisk, All Varieties®	9
Mellow Yellow®	52.8
Mountain Dew®	54.7
Mug Root Beer®	0
Nestea Sweet Iced Tea®	26.5
"No Name" formerly "Cocaine," 8.4 oz	280
Pepsi-Cola®	43.1
Pepsi One®	36–38
RC Cola®	33.7
Red Bull (8.2 oz)®	80.0
RockStar, 8.0 oz	80.0
Snapple Sweet Tea®	18.0
Sunkist Orange®	40.0
Tab	46.8
Tea Bags (Average Per 8 oz Cup)	
Black teas	40–120
Green teas	9–19
Coffee (Average Per 8 oz Cup)	
Instant	65–100
Electric percolator	80–135
Stove percolator	105
Drip	115–175
Starbuck's tall latte®	375
Starbucks grande® (470 mL)	>500
Starbucks® espresso decaffeinated (per short)	3–15.8
Starbucks® brewed decaffeinated (16 oz)	12–13.4
Starbucks Vanilla Latte 16 oz	15
Starbuck Coffee Ice	50–60
Cocoa	10–17

(*Continued*)

Table 2.2 (*Continued*) Caffeine Content (in mg) of Some Common Beverages and Medications

Medications	
Cafergot Tablet®	100.0
Darvon Compound 65®	32.4
Excedrin Extra Strength®	65.0
Fiorinal Capsules®	40.0
NoDoz Tablets®	100.0
NoDoz Maximum Strength®	200.0
Norgesic Tablets®	30.0
Common Prescription and Over-the-Counter Drugs Containing Caffeine	
Actamin Super®	65.4
Anacin Maximum Strength®	32.0
Anacin Tablets and Caplets®	32.0
Aspirin-Free Excedrin Caplets®	65.0
Headache pain relief	65.4
Cafergot suppositories (other names: Cafertrine®, Cafetrate®, Migergot®, Wigraine®)	100
Cafergot tablets (other names: Ercaf®, Ergo-Caff®, Gotamine®, Wigraine®)	100
Dristan Capsules®	16.0
Excedrin Caplets®	65.0
Excedrin Caplets Extra Strength®	65.0
Excedrin Extra Strength Caplets and Tablets®	65.0
Fiorinal with Codeine No. 3®	40
Goody's Extra Strength Tablets®	16.25
Goody's Headache Powder®	32.5
Midol Maximum Strength Caplets®	60
Midol for Cramps Maximum Strength Caplets®	32.4
Norgesic Forte, Norphadrine Forte®	60
Norgesic, Norphadrine Forte®	30
Triaminicin with Codeine Tablets®	30
Vanquish Caplets®	65.0
Vivarin®	200

Sources: Data for caffeine-containing beverages taken from Bunker and McWilliams (1979). Data on decaffeinated coffee from McCusker et al. (2006). Additional data from National Soft Drink Association and US Food and Drug Administration. Current labeling requirements do not require manufacturers to list caffeine content. Values may have changed. Data for medications taken from current PDR, WebMD, and the Cleveland Clinic (Anon, 2006). Additional data from Center for Science in the Public Interest.

Note: Caffeinated alcoholic beverages (CABs). CABs are premixed beverages that combine ethanol, caffeine, and other stimulants. The ethanol may be malt or distilled spirits based, and these beverages usually have higher ethanol content than beer (i.e., 5%–12% on average for CABs and 4%–5% for beer). The caffeine content in these beverages is usually not reported.

earlier, and, by 1690, 250 coffee houses were registered in France; by 1782, that number had risen to 1800. Some of the coffee houses were quite opulent, with marble tables and crystal chandeliers. Like the English, the French also had some doubts about the habit. Medical literature from that period contains reports both praising and condemning the effects of coffee. It was alleged that coffee caused inflammation of the liver and spleen and that it even caused kidney stones.

Suspicions that coffee drinking is unhealthy have never entirely disappeared. Even Virchow classified caffeine, along with ethanol, as an addictive substance. Lewin (1931), who generally thought that coffee drinking was a good thing, accepted reports of "delirium, vertigo, trembling, and even convulsions" as an occupational disease in coffee roasters. In modern times, epidemiologic investigations have focused on possible links between caffeine intake and myocardial infarction, sudden death, and fibrocystic disease and arrhythmias. Alleged links to cancer have never been proven (Stavric, 1988a), and the histopathologic changes associated with caffeine treatment in animals have never been confirmed in man (Strubelt et al., 1976). Interestingly, the same suspicions have never been entertained about other caffeine-containing beverages such as cocoa and certainly never about tea, even though both contain substantial amounts of caffeine. That may be because it has become increasingly apparent that tea consumption may actually protect against cancer (Hirose et al., 1999; Okabe et al., 1999).

During the 1990s, a great deal was written about the dangers of combining caffeine and ephedra/ephedrine in athletic supplements and the increased risk of such combinations for producing cardiovascular disease (Haller and Benowitz, 2000; Haller et al., 2004b). The caffeine concentrations in these products ranged from 40 to 200 mg (the equivalent of one-half to two cups of coffee) (Haller et al., 2004a). Since the beginning of the 2000s, there has been a virtual explosion of caffeine research into new areas, perhaps the best publicized being the apparent ability of caffeine consumption to modify or even reverse some of the changes associated with Parkinson's disease (Aguiar et al., 2006; Alisky, 2006; Deleu et al., 2006) and Alzheimer's disease (Dall'Igna et al., 2003). There has also been diminished interest in coffee consumption and coronary artery disease, as large epidemiologic studies have shown that no such relationship exists. The lack of correlation is attributed to the tolerance that rapidly emerges to caffeine's effects (Shi, 1997; Lopez-Garcia et al., 2006). However, data published in early 2008 strongly suggest that caffeine may exert deleterious effects on pregnant women (Savitz et al., 2008).

2.2.4 Chemical Constants and Physiochemical Properties

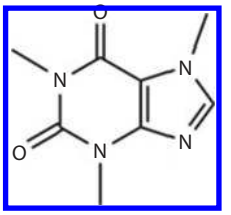
2.2.5 Sources

Depending on the type of bean, chemical extraction of roasted coffee beans yields from 8 to 20 mg of caffeine per gram of coffee (Zuskin et al., 1983). Teas made from the leaves of *Camellia sinensis* also contain caffeine. Herbal teas made by soaking other plant leaves, such as mint, in hot water do not contain caffeine, nor does it appear that they contain the antioxidants thought to prevent cancer, or any of the other recently discovered potential benefits associated with coffee and tea consumption. Green teas, such as gunpowder tea, are made from tea leaves that are heated immediately after they are picked and then rolled and crushed, thereby preserving the natural constituents of the leaves, including their color. Black teas, such as pekoe, are picked, allowed to dry, and then packaged. Fermentation partly digests some of the components of the leaf, giving black tea its reddish-brown color (Karch, 1999). The caffeine content of a cup of tea depends partly on which leaves are used and on how the leaves are brewed; however, the caffeine content of *Camellia sinensis* is not high enough to make extraction for other purposes worthwhile (Table 2.3).

Guarana seeds contain more caffeine (4%–5%) than any other plant and are the main source of commercial caffeine. In addition, the leaves contain large amounts of theobromine (Carlson and Thompson, 1998), a close relative of caffeine. Guarana

Table 2.3 Physiochemical Properties and Pharmacokinetics of Caffeine

Chemical name	1,3,7-trimethyl-1H-purine-2,6(3H,7H)-dione.
Structure of beta form	CAS 58-08-2. MW 194.19. V_d 0.5 L/kg. pK_a 0.8. Protein binding 17%–36%.
Synonyms and common names	3,7-trimethylxanthine, trimethylxanthine, theine, mateine, guaranine, methyltheobromine.
Pharmacokinetics	Bioavailability: Oral, 100%; inhalation, 60%. C_{max}: oral, 15–120 min. Half-life: 1. Healthy male adults, 2–8 h. 2. Women taking oral contraceptives, 6–10 h. 3. Pregnant women, 9–11 h. 4. In the presence of severe liver disease, 96 h. 5. Newborns, 30–80 h. 6. Premature, at birth, 65–102 h. 7. 3–4.5 months old, 14 h. 8. 5–6 months old, 3 h.
Metabolism	Metabolized by *N*-demethylation and oxidation of 8-carbon to uric acid derivatives; involving CYP1A2 isozyme.
Urine excretion	Caffeine 2%, with mostly 1-methyluric acid 22%, 1-methylxanthine 10%, 6-methyl uric acid 6,% paraxanthine 4%, theobromine 2.0%, and theophylline 1%. 5-Acetylamino-6-amino-3-methyl uracil is also excreted.
Interactions	Serotonin reuptake inhibitors, antiarrhythmic drugs such as mexiletine and propafenone, antipsychotics (clozapine), psoralens, and phenylpropanolamine all compete for CYP1A2 and have the potential to cause dangerous interactions.
Key papers	Bonati et al. (1982); Levy and Zylber-Katz (1983); Sanchez-Alcaraz et al. (1991); Magkos and Kavouras (2005); Zandvliet et al. (2005); Bchir et al. (2006); Rengelshausen et al. (2007).



also contains a number of tannins, some very similar to those found in tea (the same ones that are thought to provide beneficial antioxidant effects) (Morton, 1992). At least nine different antioxidants/tannins have been identified in the oil extracted from guarana; two of them, estragole and anethol (Benoni et al., 1996), are thought to be psychoactive. In Brazil, guarana is used to make an extremely popular carbonated soft drink. In laboratory studies, low concentrations (1.2 mg/mL) of guarana inhibit lipid peroxidation. No histopathologic changes secondary to guarana ingestion have been detected, even in animals treated with very large amounts (250–2000 mg/kg) of this drug (Mattei et al., 1998).

Two main varieties of the coffee plant are used for brewing coffee: robusta and arabica. As its name implies, the robusta plant is the hardier of the two, but at a price. Beverages produced from it do not have nearly the flavor of arabica beans, which is why

most robusta beans are used to make instant coffee or are used in the least expensive coffee blends. While robusta beans may be lacking in flavor, their caffeine content is about twice that of arabica.

The caffeine content of cocoa is substantially less than that of coffee. Cocoa trees only grow in a limited geographic area: 10° to the north and south of the equator. Accordingly, almost 70% of the world crop is grown in West Africa. The estimated annual world production is approximately 3,000,000 tons. Chocolate derived from cocoa contains a small amount of caffeine, and the limited stimulant effects possessed by this plant are mostly due to its content of theobromine and theophylline (Smit et al., 2004). Still, cocoa contains too little of these compounds for a reasonable serving to create effects in humans or at least none that are on par with coffee. A typical 28 g serving of a milk chocolate bar has about as much caffeine as a cup of decaffeinated coffee.

2.2.6 Routes of Administration

Most caffeine is consumed orally, either in beverages or in medications, especially those used to treat headache and migraine (Pradalier et al., 1985). When ingested orally, caffeine is rapidly absorbed and distributed throughout total body water (including the fetus) and reaches a peak plasma level between 30 and 75 min. Animal studies have demonstrated that the caffeine concentration in mouse brain is about 80% that of plasma (Kaplan et al., 1997) and that value probably also obtains in man. Plasma levels of caffeine after the consumption of up to about six cups of coffee per day generally ranged between 2 and 6 mg/L (Lelo et al., 1986). In another study, plasma caffeine levels peaked at about 3 mg/L after 4.2 mg/kg/day of caffeine added to decaffeinated coffee and reached 13 mg/L after 12 mg/kg/day of caffeine was added.

When a capsule containing 2 mg/kg of caffeine (corresponding roughly to two cups of coffee for an adult) was given to volunteers, the plasma level reached a value of about 3 mg/L and plateaued at 7.5 mg/L after a 4 mg/kg dose of caffeine (Benowitz et al., 1995). This mode of administration is convenient and precise, but does not actually mirror the absorption of caffeine after drinking sufficient cups of coffee to contain the same quantity of caffeine. Caffeine is also given intravenously to help prevent headache after spinal anesthesia (Yucel et al., 1999) and to treat apnea in preterm infants (Tobias, 2000). The pharmacokinetics of caffeine remains the same, regardless of the route of administration (Fredholm and Lindstrom, 1999). Caffeine can also be inhaled and is frequently added to pharmaceutical grade heroin, both on the streets and in replacement programs. The addition of caffeine results in a decreased sublimation temperature of the heroin and a slightly higher recovery after it has been smoked. Perhaps most importantly, pyrolytic decomposition of the heroin is reduced (Huizer, 1987). Caffeine is rapidly and effectively absorbed after inhalation with a bioavailability of 60%. The volume of distribution for the central compartment is estimated to be 0.65 L/kg (Zandvliet et al., 2005).

2.2.7 Metabolism

The first step in caffeine metabolism involves demethylation into dimethylxanthines, specifically paraxanthine, theobromine, and theophylline (Figure 2.6). In humans, the metabolic pathway for demethylation of caffeine into paraxanthine and 1-methylxanthine predominates (Brice and Smith, 2001). Other pathways are recognized in animals. Human

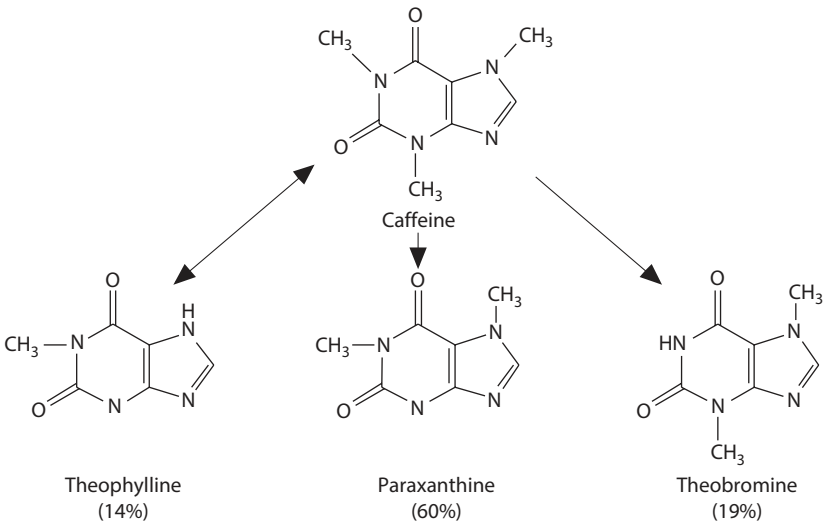


Figure 2.6 Caffeine metabolism. Assuming an average caffeine intake of roughly 500 mg/day, 60% will be excreted as paraxanthine, 20% as theobromine, and 14% as theophylline. The results may be quite different in smokers and patients with cirrhosis.

caffeine metabolism is controlled by the CYP1A2 enzyme, which is a genetically polymorphic enzyme. CYP1A2 activity is influenced by a variety of factors, especially smoking, which induces CYP1A and therefore, controls the rate of caffeine breakdown to 1-methylxanthine (Tang et al., 1991; Caubet et al., 2002). Inhibiting this enzyme prolongs caffeine's half-life and reduces clearance.

Caffeine and theophylline both convert entirely to other xanthines and are then excreted in the urine. At least 14 different caffeine metabolites have been identified in human urine (Rodopoulos and Norman, 1994). On average, 14% of ingested caffeine is excreted as theophylline and 60% as the demethylation and oxidation product paraxanthine (1,7-dimethylxanthine). Paraxanthine is not found in man but is found as a metabolite in many different species (Ullrich et al., 1992); it has sympathomimetic effects comparable to those of caffeine itself (Benowitz et al., 1995). In humans, paraxanthine is either acetylated or further demethylated to a compound devoid of sympathetic activity (Sawynok and Yaksh, 1993).

Many commonly prescribed medications are potent CYP1A2 inhibitors. It is not known whether the other ephedrine isomers are CYP1A2 inhibitors, but this interaction could have important clinical implications, resulting in higher than anticipated caffeine concentrations. It has been suggested that unsuspected interactions might inadvertently cause athletes to exceed the 12 mg/L urinary caffeine concentration limit set by the International Olympic Committee (IOC) and other sports regulatory bodies (Carrillo and Benitez, 2000).

2.2.8 Mechanisms of Action

For many years, caffeine's cardiovascular effects were assumed to be a consequence of its ability to act as a phosphodiesterase inhibitor, leading to increased formation of cyclic AMP and cyclic GMP (Wells and Kramer, 1981). That explanation is almost certainly incorrect because caffeine is a relatively nonspecific phosphodiesterase inhibitor, and very

high caffeine concentrations, substantially higher than those that result after a few cups of coffee, are required to produce measurable vasoconstriction in humans (Casiglia et al., 1992). However, even low doses of caffeine antagonize adenosine receptors present in the brain, blood vessels, kidneys, heart, gastrointestinal tract, and respiratory tract (Chou and Benowitz, 1994; Daly and Fredholm, 1998). It now appears that the stimulatory effects of caffeine are a result of its ability to block adenosine type 2A receptors. Blockade of these receptors activates adenyl cyclase, increases concentrations of cyclic AMP, and causes the closing of K^+ channels that indirectly increase calcium concentration within cells. In the brain, this leads to stimulation of GABAergic neurons that inhibit the dopaminergic reward system.

When herbal supplement makers began combining caffeine and ephedrine, some researchers expressed concern that blockade of A_2 receptors would lead to systemic and coronary artery vasoconstriction and that adenosine blockade would favor platelet aggregation. More recent work has tended not to confirm these fears, largely because the number of A_2 receptors is so great that the caffeine levels achieved with anything like normal caffeine consumption cannot block critical numbers. Most prospective cohort studies have not found coffee consumption to be associated with significantly increased risk for cardiovascular disease (Higdon and Frei, 2006).

In fact, most of the worries were misplaced, and the results of modern epidemiologic research suggest that coffee consumption may help prevent several chronic diseases, including type 2 diabetes mellitus, Parkinson's disease, and liver disease (Riksen et al., 2011), including both cirrhosis and hepatocellular carcinoma (Ruhl and Everhart, 2005; Higdon and Frei, 2006; Yamauchi et al., 2010). Adenosine antagonists, such as caffeine, are under active development as adjunctive symptomatic treatment for advanced parkinsonism (Alisky, 2006; Deleu et al., 2006; Morelli and Simola, 2010), diabetes (Yamauchi et al., 2010), and dementia (Biessels, 2010). There is preclinical evidence that A_{2A} antagonists favorably alter the course and symptoms of the disease, while epidemiologic and laboratory data suggest that A_{2A} blockade may prevent dopaminergic neuron degeneration (Xu et al., 2005). Reanalysis of data collected prospectively for a very large cancer prevention study found clear evidence of a protective effect of caffeine against the onset of parkinsonism, with an attenuating influence of hormone replacement therapy in women (Palacios et al., 2012). Some of coffee's effects have nothing to do with caffeine. For example, coffee generally stimulates the gastrointestinal tract and causes the gallbladder to contract, even when the caffeine has been removed (Boekema et al., 1999).

The underlying mechanism of caffeine-induced diuresis is not known, but it has been shown that the process does not involve increased production of atrial natriuretic factor (Nussberger et al., 1990). In a double-blind controlled study, 642 mg of caffeine given to 12 healthy volunteers led to an increase in 24 h urine excretion of 753 ± 532 mL, a corresponding negative fluid balance, and a concomitant decrease in body weight of 0.7 ± 0.4 kg. Total body water decreased by 1.1 ± 1.2 kg (or 2.7%), and urinary excretion of sodium and potassium increased by 66% and 28%, respectively (Neuhauser et al., 1997).

Under controlled laboratory conditions, caffeine itself has no effect on body mass, urine osmolality, urine specific gravity, urine color, 24 h urine volume, 24 h Na^+ and K^+ excretion, 24 h creatinine, blood urea nitrogen, serum Na^+ and K^+, serum osmolality, hematocrit, or total plasma protein, and there is no evidence of dehydration (Armstrong et al., 2005). These results are in conformity with earlier clinical studies showing that caffeine exerts no effect on the production of atrial natriuretic peptides (Nussberger et al., 1990).

2.2.9 Pharmacokinetics

A caffeine dose of 1 mg/kg (roughly equivalent to a 70 kg man drinking a cup of coffee) will produce peak plasma concentrations of 500–1000 ng/mL (Carrillo and Benitez, 2000). Caffeine is taken up mainly by lean body tissue. The same dose of caffeine will produce much higher blood levels in older than in younger adults (Massey, 1998). It will also produce plasma concentrations 20% higher than saliva, though there is excellent correlation between the two matrices (Carrillo et al., 2000). In the newborn, lean body mass is reduced in proportion to the amount of fat present (Anderson et al., 1999). The half-life of caffeine is extremely variable: during the first 3 h after ingestion, there is a 5–11-fold interindividual variation in plasma caffeine concentrations (Bonati et al., 1982). Among the factors accounting for the variation are the age of the individual, lean body mass, and CYP1A2 activity. Smoking enhances CYP1A2 activity, while some drugs decrease the rate at which caffeine is metabolized (Faber et al., 2005).

The half-life for caffeine in healthy adults is 3–7 h (Levy and Zylber-Katz, 1983), but even wider ranges have been reported (Magkos and Kavouras, 2005). In adults, but not necessarily in children, caffeine has a low volume of distribution, 0.45 L/kg. Because of the higher fat content of neonates and the hydrophilic nature of caffeine, the volume of distribution may be more than three times as high in neonates (Anderson et al., 1999) (Table 2.4). Caffeine's metabolism is saturable. Saturation occurs at a value of roughly 8000–9000 ng/mL (Mandel, 2002). Human trials have shown the caffeine kinetics to be nonlinear; clearance decreases and the elimination half-life is prolonged when larger doses (500 vs. 250 mg) of caffeine are given (Troger and Meyer, 1995; Kaplan et al., 1997).

Theophylline (1,3-methylxanthine), the caffeine metabolite, initially undergoes demethylation followed by acetylation (Troger and Meyer, 1995). Its volume of distribution is also low (0.46–0.90 L/kg) (Sanchez-Alcaraz et al., 1991). When theophylline clearance is corrected for body weight, it is substantially higher in young adults than in the elderly—0.52 mL/min/kg for those over age 56 versus 0.72–0.77 mL/min/kg in individuals aged 20–50 years. The pharmacokinetics of caffeine is essentially the same, no matter whether the caffeine is given orally or intravenously (Fredholm et al., 1999).

Over the last several years, caffeine has become increasingly used as a probe to assess CYP1A2 activity. Caffeine clearance, 3-*N*-demethylation, measurement of the amount of ^{13}C-caffeine expired in the breath, and calculation of the ratio of different xanthine metabolites can all be used to indirectly assess CYP1A2 activity. The ability to do so is of more than academic interest. A number of drugs besides caffeine, some potentially quite toxic (phenacetin, clozapine, imipramine, tacrine), are metabolized by this same enzyme system, and CYP1A2 activity is deficient in part of the human population (Spigset et al., 1999).

Table 2.4 Half-Life of Caffeine versus Age

Age	Half-Life of Caffeine (HR)
Premature, at birth	65–102
Term, at birth	82
3–4.5 months old	14.4
5–6 months old	2.6
Adult	3–7.5

2.2.10 Tissue Concentrations

A healthy adult drinking two cups of very strong coffee would be expected to have urine caffeine levels no greater than 3–6 mg/mL, which is not very much different than levels seen in heroin smokers who are "chasing the dragon" (Zandvliet et al., 2005). Plasma measurements made 1 h after drinking two cups of coffee showed a peak caffeine value of 5.3 mg/mL (Marks and Kelly, 1973), though peak levels can be expected to occur anywhere from 30 to 45 min after ingestion. The clearance rates for both theophylline and paraxanthine decrease in chronic users. Tissue measurements in rats have shown that, after dosing with caffeine, concentrations of caffeine and theophylline are equal in most tissues except the brain, where caffeine levels are 25% higher than theophylline levels (Stahle et al., 1991). If this is also the case in humans, it might explain the different clinical profiles of theophylline and caffeine.

What really matters to caffeine drinkers is getting the caffeine to the A_1 adenosine receptors where it may act to provide the desired effects. This issue has been studied by using the technique of modern positron-emission tomography scanning. Measurements were made in 36 healthy volunteers, aged 22–74 years, and compared. There was no significant association between regional uptake, age, gender, caffeine consumption, or sleep duration and adenosine receptor binding, but there was a significant age-dependent decrease of adenosine receptor binding in all regions of the brain except the cingulate gyrus. Declines ranged from –17% (striatum) up to –34% in the postcentral gyrus. The average cortical decline was –23%. The decrease in uptake was not affected by gender, caffeine consumption, or sleep duration. Although as yet unproven, researchers speculated that the decrease in A_1 binding could be a general marker for neurodegenerative disease (Meyer et al., 2006).

Newborns, like adults, can convert caffeine to theophylline and theophylline to caffeine, but the direction and degree of conversion are not always predictable. Measurements of cord blood caffeine levels in children born to cocaine-abusing mothers have shown that these women are likely to be abusing caffeine and nicotine as well; caffeine may be present in substantial concentrations (up to 10 mg/L) (Dempsey et al., 1998). In three newborns treated with therapeutic doses of intravenous aminophylline, the highest levels were observed in the blood and then the brain. Decreasing levels were found in the heart, liver, lung, and kidney. Brain theophylline levels ranged from 6 to 30 mg/g, while caffeine levels ranged from 2.1 to 3.7 mg/g. Caffeine can be detected in most biofluids, including saliva, semen, and breast milk (Bonati et al., 1982), but levels have not been systematically studied.

Ingestion of even modest amounts of caffeine by naïve mothers can produce significant effects on maternal and fetal circulation (Miller et al., 1994). Infants born to heavy coffee drinkers have high caffeine levels at birth (Khanna and Somani, 1984) and generally manifest intrauterine growth retardation. A recent large epidemiologic investigation ($n = 1606$) measured the association between caffeine and its primary metabolites in umbilical cord blood and attempted to correlate them with intrauterine growth restriction. Using an adjusted model including caffeine only, levels in all growth quartiles were associated with risk of intrauterine growth restriction. In adjusted analyses including paraxanthine and caffeine, serum paraxanthine levels in the highest quartile were associated with increased risk of the same problem. The findings suggest that CYP1A2 metabolic activity may play a key role in growth retardation. No associations were observed between caffeine or any metabolites and/or preterm delivery (Grosso et al., 2006).

Substantially, similar results have been obtained in other recent studies. Children born to coffee-drinking mothers who did not smoke had statistically significant lower birth weights and smaller placentas ($p < 0.05$), but there was no difference between groups according to body lengths, head circumferences, and diameters of placentas. On the other hand, birth weight and placenta size in pregnant smokers ($p < 0.05$) were even lower (Balat et al., 2003).

2.2.11 Toxicity by Organ System

Reactions to low doses of caffeine are widely variable and unpredictable because of the interconversion of theophylline and caffeine in humans. The ratio of plasma theophylline to caffeine after caffeine administration is 8:6, and it is not clear whether toxic reactions, when they are observed, are really the result of caffeine or theophylline excess. After theophylline administration, the ratio of theophylline to caffeine is nearly the same (Stavric, 1988b).

2.2.11.1 Neurologic

A 250 mg dose of caffeine (approximately 2.5 cups of coffee) reduces cerebral blood flow for 90 min. The decrease in cerebral flow is unexplained. It is not due to changes in the general circulation or in CO_2 levels, but it might be the result of the ability of caffeine to block adenosine receptors (see Section 2.2.8). Adenosine is a powerful cerebral vasodilator, and it may be that adenosine receptor blockade results in decreased cerebral flow. Interactions with the adenosine receptor have also been suggested as a possible mechanism in caffeine-related seizures, although this suggestion also remains unproven (Morgan and Durcan, 1990), as does the role of adenosine receptors in caffeine-related hypertension (Nurminen et al., 1999). These earlier studies have been confirmed in more recent studies done with functional magnetic resonance imaging. Because chronic caffeine use causes an upregulation of adenosine receptors, the differential effects of caffeine observed in occasional and regular coffee users are to be expected. The blood flow decrease in the visual cortex is significantly greater in heavy coffee drinkers than in more modest users. In addition, the magnitude of the change is significantly correlated with caffeine consumption. This correlation is thought to be the result of upregulation of adenosine receptors in high users (Laurienti et al., 2002).

The elderly are more sensitive to the psychological effects of caffeine, including increased alertness and improved performance on certain psychological tests (Massey, 1998; Kallus et al., 2005). Older adults also seem to be more likely to experience insomnia after consuming caffeine than are younger individuals (Brezinova, 1976). The suggestion has also been made that a caffeine dependence syndrome exists and that this syndrome meets all the generic criteria for substance dependence, including the fact that affected individuals continue to use caffeine in spite of persistent problems related to its use (Holtzman, 1990). In one controlled study, dependence was diagnosed in 16 of 99 individuals who were evaluated. The median daily caffeine consumption in this group was only 357 mg/day (Strain et al., 1994).

Since this observation was first published, caffeine addiction has been added as an official diagnosis in ICDM 9. This decision is disputed by many and is not supported by any convincing body of experimental evidence. For example, other abused drugs lead to predictable increases in cerebral function and dopamine release in the shell of the nucleus accumbens. Caffeine does not. Except in massive doses, caffeine does not cause an increase in nucleus accumbens glucose utilization, as do drugs such as heroin, cocaine, and methamphetamine (Fredholm et al., 1999; Nehlig, 1999). All of these observations strongly suggest that caffeine

does not act on the dopaminergic structures related to addiction, nor does it improve performance by alleviating any symptoms of withdrawal (Hewlett and Smith, 2006).

The FDA has warned that immensely popular caffeine and ethanol-containing drinks act synergistically to produce toxicity. These combination drinks first appeared on U.S. markets in 2007, and almost immediately anecdotal reports of toxicity and even death began to appear. Government regulators contend that when "alcoholic beverages are mixed with energy drinks, a popular practice among youth, the caffeine in these drinks can mask the depressant effects of alcohol" (presumably because of the risk of ethanol poisoning). Whether or not this fear is founded on actual fact is not known. At the same time, caffeine has no effect on the metabolism of ethanol by the liver and thus does not reduce breath ethanol concentrations or reduce the risk of ethanol-attributable harms (Centers for Disease Control and Prevention, 2010). The Centers for Disease Control report referenced previously cites only one source, and that was published in 2006. The authors of that study found no evidence of toxicity, and not much evidence that symptoms of ethanol intoxication were reduced in the presence of caffeine (Ferreira et al., 2006).

Neurologic researchers are now focused, to a very large degree, on the possibility of treating, or at least mitigating, the symptoms of Alzheimer's and Parkinson's disease with caffeine. Epidemiologic studies have conclusively shown that a higher coffee and caffeine intake is associated with a significantly lower incidence of parkinsonism, probably as a result of the caffeine and not of any of the other compounds contained in that drink (Ross et al., 2000). Recent studies have shown that administration of caffeine shortens the time until maximal plasma concentrations of levodopa are reached, decreases the latency to levodopa walking and tapping motor response, and increases the magnitude of walking response. Caffeine administered before levodopa may improve its pharmacokinetics in some parkinsonian patients (Deleu et al., 2006). Because adenosine A_1 and A_{2A} receptors are expressed in the basal ganglia (structures involved in motor control, including Parkinson's disease) and because caffeine acts as an antagonist to both types of receptors, it is possible that caffeine's stimulating effects are exerted via a particular group of projection neurons located in the striatum—the main area that receives input from the basal ganglia, cells expressing very high levels of adenosine A_{2A} receptors. These receptors are involved in many intracellular processes, and alterations in just one of them may be sufficient to help patients with Parkinson's disease (Chen, 2003; Fisone et al., 2004).

The other area of intense interest is Alzheimer's disease, where adenosine receptors also seem to play a role. In laboratory animals, the pharmacologic blockade or gene disruption of adenosine A_{2A} receptors confers neuroprotection in several different neurotoxic brain disorders. These disorders can be reversed either by giving coffee or a specific A_{2A} antagonist, suggesting that A_{2A} is the molecular target responsible for the observed beneficial effects of caffeine consumption in the development of Alzheimer's disease (Dall'Igna et al., 2003).

2.2.11.2 Cardiovascular

Caffeine, regardless of the source, acutely raises blood pressure in naïve individuals, but only for a short time and not to a very great degree (Cameron et al., 1990). When a large (44,005 men and 84,488 women) cohort of American coffee drinkers was followed over a 20-year period, there were 2173 cases of myocardial infarction among the men (724 fatal) and 2254 (693 fatal) among the women. Results of the study strongly suggest that individuals who drank two cups of coffee per day experienced no increase in risk for heart attack.

Intake of decaffeinated coffee, tea, and the total caffeine intake were all completely unrelated to the occurrence of coronary artery disease. Even individuals who drank six or more cups of coffee per day did not experience any significant risk in coronary artery disease. At the same time, neither caffeinated nor decaffeinated coffee had any effect on individual lipid profile. It appears that tolerance to caffeine's vascular effects emerges so quickly that caffeine's undesirable effects (such as slight elevation in pulse and blood pressure) are only transient. Another possibility not to be dismissed is that coffee is a very rich source of antioxidants; it is, in fact, the principal antioxidant in most Americans' diet. It may well be that any damage done by the caffeine is more than offset by the protective antioxidants contained in coffee.

Results are hard to predict when physiologic studies are done with individuals who consume caffeine on a regular basis, presumably because of tolerance (James, 1997). Evidence exists that increasing age is associated with increasing response to the pressor effects of caffeine (Massey, 1998). Some epidemiologic studies suggest that regular coffee consumption may be harmful to those with established hypertension (Nurminen et al., 1999). Even if coffee intake is not associated with coronary artery disease (see earlier), that does not mean that it may not be associated with the occurrence of sudden cardiac death. Of course, a very large intake of caffeine by individuals with coronary artery disease substantially increases the risk for sudden cardiac death (de Vreede-Swagemakers et al., 1999). In some cases, death may be due to the presence of an abnormal ryanodine (RyR2) receptor. Point mutations in *RyR2* lead to abnormal Ca^{2+} release following cardiac stimulation that, in turn, can lead to the occurrence of sudden cardiac death (Thomas et al., 2005). In any individual case, such a nexus could only be proven by DNA sequencing.

The results of early studies suggested that caffeine caused the release of catecholamines from the adrenal medulla (Robertson et al., 1978), but the results of more recent research suggest that caffeine-related catecholamine perturbations are minimal. Human volunteers given a mean dose of 250 mg of caffeine exhibited only insignificant increases in catecholamines (Cameron et al., 1990). Rises did occur, but were so trivial that the clinical significance, if any, would have been negligible. Table 2.5 shows catecholamine levels in a number of situations.

Table 2.5 Catecholamine Concentrations

Activity/Condition	Ephedrine (pg/mL)	Norephedrine (pg/mL)
Resting	35 (Eisenhofer et al., 2005)	200–300 (Eisenhofer et al., 2005)
Normal exercise	700 (Bell et al., 2001)	299–300 (Bell et al., 2001)
	Resting epinephrine not measured	Norepinephrine, mean 204 ± 102 pg/mL, range 97–953/pg/mL[a] (Ghimire et al., 2012)
Exercise and ephedrine	700 (Pott et al., 1996; Bell et al., 2001)	300 (Bell et al., 2001)
Heart failure	35–75 (Yan et al., 2005)	500–800 (Yan et al., 2005)
Pheochromocytoma	35 (Eisenhofer et al., 2005)	3000–5000 (Eisenhofer et al., 2005)
Cocaine	30–40 (Sofuoglu et al., 2001)	1000–2000 (Sofuoglu et al., 2001)
Cardiac arrest	10,000–100,000 (Wortsman et al., 1984)	500–600 (Wortsman et al., 1984)

[a] The observed values demonstrate wide interindividual variation. Resting norepinephrine concentrations were particularly associated with variants of four genes: *CYB561, VMAT2, CHGA*, and *PNMT*. Postexercise plasma concentrations related to three different variants of *PNMT* and *COMT*. There can be no way to interpret these results except to suppose that the great interindividual variation seen after exercise is, in some way, genetically determined. (Data adapted from Ghimire, L.V. et al., *Pharmacogenet Genomics*, 22(4), 254, 2012.)

All methylxanthines cause the release of calcium ions from the sarcoplasmic reticulum into the cytoplasm, where the calcium acts as a second messenger, altering myofilament contraction and electrical conduction. Some methylxanthines exert more potent effects than others. The differences in potency appear to be a function of differences in membrane permeability to the different methylxanthines (Donoso et al., 1994). A relationship between caffeine intake and ventricular ectopy has always been presumed, but electrophysiologic studies of patients with recurrent ventricular tachycardia have failed to confirm any such action. In fact, some patients with ventricular ectopy who had been treated with caffeine had fewer extra heartbeats after drinking coffee than at baseline (Chelsky et al., 1990). Signal-averaged electrocardiograms (EKGs) of normal subjects before and after administration of 5 mg/kg of caffeine show small but statistically significant prolongation of the signal-averaged QRS complex. The finding is consistent with, but far from proof of, the notion that excessive caffeine intake might be a risk factor for serious arrhythmias (Donnerstein et al., 1998). Similarly, dogs given caffeine intravenously can be made to fibrillate, but only with massive doses of caffeine. After low doses, comparable to those seen in coffee drinkers, either no arrhythmias or only inconsequential arrhythmias occurred, suggesting that the arrhythmogenicity of caffeine is dose related (Mehta et al., 2004).

Cardiac alterations similar to those reported in animals have not been mentioned in the few published human autopsies. In a case of one overdose, with a postmortem caffeine level of 113.5 mg/L and clinical evidence of acute heart failure, there was right atrial dilation, acute pulmonary edema, and passive congestion of the liver, but no specific cardiac lesion could be identified (Bryant, 1981). No anatomic basis could be found in a second case report where there were even higher caffeine levels (181 mg/L), pulmonary edema, and passive congestion of the liver, but the heart was not specifically described (Alstott et al., 1973). Even though heart failure occurred in a 1860 g 31-week-old child who was accidentally given a caffeine overdose as a treatment for apnea, the child nonetheless survived. Its initial serum concentration was 2175 mg/L 36.5 h after dosing. Toxic manifestations included heart failure, pulmonary edema, gastric dilation, metabolic acidosis, and hyperglycemia. Nonetheless, the child made an uneventful recovery (Anderson et al., 1999).

Several case reports of alleged caffeine-related infarction and arrhythmia have been published, but it is difficult to know how they should be interpreted. One case involved a 20-year-old bulimic woman who ingested 20 g of caffeine in a suicide attempt. After being evaluated and discharged from the emergency room, she was readmitted with EKG changes and ultimately found to have sustained a subendocardial infarction. Angiography was not performed (Forman et al., 1997).

More recently a 31-year-old man committed suicide with an unknown dose of diet supplement. He was found to have a caffeine concentration of 170 mg/L, in femoral blood. He was not found to have evidence of myoglobinuria, glycosuria, or ketonuria (Bonsignore et al., 2014). In the only case series to be published, the mean caffeine was 140 mg/mL. Two of the patients described had been classified as suicides, while the manner of death in the other six was undetermined (Banerjee et al., 2014). In another documented suicide, a 39-year-old man orally self-administered approximately 12 g of pure anhydrous caffeine. Autopsy blood caffeine levels were 350 mg/L.

2.2.11.3 Hematologic

Although the claim is still disputed, some reports have shown that chronic caffeine consumption may lead to a reduction in platelet aggregability due to upregulation of A_{2A}

adenosine receptors located on the platelet surface, disrupting normal platelet aggregation and thrombogenesis (Biaggioni et al., 1991). Others have found no such evidence (Cavalcante et al., 2000). In the most recent studies of the subject, mean platelet aggregability after exposure to epinephrine was 11.8% ± 5.7%, significantly lower than in the nontreated group (85.7% ± 9.5%, $p < 0.01$). There were no significant differences in the mean values of collagen- or ristocetin-induced platelet aggregability between caffeine-treated and nontreated groups. Based on this study, it seems likely that caffeine selectively inhibits platelet aggregability, both to epinephrine and ADP, and disturbs the release of endogenous ADP from platelets in response to exogenous ADP (Choi, 2003).

2.2.11.4 Ergogenic Effects

Caffeine is frequently used by athletes as an ergogenic aid. It improves performance and endurance during prolonged, exhaustive exercise. When caffeine is consumed before prolonged running or cycling (30–60 min), the time to exhaustion is improved by 20%–50% at 60%–80% of VO_{2max}. In one controlled study, elite marathon runners given 9 mg/kg of caffeine before testing were able to increase their time on a treadmill by an average of 70% (Graham and Spriet, 1991). This improvement was achieved without evidence of toxicity and without exceeding the requirement of the IOC that testing revealed no more than 12 mg/mL of caffeine or the National Collegiate Athletic Association's even more generous limit of 15 mg/mL. In short-term competition, with intense aerobic exercise at greater than 90% of VO_{2max}, improved time to exhaustion has been repeatedly confirmed, though the performance increment has not been so great (Magkos and Kavouras, 2005).

Caffeine improves concentration, reduces fatigue, and enhances alertness. Habitual intake does not diminish caffeine's ergogenic properties. Several mechanisms have been proposed to explain the physiologic effects of caffeine but adenosine receptor antagonism most likely accounts for the primary mode of action. Caffeine is relatively safe and has no known effect as measured by maximal exercise capacity testing, nor does it cause significant dehydration or electrolyte imbalance during exercise. Routine caffeine consumption may cause tolerance or dependence, and abrupt discontinuation is said to produce irritability, mood shifts, headache, drowsiness, and fatigue. Major sport governing bodies ban excessive use of caffeine, but current monitoring techniques are inadequate, and ethical dilemmas persist regarding caffeine intake by athletes (Paluska, 2003).

2.2.11.5 Maternal/Fetal Effects

Caffeine and theobromine cross the placental and blood–brain barriers. Caffeine ingested during gestation does cause a dose-dependent decrease in body weight, but only when large doses of coffee have been consumed (>7 cups/day of coffee); it has no effect at moderate doses, and thus appears not to have the potential to cause toxicity (Eteng et al., 1997). Investigators do not agree on the quantities of the methylxanthine found in breast milk, but caffeine does not change breast milk composition and, rather, stimulates milk production. Maternal consumption of moderate amounts of caffeine during gestation and lactation has no measurable consequences on the fetus and newborn infant.

Lingering doubts exist as to whether the consumption of coffee and caffeine is associated with an increased risk of miscarriage. In the most recent study to be published on the subject, women were recruited before or early in pregnancy and interviewed regarding sources of caffeine. The interview included an assessment of changes that had been noted by the mothers during the perinatal period. Of the 2407 women enrolled, 258 lost their child.

The researchers examined the relationship of coffee and caffeine to pregnancy loss occurring within the first 20 weeks of gestation. The authors found that caffeine consumption was unrelated to total miscarriage risk. The authors concluded that there was very little indication of possible harmful effects of caffeine on miscarriage, at least among those women drinking moderate amounts of coffee. They also concluded that their result supported a reporting bias among women with losses and that their results were more indicative of exposure misclassification and unmeasured heterogeneity of pregnancy losses (Savitz et al., 2008). This finding would be in agreement with the findings of other scientists who have speculated that caffeine only appears to increase miscarriage risk because women with morning sickness, who are more likely to carry a pregnancy to term, avoid coffee and other drinks that contain it, giving the false appearance of a causal relationship.

Epidemiologic data published in early 2008 strongly suggested that caffeine may exert deleterious effects on pregnant women (Savitz et al., 2008), but this conclusion is hotly contested by the American College of Obstetrics and Gynecology that, based on the best available evidence, concluded

> Moderate caffeine consumption (less than 200 mg/day) does not appear to be a major contributing factor in miscarriage or preterm birth. The relationship of caffeine to growth restriction remains undetermined. A final conclusion cannot be made at this time as to whether there is a correlation between high caffeine intake and miscarriage.
>
> **(ACOG, 2010)**

Two special situations are of clinical and forensic interest. The CYP1A2 system is immature in infants (Tanaka, 1998), and the plasma half-life of caffeine is 17 times longer than in healthy adults (Labow, 1983). Infants being treated with aminophylline run a real risk of toxicity from caffeine, which continues to accumulate in their blood as aminophylline is converted to caffeine. Similar results can occur in patients with hepatic insufficiency or decreased cardiac output (Lacroix et al., 1985; Bechtel et al., 2000). Treatment with aminophylline under these circumstances runs the risk of caffeine toxicity. Measurement of both theophylline and caffeine levels in individuals at risk would be prudent.

Altered caffeine metabolism is also observed in children with cystic fibrosis, but the alterations remain poorly characterized. That disease is caused by defects in the gene for cystic fibrosis transmembrane conductance regulator (CFTR), which encodes for a chloride channel and is regulated by cyclic adenosine monophosphate (cAMP). Mutations in the gene for CFTR (*CFTR*) result in abnormalities of cAMP-regulated chloride transport across epithelial cells on mucosal surfaces. The mucus that is produced is abnormally thickened and susceptible to bacterial infection. There is mounting evidence that caffeine, if not some other designer xanthine, may help reverse the problem (Bulteau et al., 2000).

Given the degree of controversy, it would seem prudent for pregnant mothers to consume coffee and caffeinated beverages in moderation, especially because of the prolonged half-life of caffeine both during the last trimester of pregnancy and in the newborn infant (Nehlig and Debry, 1994).

2.2.12 Autopsy Studies

A 1980 case report described two patients who expired after repeatedly using coffee enemas. Both had underlying malignancies and both appeared to have succumbed to fluid and electrolyte abnormalities, not to any toxic effect of caffeine. In fact, both of these women had negligible

caffeine levels at the time of death (Eisele and Reay, 1980). Blood concentrations in cases of fatal intoxication have ranged from 79 to 1560 mg/L (McGee, 1980; Mrvos et al., 1989). In 1985, Garriott reported on five fatalities—three cases of combined caffeine and ephedrine and two cases of caffeine only. Blood concentrations ranged from 130 to 344 mg/L. The report did not comment on histologic findings, if any (Garriott et al., 1985). Another case report describes a 22-year-old woman who committed suicide by taking an unknown number of caffeine tablets. Death appeared to have been the result of cardiac arrhythmia. Blood obtained during attempted resuscitation had an extraordinarily high caffeine concentration of 1560 mg/L. As is true in experimental animals, postmortem findings in this case consisted mainly of pulmonary edema and visceral congestion (Dimaio and Garriott, 1974). Mrvos described a 19-year-old woman who also died of a ventricular arrhythmia. At autopsy, her caffeine blood level was 181 mg/L, and no histopathologic alterations could be identified (Mrvos et al., 1989).

The more recently reported cases have only contained toxicologic data. Four new cases were reported in 2004—two suicides and two causes undetermined. All four victims had psychiatric or polydrug histories. No autopsy findings were reported, but blood caffeine ranged from 153 to 200 mg/L with many other drugs present (Holmgren et al., 2004). More recently, Kerrigan and Lindsey (2005) described two more cases. One decedent was a polydrug user and the other an obese diabetic. Autopsy findings were not discussed, but the blood caffeine level was 597 mg/L in one and 192 mg/L in the other (Kerrigan and Lindsey, 2005). The failure to demonstrate myocardial lesions is consistent with the fact that caffeine toxicity is not associated with marked elevations in circulating catecholamines.

Other studies where caffeine has been the sole agent have all reported caffeine concentrations in comparable ranges. In an 81-year-old female suicide, caffeine concentrations in the heart and stomach were almost identical (180 and 190 mg/L, respectively) (Reisselmann et al., 1999). As with all of the abused drugs, tolerance occurs (although the mechanism is not clear), and high caffeine levels have been recorded in patients who survived massive caffeine overdoses. Blood levels of 200 mg/L were recorded in a woman who took 24 g of caffeine in an unsuccessful suicide attempt. Her theophylline level was 17.2 mg/L (Benowitz et al., 1982). Another report described the case of a 27-year-old man who regularly ingested coffee grounds in order to get "high." On one occasion, he doubled his usual dose and swallowed half a kilogram of ground coffee. He arrived at the hospital comatose, febrile, hypertensive, tachycardic, and seizing. He survived but required intense treatment with beta blockers and anticonvulsants. His caffeine blood concentration was 29 mg/L (Wurl, 1994). The most recent report described a 39-year-old man who self-administered approximately 12 g of pure anhydrous caffeine. Except for moderate pulmonary edema, the autopsy was said to be negative. The blood caffeine level was 350 mg/L anhydrous (Jabbar and Hanly, 2014).

2.2.13 Analytic Considerations

Since blood (and other fluids such as urine) has relatively high concentrations and the molecule is stable and readily extractable, the measurement of caffeine in biofluids is straightforward although it is not commonly performed because caffeine is so ubiquitous and is relatively nontoxic. Unless there is a need to prove caffeine consumption, blood concentrations under 10 mg/L (and possibly even as high as 20 mg/L) need not be reported. It can readily be detected by high-performance liquid chromatography using photodiode array

detection (Drummer et al., 1993) or by conventional gas chromatography (GC) (Drummer et al., 1994). More sophisticated methods using tandem liquid chromatography–mass spectrometry (LC–MS) are available that can also detect related xanthines, including metabolites, in a variety of specimens (Ptolemy et al., 2010).

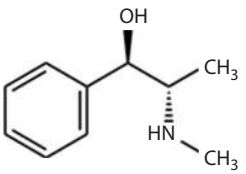
2.3 Ephedrine

2.3.1 General

In spite of the complete absence of controlled clinical trials, the FDA concluded that ephedrine-containing food supplements were too dangerous to remain on the market, and all over-the-counter products containing ephedrine were banned in April 2004 (Table 2.6). However, the FDA continues to allow prescription use of ephedrine, and possession of ephedrine is not illegal. Although it is sometimes used to treat narcolepsy and attention deficit disorder, the main use for ephedrine today is in the production of methamphetamine. The latter can be synthesized from ephedrine via reduction of chloroephedrine with hypophosphorous acid. International controls on ephedrine have been increased in an effort to control methamphetamine production. As a consequence, illicit methamphetamine is now made using pseudoephedrine as the starting material.

Table 2.6 Physiochemical Properties and Pharmacokinetics of Ephedrine

Ephedrine	
Chemical name	α[1-(methylamino)ethyl]benzene-methanol.
Structure of beta form	Usually as anhydrous substance, hydrochloride, or sulfate salts. CAS 299-42-3. MW 165.23. V_d 2–5 L/kg. pK_a 9.6.
Synonyms and common names	Isomeric forms of ephedrine include (±)-ephedrine and (±)-pseudoephedrine. The two naturally occurring isomers are (−)-ephedrine and (+)-pseudoephedrine; Primatene, Quadrinal.
Pharmacokinetics	C_{max}: Ephedrine: 53–139 ng/mL, mean 79 ng/mL after 22 mg. Pseudoephedrine: 116–649 ng/mL, mean 447 after 129 mg. Half-life 4–12 h
Metabolism	Ephedrine is mostly unmetabolized, but minor amounts undergo *N*-demethylation to norephedrine and some hydroxylation and conjugation. The enzyme responsible is not known.
Urine excretion	Ephedrine 70%–80%, norephedrine 4%, depending on urine pH.
Interactions	May precipitate acute angle glaucoma.
Postmortem artifacts	Not likely to change concentrations substantially since tissue concentrations similar to blood and low V_d.
Key papers	Wilkinson and Beckett (1968a,b); Pickup et al., (1976); Dickerson et al., (1978); Simons et al. (1996); Lachkar and Bouassida (2007).



2.3.2 Epidemiology

Ephedrine can be extracted from plants (primarily grown in Pakistani and China), or it can be synthesized, although most of the drug for sale is made from natural products. In recent years, Chinese production of ephedra extract powder (6%, 8%, 10% strengths) has been increasing. Most of this output is or was previously exported to the United States, though now diversion to the Mexican superlabs or other illicit methamphetamine producers, such as Burma, is more likely.

Since the dismantling of the DAWN report, there is no single source that can be relied upon for epidemiologic information. The U.S. government's National Drug Intelligence Center no longer follows trends in ephedrine production, except as they relate to the use of ephedrine as a methamphetamine precursor. Ephedrine is not mentioned in the U.S. Department of Justice annual report on drug intelligence, nor does it rate a mention in the National Survey on Drug Use and Health for 2006.

2.3.3 History

Ephedra plants have been identified at European Neanderthal burial sites from 60,000 B.C. (Lietava, 1992). Traditional Chinese healers used ephedra extracts thousands of years before Pliny, and the ancient Romans accurately described both the ephedra plant and its medical uses. Chinese texts from the fifteenth century recommended ephedra as an antipyretic and antitussive. At about the same time that the Chinese began using ephedra, Russian herbalists used ephedra extracts to treat joint pain, and recent laboratory studies confirm that ephedra might be useful for that purpose (Ling et al., 1995). In the 1600s, Indians and Spaniards in the American Southwest used ephedra as a treatment for venereal disease (Grinspoon and Hedblom, 1975). Settlers in the American West brewed ephedra teas, which were referred to by a variety of names including teamsters' tea, Mormon tea, and chaparral tea (Max, 1991).

The modern rediscovery of ephedrine can be attributed to the work of Nagayoshi Nagai, a Japanese-born, German-trained chemist who first isolated and crystallized ephedrine in 1885 (Holmstedt, 1991). Nagai's original observations were confirmed by Merck chemists, who thought that ephedrine might have commercial value, but sales were never very great, and ephedrine production was all but abandoned until 1930, when Chen and Schmidt published a paper recommending ephedrine as a primary treatment for asthma. Following the publication of Chen and Schmidt's report, ephedrine quickly replaced epinephrine as the first-line treatment for asthma.

Ephedrine became such a popular drug that there were concerns that demand would exceed supply. The possibility of an ephedrine shortage fostered research on methods to synthesize it. Amphetamines were created largely as a by-product of those efforts. The anticipated ephedrine shortage never emerged, but ironically ephedrine sales soared during the 1990s because ephedrine was the preferred precursor for use in the illicit manufacture of methamphetamine. Because government controls now limit the use of ephedrine for methamphetamine production, ephedrine largely has been replaced by its enantiomers, phenylpropanolamine (now withdrawn from the market) and pseudoephedrine, as the precursors of choice in clandestine laboratories.

In addition to being an effective bronchodilator, ephedrine in large doses is a potent CNS stimulant (Martin et al., 1971). Ephedrine injections called *philopon* (which means

"love of work") were given to Japanese kamikaze pilots during World War II. During the 1940s, the Japanese government distributed ephedrine pills to almost anyone who wanted them (in hopes of reducing combat fatigue and increasing industrial output). After 1945, large stocks of the drug, either looted from military supplies or sold by the army in hopes of raising cash, flooded the market. In 1952, ephedra was finally made illegal and immediately became a revenue generator for the criminal underground (yakuza gangs). During the epidemic of the 1950s, abusers in Japan injected themselves with ephedrine, then called *hiropon*, in much the same way that methamphetamine is injected today (Deverall, 1954).

Filipinos have, for many years, smoked a mixture of ephedrine and caffeine called *shabu* (in Japan the same word is used to describe amphetamines in general). In the late 1980s, shabu smoking gave way to the practice of smoking methamphetamine ("ice"). In what is perhaps a tribute to the past, some "ice" is sold under the *hiropon* name today. After the passage of the Dietary Supplement Health and Education Act in 1994, hundreds of "food supplement" producers came into being. Mainly they sold ephedrine combined with caffeine. That industry no longer exists, and ephedrine has been largely replaced by more effective decongestants and treatments for asthma, but it is still widely used for the prophylaxis and treatment of hypotension caused by spinal anesthesia (Flordal and Svensson, 1992; Yap et al., 1998).

2.3.4 Sources

Ephedrine (*ephèdre du Valais* in French and *Walliser Meerträubchen* in German) can be extracted from a group of closely related species of plants that grow in Asia, Western Europe, Southeastern Europe, and even the New World. The alkaloid content of these plants varies quite considerably. The best known species, *Ephedra sinica* (average 1.3% alkaloid content) and *E. equisetina* (average 2.2% alkaloid content), are collectively known as *ma huang* and are grown mainly in China, Northern India, and Pakistan (Cui et al., 1991). *E. gerardiana*, *E. intermedia*, and *E. major* grow in Southwest Asia, while other members of the family Ephedraceae can be found in Europe and the United States (*E. distachya*, *E. vulgaris*).

The most common Chinese cultivar (called China 3) contains 1.39% ephedrine, 0.361% pseudoephedrine, and 0.069% methylephedrine (Sagara et al., 1983). This mix is fairly typical for commercially grown ephedra plants (Zhang et al., 1989; Cui et al., 1991; Gurley et al., 1998). Only *L*-ephedrine occurs naturally.

Ephedrine can now be produced synthetically (benzaldehyde is fermented with brewer's yeast, followed by reductive condensation with methylamine, yielding pure (–)-ephedrine), but there is no evidence that clandestine drug makers are utilizing this approach (Dewick, 1997).

2.3.5 Routes of Administration

Ephedra may be taken orally, injected, or smoked. The last route is reserved for abusers, primarily in the Philippines, where the practice has been popular for many years.

2.3.6 Pharmacokinetics

No data on the pharmacokinetics of smoked ephedra or smoked pure ephedrine have been published. Peak ephedrine levels after an oral dose of 400 mg of *ma huang* (equivalent to 20 mg of pure ephedrine) were 81 ng/mL (White et al., 1997; Table 2.7). Very nearly the

Table 2.7 Pharmacokinetic Parameters of Oral Ephedrine

Dosage Form	Dose (mg)	Ka (h^{-1})	T_{lag} (h)	AUC (ng·h/mL)	$t_{1/2}$ (h)	V_{ss}/F (L)	CI/F (L/h)	t_{max} (h)	C_{max} (ng/mL)
Ephedrine tablet	20.0	1.73	0.38	638	5.74	213.5	28.7	1.69	73.9

Source: Data adapted from White, L.M. et al., *J. Clin. Pharmacol.*, 37, 116, 1997.

Note: Ka, absorption rate constant; T_{lag}, lag time; AUC, area under the concentration–time curve; $t_{1/2}$, elimination half-life; V_{ss}/F, apparent volume of distribution at steady state; CI/F, apparent clearance; t_{max}, time to reach maximum concentration; and C_{max}, maximum plasma concentration.

same peak ephedrine levels were seen after giving an equivalent amount of pure ephedrine (25 mg) or the equivalent amount of ephedrine given in combination with other botanicals (Gurley et al., 1998).

In a separate study, 50 mg of ephedrine given orally to 6 healthy 21-year-old women produced mean peak plasma concentrations of 168 ng/mL (Vanakoski et al., 1993). The results are comparable to those obtained in studies done nearly 30 years earlier (Wilkinson and Beckett, 1968a). In a study performed to assess the effectiveness of caffeine/ephedrine combinations as performance-enhancing agents in combat troops, volunteers ($n = 20$) were treated with 375 mg of caffeine, 75 mg of ephedrine, 375 mg of caffeine combined with 75 mg of ephedrine, or placebo. Modest elevations in pulse and blood pressure were seen in all the treatment groups, with peak effects occurring 1–2 h postadministration. Maximal ephedrine concentrations of approximately 300 ng/mL occurred at 3 h. Maximal caffeine concentrations were also peaked at 3 h and ranged from 7 to 8 mg/L. Drug concentrations when the two agents were taken together were no higher than when they were taken separately, and there was no significant measurable increase in catecholamine levels (Bell et al., 1998, 2001, 2002; Jacobs et al., 2003) (Figure 2.7).

Methylephedrine is a minor component of most ephedra plants, but in Japan, it is produced synthetically and used in cough and cold remedies. One of these products (called BRON) is very widely used (Tokunaga et al., 1989; Ishigooka et al., 1991; Levine et al., 1993; Nakahara and Kikura, 1997). Blood concentrations of methylephedrine in patients taking BRON for legitimate therapeutic purposes are usually less than 0.3 mg/L (Kunsman et al., 1998). Higher concentrations appear to produce toxic effects, usually psychiatric.

2.3.7 Metabolism and Pharmacology

Ephedrine mainly stimulates β_1 and β_3 receptors. The net result of β_1 stimulation is a modest, somewhat unpredictable increase in pulse and blood pressure. The degree of increase is somewhat unpredictable because of concomitant, but variable, increases in peripheral resistance—the phenomenon is known as *diastolic runoff* (Webb and Shipton, 1998). In some situations, administration of ephedrine may result in decreased systemic blood pressure, simply because β-induced vasodilation occurs concurrently in the extremities.

Ephedra extracts contain a complex mixture of phenylpropanolamines (Rothman et al., 2003; Ma et al., 2007) with several isomers including (+)- and (–)-ephedrine and (+)- and (–)-pseudoephedrine. In fact, all of the agonists have their highest affinities as

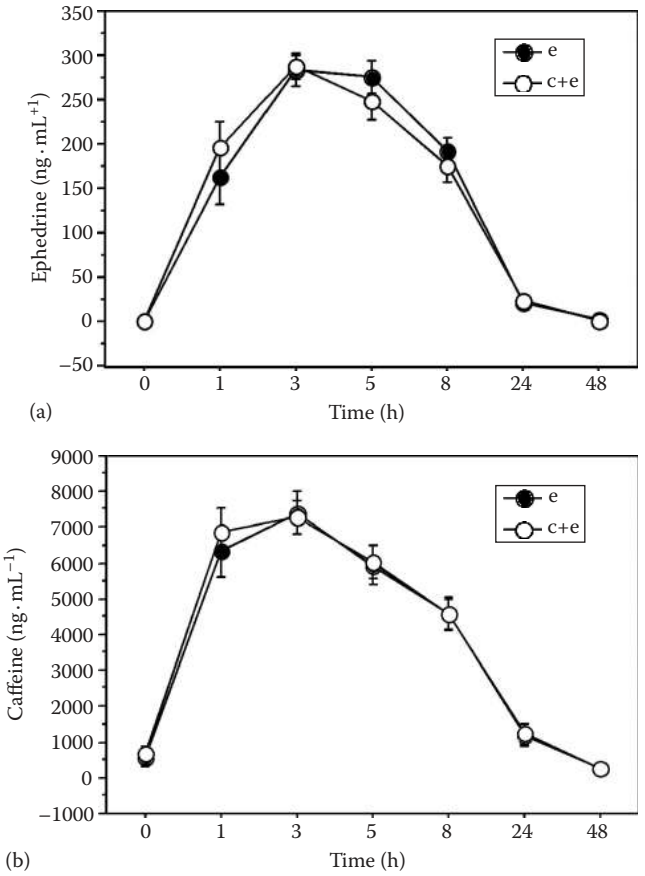


Figure 2.7 (a) Plasma ephedrine levels after ephedrine and caffeine/ephedrine ingestion. The level of ephedrine in the plasma after ephedrine alone and after ephedrine combined with caffeine had no effect on clearance rates. Peak levels occurred between 2 and 4 h after drug ingestion. (b) Caffeine concentrations peaked at between 1 and 2 h, regardless of whether the caffeine was given alone or in combination with ephedrine. (Courtesy of Douglas Bell, Defense and Civil Institute of Environmental Medicine, the Department of National Defense of Canada, Toronto, Ontario.)

norepinephrine transporter substrates. Most of the compounds also have modest activity as dopamine transporter substrates. None of the tested compounds displayed any functional activity at cloned human α_{1A}-adrenergic or cloned human α_2-adrenergic receptors (Rothman et al., 2003). Taken together, these results are consistent with the notion that the main cardiovascular actions of ephedrine and related phenylpropanolamines are due to an indirect sympathomimetic action (Roth et al., 2004).

Ephedrine is one of the few naturally occurring drugs that is an agonist at β_3 receptors. This receptor mediates various pharmacologic and physiologic effects such as lipolysis, thermogenesis, and relaxation of the urinary bladder. Investigations continue into the possibility that the β_3-androgen receptor (AR) might be one way to cure obesity, type 2 diabetes mellitus, and frequent urination (Sawa and Harada, 2006). Investigation is difficult because the β_3-AR gene is highly polymorphic, making it impossible to predict just what effect β_3 agonists will exert upon patients. It has been reported that one of

these mutations is correlated with the occurrence of diabetes and hypertension in obese individuals. Other studies suggest a relationship between polymorphism and carcinogenesis (Babol and Blasiak, 2005). Still other clinical studies have produced results suggesting that ephedrine can produce peroxisome proliferators (and therefore, weight loss) via β_3 stimulation (Bogacka et al., 2007).

Ephedrine's usefulness as a bronchodilator is limited by the number of β receptors located on the bronchi. The number of β receptors located on human lymphocytes correlates with the number found in the lungs, and it has been observed that they decrease rapidly after the administration of ephedrine; the density of β binding sites drops to 50% of normal after 8 days of treatment and returns to normal 5–7 days after the drug has been withdrawn (Neve and Molinoff, 1986). The downregulation of receptors renders ephedrine useless as a bronchodilator after a few weeks, which explains why it is no longer used as a first-line drug in the treatment of asthma.

Each of the ephedrine isomers has different pharmacokinetic and toxicokinetic profiles. Phenylpropanolamine, now withdrawn from the U.S. OTC market, is readily and completely absorbed, but pseudoephedrine (the sale of which is now much restricted in hopes of limiting methamphetamine manufacture) is subject to gut-wall metabolism and has a bioavailability of only 38% (Kanfer et al., 1993). Pure ephedrine is well absorbed from the stomach, but absorption is slower when ephedrine is given along with other botanicals rather than when it is given in its pure form (Gurley, 2000). Peak plasma concentrations occur 2.5–3 h after administration. The volume of distribution of ephedrine appears to be the same whether or not pure drug is given or equivalent amounts are given in ephedra mixtures: 2.5–3.0 L/kg (Gurley et al., 1998).

Ephedrine is eliminated in the urine largely as unchanged drug, with a half-life of about 3–6 h. Peak concentrations for the other enantiomers, specifically phenylpropanolamine and pseudoephedrine, are shorter (0.5 and 2 h, respectively) than for ephedrine, but both drugs are extensively distributed into extravascular sites, with apparent volumes of distribution that are greater than that of ephedrine (2.6 L/kg for phenylpropanolamine and 5.0 L/kg for pseudoephedrine). No protein-binding data in humans are available. Urinary excretion of all three enantiomers is pH dependent. Excretion may be much more rapid in children, and a greater dosage may be required to achieve therapeutic effects. Unlike amphetamines, acidification of the urine has no effect on ephedrine excretion (Wilkinson and Beckett, 1968a).

Patients with renal impairment are at special risk for toxicity because ephedrine isomers may accumulate (Kanfer et al., 1993). None of the enantiomers is easily removed by dialysis, and the only treatment is supportive, using pharmacologic antagonists to counter the α- and β-adrenergic effects of these drugs (Lyon and Turney, 1996). Because excretion is pH dependent, patients with renal tubular acidosis, a rare disorder, are also at particular risk (Brater, 1980).

Traditional Chinese herbalists have always claimed that *ma huang* could be used to treat arthritis, and, although Western-style clinical trials are still lacking, it appears that these claims could have a scientific foundation. One problem in establishing efficacy is that traditional herbalists usually combine ephedra with other herbs, or even acupuncture. In animal models of arthritis, mRNA expression of TNF-α and interleukin-6 genes, which are stimulated in arthritic rat joints, returns to normal levels after treatment with ephedrine (Yeom et al., 2006).

2.3.8 Toxicity by Organ System

Judged by reports in the peer-reviewed literature alone, the most frequent complications of ephedrine abuse appear to be behavioral. However, case reports describing toxicity in nonabusers are simply too few to permit generalizations. A large collection of controlled clinical ephedrine trials were undertaken in the 1960s and 1970s when drug makers began to introduce new, more effective antiasthmatics. The side effect profile that emerged was not very different than that of pseudoephedrine—generally benign.

2.3.8.1 Neurologic

Ephedrine-induced psychosis has been reported with some regularity (Herridge and a'Brook, 1968; Roxanas and Spalding, 1977; Whitehouse and Duncan, 1987; Ishigooka et al., 1991; Capwell, 1995; Doyle and Kargin, 1996; Jacobs and Hirsch, 2000; Boerth and Caley, 2003; Kim and LeBourgeois, 2004; Miller, 2005). Ephedrine psychosis closely resembles amphetamine psychosis: paranoia with delusions of persecution and auditory and visual hallucinations, but consciousness remains unclouded. Typically, patients with ephedrine psychosis will have to ingest more than 1000 mg/day. Recovery is rapid after the drug is withdrawn (Kalix, 1991; Nakatani and Hara, 1998). The results of neurochemical studies suggest that the basis for ephedrine-induced behavioral changes may be altered dopamine metabolism.

Intracranial hemorrhage has been reported but almost always in the setting of drug overdose (Loizou et al., 1982; Wooten et al., 1983; Stoessl et al., 1985; Glick et al., 1987; Forman et al., 1989; Bruno et al., 1993; Baker et al., 2005; Kendirli et al., 2006; Kunieda et al., 2006). Only a handful of peer-reviewed reports describing cerebral infarction in ephedrine users have been published, and most contain so little detail that causation assessment is impossible (Gorey et al., 1992; Haller and Benowitz, 2000; Vahedi et al., 2000; du Boisgueheneuc et al., 2001; Chen et al., 2004).

2.3.8.2 Renal

Chronic ephedrine use has occasionally been implicated as the cause of renal calculi (Schweisheimer, 1976; Powell et al., 1998; Assimos et al., 1999; Hoffman et al., 2003; Bennett et al., 2004; Song et al., 2005). A large commercial laboratory that analyzes kidney stones found that 200 of 166,466 stones (0.064%) contained either ephedrine or pseudoephedrine (Blau, 1998). Unfortunately, the analytic technique utilized by that laboratory could not distinguish between ephedrine and pseudoephedrine. Except for the possibility of renal stones, no direct effect on the kidney or altered renal function has ever been demonstrated, nor is there any evidence that ephedrine exerts diuretic effects. Urinary retention can occur as a consequence of drug overdose (Glidden and DiBona, 1977; Lindberg, 1988) but has not been reported when recommended doses are consumed.

2.3.8.3 Cardiovascular

Even though it has never been observed by anesthesiologists, who use the drug frequently, it has been suggested that ephedrine causes dangerous QT prolongation. Researchers, using a double-blind controlled study design, found that participants who received ephedrine/caffeine mixtures were more likely to experience a QTc interval increase of at least 30 ms versus placebo (8 individuals—53.3%), which would place them at higher risk of

developing torsades de pointes (McBride et al., 2004). A later study showed that the Bazett correction (the formula used most often by hospital computers to correct the QT interval for rate) overestimates the corrected QT interval: the higher the heart rate increase, the greater the increase in calculated QT interval duration (Milic et al., 2006). Intraspinal ephedrine has no effect on QTc (Sen et al., 2006), and there is evidence that in severely preeclamptic women, spinal anesthesia may in fact normalize the QT interval (Sen et al., 2006). Thus, it appears that concerns over ephedrine-related QT prolongation were actually based on a mathematical artifact.

It has also been suggested that ephedrine causes increased plasma catecholamine concentrations. However, catecholamine concentrations in exercising ephedrine users are not altered by catecholamine administration.

A handful of case reports describe heart failure in ephedrine abusers. One was a 35-year-old asthmatic taking 4000 mg of ephedrine per day and "liberal doses of prednisolone" for 14 years. Another involved a woman who had been abusing ephedrine (300–600 mg/day) for 10 years, and a third case involved a 28-year-old, cigarette-smoker, 321 pound woman taking 2000 mg of ephedrine every day for 8 years (To et al., 1980; Gaulteri, 1996; Schafers et al., 1998; Naik and Freudenberger, 2004; Mark et al., 2005). The difficulty in interpreting these reports is that histologic findings were not described and angiography was not performed, making it virtually impossible to establish the diagnosis of cardiomyopathy.

Similar considerations apply to the possible relationship, if any, between myocardial infarction and ephedrine use. One case report describes a 25-year-old man who injected himself intravenously with an unknown amount of what he believed was amphetamine. It was, in fact, ephedrine, and he sustained a posterior wall infarction. Blood ephedrine concentrations were not determined (Cockings and Brown, 1997). No case report in the peer-reviewed literature has ever linked the use of ephedrine to actual clinical episodes of myocardial infarction. That is not the case, however, for ephedrine isomers. Myocardial necrosis and arrhythmias have been reported both in humans and experimental animals after administration of phenylpropanolamine (Pentel et al., 1982) and, more rarely, after pseudoephedrine (Wiener et al., 1990). The relative paucity of ephedrine-related infarcts (Forte et al., 2006) but the much more common occurrence of infarction with the other ephedrine isomers may be explained by the fact that the latter are both more effective α agonists than ephedrine.

If, in fact, any clinical trial were to link ephedrine use to myocardial infarction, the connection would probably have to do with increased calmodulin kinase II activity. Increased activity of this enzyme is linked to arrhythmias (Wu et al., 2002; Kirchhof et al., 2004), sudden death, and mechanical dysfunction (Zhang et al., 2003). Unlike cocaine, which causes increased production of calmodulin kinase II, leading to myocardial hypertrophy and elevated intracytosolic calcium, thereby increasing the likelihood of lethal arrhythmia (Henning and Cuevas, 2006), ephedrine has never been demonstrated to have any such effect.

2.3.8.4 Gastrointestinal

Chronic ephedrine abusers are prone to develop hepatic steatosis. In the only published autopsy series, fatty liver infiltrates were found in approximately one-fifth of the decedents, but since almost all the decedents were polydrug abusers, attributing causation is impossible (Blechman et al., 2004), especially as there is evidence that, in animal studies at least, ephedrine may be hepatoprotective (Yamada et al., 2008).

2.3.8.5 Dermatologic

The use of ephedrine (Catlin et al., 1993; Villas Martinez et al., 1993) and its enantiomers is occasionally associated with the occurrence of nonpigmented fixed drug eruptions (Tomb et al., 1991; Garcia Ortiz et al., 1997; Anibarro and Seoane, 1998; Vega et al., 1998; Moreno-Escobosa et al., 2002). Similar eruptions have been reported in cocaine users (Hofbauer et al., 1999).

2.3.9 Drug Testing

The IOC, while not entirely banning ephedrine consumption, has ruled that urine levels of over 1000 ng/mL indicate abuse and are grounds for disqualification. The 1000 ng/mL level set by the IOC is probably unrealistically low, since ephedrine is still used as a nasal decongestant in Europe and other countries. IOC rules consider each ephedrine enantiomer separately. However, under its new rules, the World Anti-Doping Agency (WADA) can issue therapeutic use exceptions (TUEs). In order to obtain such an exemption, pulmonary function testing is required. It seems unlikely that ephedra users would ever be granted a TUE since healthy volunteers given realistic doses of ephedrine-containing nasal spray (roughly 14 mg) were found to have urine levels ranging from 0.09 to 1.65 mg/mL (Lefebvre et al., 1992).

Occasionally, innocent nonabusers may find themselves falsely accused of ephedra abuse. A Dutch professional cyclist who thought he was using a perfectly legal, ephedra-containing food supplement found to his surprise that he was taking cathine, a weak stimulant present in both khat and ephedra (Ros et al., 1999). When tested after a competition, the cyclist's urine was found to contain 20 mg/L of norpseudoephedrine (cathine). No species of ephedra contains more norpseudoephedrine than ephedrine, and most contain substantially less. Obviously, the makers of the supplement had been spiking their product with norpseudoephedrine. Ignorance of the law is not a sufficient excuse for the IOC or WADA, since both organizations enforce a policy of strict liability.

2.3.10 Postmortem Tissue Measurements and Autopsy Findings

There is only one controlled study of the postmortem toxicology findings in ephedrine-related deaths. The Office of the San Francisco Medical Examiner undertook a review of all cases from 1994 to 2001, where ephedrine or any of its isomers were detected (Blechman et al., 2004). The anatomic findings in ephedra-positive cases were compared to those in a control group of drug-free trauma victims. Of 127 ephedra cases identified, 33 were due to trauma. Decedents were mostly male (80.3%) and mostly Caucasian (59%).

Blood ephedrine concentrations were <0.49 mg/L in 50% of the cases, but ranged from 0.07 to 11.73 mg/L in trauma victims and 0.02 to 12.35 mg/L in nontrauma cases. Norephedrine was present in the blood of 22.8% (mean of 1.81 mg/L, SD 3.14 mg/L) and in the urine of 36.2% (mean of 15.6 mg/L, SD 21.50 mg/L). Pseudoephedrine was present in the blood of 6.3% (8/127). More than 88% (113/127) of the decedents also tested positive for other drugs, the most common being cocaine (or its metabolites) and morphine. The most frequent pathologic diagnoses were hepatic steatosis (27/127) and nephrosclerosis (22/127). Left ventricular hypertrophy was very common, and coronary artery disease was detected in nearly one-third of the cases. The most common abnormalities in ephedra-related deaths were those generally associated with chronic stimulant abuse. There were no cases of heat stroke and no cases of rhabdomyolysis. In most instances, norephedrine was not detected, suggesting it plays no role in ephedrine toxicity.

2.3.11 Analytic Considerations

The drug is readily measured using conventional chromatographic assays and is usually incorporated into MS methods with the amphetamines. GC–MS has been the most common, using heptafluorobutyric anhydride to produce useful mass spectral definition (Gunn et al., 2010). Separation of herbal phenalkylamines and methcathinone by tandem LC–MS has also been published (Beyer et al., 2007). Chiral separation of enantiomers by GC–MS is also possible (Drake et al., 2011).

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Synthetic Stimulants 3

"Amphetamine" is a term used to denote a class of chemicals that share a common structural backbone. They are structurally related to naturally occurring compounds such as ephedrine (e.g., *Ephedra* spp.) and the ephedrine diastereomer pseudoephedrine. Amphetamines are also chemically related to the endogenous catecholamines, norepinephrine (NE) and epinephrine, although amphetamines themselves are not considered catecholamines. Mixtures of amphetamine salts are widely used to treat attention deficit/hyperactivity disorder (ADHD). Methamphetamine (MA), although it does have some very limited clinical applications, is primarily a drug of abuse.

Many amphetamine analogs have been created and are readily available worldwide. The most widely known are 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine, 3,4-methylenedioxyethylamphetamine (MDEA), and *p*-methoxyamphetamine (PMA). Numerous analogs such as "2C-B" have been produced and all have strong stimulant activities. Several piperazine and pyrrolidinophenone derivatives also mimic the actions of amphetamines. These are discussed here. The increasingly popular new drug mephedrone (4-methylmethcathinone) belongs to a series of beta-keto amphetamine derivatives based on cathinone, a naturally occurring amphetamine found in the khat plant. Other members of this group include butylone (bk-MBDB [1,3-benzodioxolyl-*N*-methylbutanamine]) and methylone (bk-MDMA).

In the United States, amphetamine salts are combined to form a proprietary drug called Adderall, the single most popular drug used to treat ADHD. Dextroamphetamine abuse per se is uncommon in the United States, but still popular in Europe. Use of MA is growing very rapidly in Eastern Europe. In the United Kingdom, methamphetamine is spelled metamfetamine, and dextroamphetamine is spelled dexamfetamine. In terms of absolute numbers, cocaine is likely to be the most popularly abused drug, but its geographic distribution means that in many countries, after cannabis, the second most common illicit drug is not cocaine at all, but MA.

3.1 Amphetamines

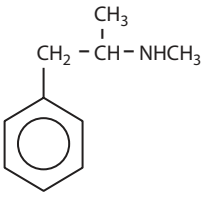
3.1.1 Physical, Chemical, and Pharmacokinetic Properties

The two amphetamines of most interest are MA and amphetamine. Both share similar chemical and pharmacokinetic properties. These are summarized in Tables 3.1 and 3.2.

3.1.2 Incidence and Availability

In the United States, the use of MA has increased dramatically since the 1990s, when fewer than a thousand deaths were reported (Kissin et al., 2000), but there is no very good way to track that increase. Ever since the U.S. government reformatted the system used to track

Table 3.1 Physiochemical Properties and Pharmacokinetics of Methamphetamine

Chemical name	*N*-α-dimethylbenzeneethanamine, *d*-*N*-α-dimethylphenethylamine, and *d*-deoxyephedrine	
Physiochemical properties, structure, and form	Free base and hydrochloride salt CAS 537-46-2 MW 149.2 (base) pK_a 10	
Synonyms	(+)-(*S*)-deoxyephedrine; (+)-methamphetamine; (+)-methylamphetamine; (+)-*N*-methylamphetamine; (*S*)-*N*, α-dimethylbenzeneethanamine; 1-phenyl-2-methyl-amino-propan (German); benzeneethanamine; *N*-α-dimethyl-, *d*-(*S*)-methamphetamine-amine; *d*-1-phenyl-2-methylaminopropan; *d*-1-phenyl-2-methylaminopropane; *d*-de-oxyephedrine; *d*-desoxyephedrine; *d*-methamphetamine; *d*-methylamphetamine; *d*-*N*, α-dimethylphenethylamine; *d*-*N*-methylamphetamine; *d*-phenylisopropylmethyl-amine; *L*-methamphetamine; methamphetamine; methyl-β-phenylisopropylamine; methylamphetamine; *N*-methylamphetamine; Norodin; (*S*)-(+)-methamphetamine	
Brand names	Desoxyn (Abbot, USA), Methampex (Lemmon, USA), Methedrine (Wellcome, UK), Pervitin (Trenker Bldg.), Temmler (Germany)	
Pharmacokinetic parameters	Bioavailability: Oral: 67% (Cook et al., 1993) Smoked: 90% (Cook et al., 1993) C_{max} (oral) 10 mg 14–90 ng/mL (Cook et al., 1993; Schepers et al., 2003; Huestis and Cone, 2007) 20 mg 26–320 ng/mL (Cook et al. 1993; Schepers et al., 2003; Huestis and Cone, 2007) C_{max} (smoked) 22 mg 47 ng/mL (Perez-Reyes et al., 1991) T_{max} 2–8 h after oral administration, 2–3 h after smoking, and 3–4 h after nasal administration V_d 3–4 L/kg Protein binding: 10%–20%	
Common blood concentrations in drug users	Methamphetamine: up to ~0.1 mg/L Amphetamine metabolite: usually about 1/5 of parent	
Blood terminal elimination half-life	Methamphetamine Amphetamine Likely to reduce with advanced age, and liver and kidney disease, and affected by urine pH	10–30 h 4–15 h
Metabolism	Amphetamine, *p*-hydroxymethamphetamine; *l*-methamphetamine is a metabolite of selegiline, fenethylline, and some other legally available drugs.	
Urinary excretion	Most is excreted in 2–4 days depending on doses, urine pH, and analytic cutoff (Schepers et al., 2003). Methamphetamine Amphetamine *p*-hydroxymethamphetamine	 35%–45% 4%–7% 15%

(*Continued*)

Table 3.1 (*Continued*) Physiochemical Properties and Pharmacokinetics of Methamphetamine

Postmortem artifacts	Blood concentrations can approximately double after death, even in peripheral blood samples.
Interactions	Methamphetamine induces production of CYP2C6, which could have undesirable effects when certain other drugs are taken (e.g., codeine and many of the antidepressants), and some of these interactions are unpredictable because in most situations it is not known whether the individual is a slow, rapid, or ultrarapid metabolizer (the last term used to describe a person who carries multiple copies of CYP2C6).
Published reviews	Barnett et al. (1981), Cone (1995)

drug deaths (the medical component of the Drug Abuse Warning Network [DAWN] report), it has become impossible to determine on a countrywide basis how much MA is actually being produced or how much is used, let alone how many users become ill, or even how many die. This difficulty is compounded by the fluid state of the illicit drug market and government delays in reporting times.

MA and amphetamine together account for most of the illicit amphetamine produced. The United Nations Office on Drugs and Crime (UNODC) estimates that in 2009, production amounted to 353 tons (UNODC, 2012).

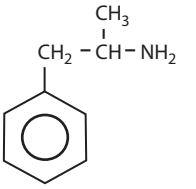
The amount of MDMA on the streets is much smaller. The UN estimates that annual MDMA production amounts to less than 126 metric tons per year. There is some evidence that MA production began to decline in 2001–2003, probably as a result of new, readily available designer drugs. The new increases that have been reported by various international agencies are thought to reflect a worldwide increase in MDMA production. At the same time, record quantities of amphetamine precursors have been seized, and an increasing number of clandestine laboratories are being discovered every year. These developments suggest that production of both amphetamine and MDMA is, at present, increasing worldwide. The UN attributes this increase both to the attractiveness of the drug among young people and the high profit realized by criminal suppliers.

The apparent upward trend in the popularity of MDMA use worldwide seems genuine. Production may well have declined in the Netherlands (the world's largest ecstasy-producing country), and evidence points to decreasing consumption in the United States. Elsewhere, the situation is not so encouraging. The 2013 edition of the *World Drug Report* (UNODC, 2013b), which lumps all amphetamine-type drugs, including ecstasy, together as "amphetamine-type drugs" or amphetamine-type stimulants (ATS), reports that in 2011, an estimated 0.7% of the global population aged 15–64, or 33.8 million people, had used ATS in the preceding year. The prevalence of "ecstasy" in 2011 (19.4 million, or 0.4% of the population) was lower than in 2009.

3.1.3 Production

Unlike heroin and cocaine production, illicit amphetamine production is decentralized, making it impossible to track global production trends. The UN estimates that in 2008, amphetamine production (all types) was in the range of 161–588 metric tons. Whatever the exact numbers, the amount of MA being produced is clearly increasing.

Table 3.2 Physiochemical Properties and Pharmacokinetics of Amphetamine

Chemical name	1-phenylpropan-2-amine
Physiochemical properties, structure, and form	Amphetamine sulfate, very soluble in water; also as phosphate salt CAS 300-62-9 (base) MW 135.2 (base) p*K*a 9.9 
Synonyms	1-(+/−)-benzedrine, (+/−)-desoxynorephedrine, (+/−)-β-phenylisopropyl-amine, 1-methyl-2-phenylethylamine, 1-phenyl-2-aminopropane, 3-methoxy-α-methylbenzeneethanamine, 3-methoxyamphetamine, 3-methoxyphenylisopropylamine, actedron, adipan, allodene, α-methylbenzeneethanamine, amphetamine (narcotics), amphetamine base, amphetamine sulfate, anorexide, anorexine, benzebar, benzedrine, benzolone, β-aminopropylbenzene, dl-α-methylphenethylamine, *dl*-amphetamine, dl-benzedrine, desoxyn, dexampex, dexedrine, dextroamphetamine sulfate, dextrostat, *dl*-1-phenyl-2-aminopropane, elastonon, fenamin, fenylo-izopropylaminyl, ferndex, finam, isoamycin, isoamyne, isomyn, mecodrin, methampex, *m*-methoxy-α-methylphenethylamine, *m*-methoxyamphetamine, [1-(3-methoxyphenyl)-2-propylamine, norephedrane, norephedrine, deoxy-novydrine, oktedrin, ortedrine, paredrine, percomon, phenamine, phenedrine, phenylisopropylamine, profamina, propisamine, psychedrine, racemic-desoxynor-ephedrine, raphetamine, rhinalator, simpatedrin, simpatina, sympamin, sympamine, sympatedrine, weckamine
Brand names	Adderall (amphetamine + dextroamphetamine), Adderall XR 10–25 mg (amphetamine aspartate + amphetamine sulfate dextroamphetamine saccharate + dextroamphetamine sulfate); Benzedrine, Dexedrine, Dextrostat, Desoxyn, ProCentra, Vyvanse
Pharmacokinetic parameters	Bioavailability: Oral: 67.2 ± 3% Smoked: 90.3%; appears to be greater when stomach is empty (Wan et al., 1978) C_{max} (oral) 10 mg: 14.5–33.8 ng/mL (Schepers et al., 2003) C_{max} (smoked) 22 mg: 47.1 ± 5.6 (Perez-Reyes et al., 1991) T_{max} (oral): 2–8 h (Schepers et al., 2003) T_{max} (smoked): 2.5 ± 5 h (Perez-Reyes et al., 1991) V_d 3–5 L/kg Protein binding 23%–26% (Franksson and Angaard, 1970)
Common blood concentrations in drug users	Amphetamine: up to ~0.1 mg/L
Blood terminal elimination half-life	4–30 h (Perez-Reyes et al., 1991; Cook et al., 1993; Schepers et al., 2003; Mendelson et al., 2006). The mean elimination half-life ($t_{½}$) for *d*-amphetamine was shorter than the $t_{½}$ of the *l* isomer (9.8–11 h vs. 11.5–13.8 h) and is likely to be reduced with advanced age and liver and kidney disease and is markedly affected by urine pH.

(*Continued*)

Table 3.2 (*Continued*) Physiochemical Properties and Pharmacokinetics of Amphetamine

Metabolism	Deamination, oxidation, hydroxylation, conjugation producing norephedrine and hippuric acid. P450 enzymes CYP2D and CYP3A form 4-hydroxyamphetamine (and subsequent conjugates) and amphetamine (Dostalek et al., 2007). Amphetamine is also a metabolite of the antiparkinson drug selegiline, clobenzorex, fenproporex, fenethylline, mefenorex, and benzphetamine.
Urinary excretion	Most is excreted in 2–4 days depending on doses, urine pH, and analytic cutoff. Approximately 3%–60% of amphetamine is excreted unchanged.
Postmortem artifacts	Blood concentrations can approximately double after death, even for peripheral blood.
Interactions	Amphetamines as a group are substrates for both P-gp and CYP3A4. They may alter the transport and metabolism of protease inhibitors and nonnucleoside reverse transcriptase inhibitors if administered in combination with azole antifungals, macrolide, and fluoroquinolone antibiotics, statins, some cardiovascular agents, immune modulators, and even other abused drugs, in particular benzodiazepines, cocaine, lysergic acid diethylamide, marijuana, amphetamine (meth), 3,4-methylenedioxymethamphetamine, and some opiates (Auret et al., 2006).
Published reviews	Kraemer and Maurer (2002), Drummer (2004), Bortolotti et al. (2012)

The U.S. government maintains a database called System to Retrieve Information from Drug Evidence (STRIDE) comprised of all drug exhibits submitted to its laboratories. The database itself is not intended to reflect market trends but does serve as a surrogate because it accurately tracks prices and purity. The STRIDE data show that from January 2007 through September 2009, the price per gram of pure MA decreased by 13%, from $147 to $127 while, at the same time, purity increased by 12%, from 57% to 69% (U.S. Department of Justice, 2010). These changes are illustrated in Figure 3.1.

In 2007, after many years of ignoring international law, Mexico finally imposed import restrictions on pseudoephedrine and ephedrine (the precursors used for making MA; see Figure 3.2). This led to a sharp decline in Mexican MA production in 2007 and 2008. Unfortunately, the decline was only transient since Mexican production exceeded previous levels in 2009. During that same period, production in the United States remained stable. The number of MA laboratory seizures in the United States in 2009 exceeded that for 2008. Legitimate medical indications for MA and amphetamine have all but disappeared, mostly because amphetamine salts, used in the treatment of ADHD, have largely been replaced by methylphenidate. However, while legitimate medical use has decreased, there has been a recent dramatic rise in the number of illicit MA laboratories operating around the world. High levels of production in Mexico, along with an increase in the number of domestic manufacturing operations, have combined to make MA readily available throughout the United States. Law enforcement and intelligence reporting, as well as seizure, price, and purity data, all indicate that the availability of MA in general is increasing in markets in every region of the United States, while MA prices have declined steadily since peaking in 2007; purity levels have increased concurrently. Mexico remains the primary source of

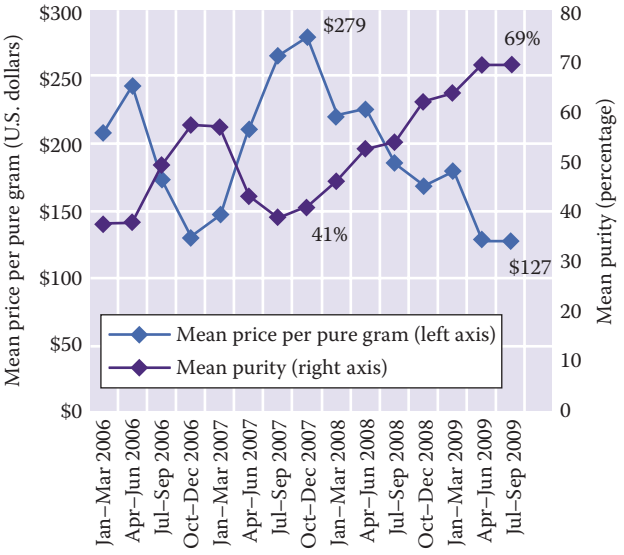


Figure 3.1 Methamphetamine prices and purity 2006–2009 in the United States (STRIDE). *Note:* STRIDE is a database of drug exhibits maintained by the U.S. Drug Enforcement Administration. The values reported here represent averages of all methamphetamine purchases in the database. Although not collected as a representative sample of the U.S. market, STRIDE data reflect the best information available on changes in methamphetamine price and purity in the U.S. market. (From U.S. Department of Justice, *National Drug Threat Assessment 2010*, National Drug Intelligence Center, 2010.)

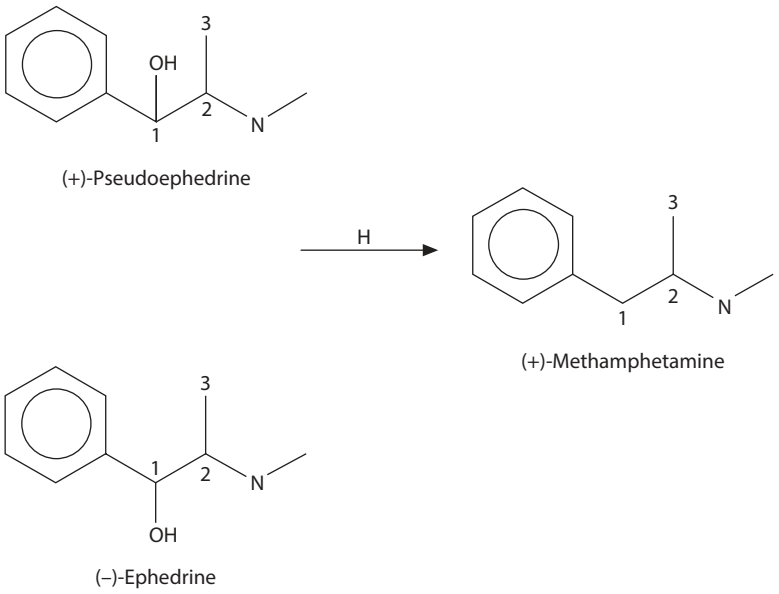


Figure 3.2 Synthesis of MA. The most popular formula for making MA starts with ephedrine and uses red phosphorus as a catalyst. Ephedrine used to be inexpensive and easily available, but now its sales are controlled and availability limited.

the U.S. MA supply. Manufacturers are maintaining high levels of production, somehow adapting to strong government of Mexico precursor chemical control laws.

3.1.4 Epidemiology

In 2011, there were 670,000 persons aged 12 or older who had used cocaine for the first time within the past 12 months; this averages to approximately 1,800 initiates per day. This estimate was similar to the number in 2010 (642,000), 2009 (623,000), and 2008 (724,000). The annual number of cocaine initiates declined from 1.0 million in 2002 to 670,000 in 2011. The number of initiates of crack cocaine declined during this period from 337,000 to 76,000. In 2011, most (74.7%) of the 0.7 million recent cocaine initiates were 18 or older when they first used. The average age at first use among recent initiates aged 12–49 was 20.1 years. The average age estimates have remained fairly stable since 2002 (Substance Abuse and Mental Health Services Administration, 2012). The U.S. DAWN survey estimates that of the 2.1 million drug-related emergency room visits, slightly over 21% of all visits (Table 3.3) involved the use of illicit drugs (Monitoring the Future, 2011; Center for Behavioral Health Statistics and Quality, 2013).

These results confirm the findings of the UN and European Monitoring Centre for Drugs and Drug Addiction (EMCADDA) that indicate that the use of all types of amphetamines is decreasing, at least within the United States (National Survey on Drug Use and Health, 2010). More than one quarter (27.6%) of MA-related emergency room visits during 2008 involved MA combined with one other drug, and 34.2% involved MA combined with two or more other drugs (Figure 3.3). Almost one quarter of the MA-related emergency room visits also involved alcohol (24.0%) or marijuana (22.9%). Of all MA-involved emergency room visits, 6 in 10 (60.0%) resulted in patients being treated and released.

Admissions to substance abuse treatment for primary MA abuse were more than twice as likely in 2007 to involve those aged 40 or older (23%) compared with admissions in 1997 when they amounted to only 10%. The percentage of primary MA admissions that were Hispanic more than doubled from 9% in 1997 to 21% in 2007. The percentage of MA smokers increased each year from 1997 to 2007—from 27% in 1997 to 67% in 2007.

Of the 1.8 million admissions to substance abuse treatment in 2005, 169,500 admissions were primarily for MA and amphetamine abuse, representing 9% of all admissions. In addition, more than 80,000 admissions were for secondary or tertiary MA/amphetamine abuse, representing an additional 4% of all admissions. Sixty-six percent of primary MA/amphetamine admissions reported use of other substances, including marijuana

Table 3.3 U.S. Government Estimate of Individuals Hospitalized for Drug Abuse

	2005–2009				
	2005	2006	2007	2008	2009
Cocaine	267,922	265,969	249,980	227,786	183,932
Heroin	260,591	268,731	262,579	281,159	282,212
Marijuana	302,783	304,123	305,038	341,622	354,159
Methamphetamine	154,358	152,516	139,267	119,447	108,229

Sources: Treatment Episode Data Set 2009; U.S. Department of Justice, *National Drug Threat Assessment 2011*, National Drug Intelligence Center, 2011.

Note: The number of individuals hospitalized from 2006 to 2009 is sorted by drug. Note that in 2005, the number of cases involving cocaine was nearly double the number due to methamphetamine. By 2009, they were no longer very different.

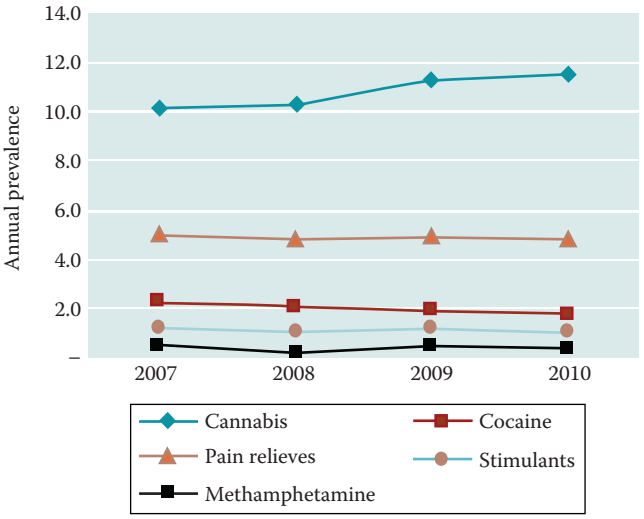


Figure 3.3 Annual prevalence of illicit drug use among the population aged 12 and over in the United States, 2007–2010 (percentage). (From Department of Health and Human Services, Substance Abuse and Mental Health Services Administration, *Results from the 2010 National Survey on Drug Use and Health: Detailed Tables*, Substance Abuse and Mental Health Services Administration, Rockville, MD, 2011.)

(41%), alcohol (34%), and cocaine (10%). In 2005, those who were admitted primarily because of MA/amphetamine were, on average, 3 years younger than admissions in which other substances were the primary drug of use (31 years vs. 34 years). Conversely, primary MA/amphetamine admissions were an average of 3 years older than other admissions when they first used their primary substance (21 years vs. 18 years). Taken together, these findings indicate that, on average, each of these individuals had used their primary drug for 6 years before admission to treatment.

Primary MA and amphetamine admissions are more likely to involve female users, while the opposite holds true with other abused drugs (46 vs. 31%). Nearly three quarters of primary MA and amphetamine admissions were white (71%) compared with 58% of other admissions. Hispanic admissions also accounted for a higher proportion of primary MA and amphetamine admissions than of other admissions (18 vs. 13%). In contrast, black admissions accounted for a greater proportion of admissions for substances other than MA/amphetamine admissions (24 vs. 3%) (National Survey on Drug Use and Health, 2010).

3.1.5 History

In the 1920s, ephedrine was the principal drug used to treat asthma, and there was concern that there might be an insufficient supply of naturally occurring ephedrine to meet the needs of asthma sufferers. Laboratories around the world attempted to create a synthetic form. A graduate student at the University of California at Los Angeles, Gordon Alles, was assigned the task of synthesizing amphetamine as his thesis project. Alles reviewed the older literature and discovered previous research carried out by an Italian chemist named Edeleano, who had synthesized the basic phenylisopropylamine molecule in 1887. Alles took the phenylisopropylamine molecule as a starting point from which to synthesize ephedrine. He never succeeded, at least not in his attempts to produce ephedrine. He did, however, produce

a molecule called phenylisopropylamine, later named dextroamphetamine. He gave samples to laboratory animals and, when he saw little evidence of toxicity, tried it on himself. The mood-altering properties of this novel molecule quickly became apparent.

A Japanese chemist named Ogata had also been trying to synthesize ephedrine, but Ogata ended up producing a different amphetamine, *d*-phenyl-isopropylmethylamine hydrochloride, later known as MA. Hermann Emde finally managed to synthesize ephedrine in 1929, but the anticipated ephedrine shortage never occurred.

Ogata licensed his process for making MA to the Burroughs Wellcome Company, which sold MA in the United States under the brand name Methedrine® until it was taken off the market in 1968. In 1932, the Smith Kline & French company sold a nasal inhaler containing Benzedrine®, their patented name for racemic β-phenylisopropylamine (*d*,*l*-amphetamine). The inhaler effectively relieved nasal congestion as well as alleviating drowsiness and fatigue. The latter quality, along with exaggerated claims by both drug manufacturers and the popular press, led to widespread interest and even more widespread abuse.

The medical community responded to the introduction of amphetamine in almost exactly the same way it had responded to the introduction of cocaine 50 years earlier. Amphetamines were recommended for the same assortment of unrelated conditions that had been treated with cocaine when it was first introduced. Given what is known today, some of the earlier recommended uses for the amphetamines appear bizarre. For example, amphetamine was recommended as a "valuable adjunct" in the treatment of seizures and schizophrenia. Bearing in mind that amphetamine-induced psychosis is still thought by some (Bell, 1965; Janowsky and Risch, 1979; Murray et al., 2013) to be a useful model for the study of schizophrenia, it is difficult to imagine what type of improvement clinicians were observing!

Amphetamines were also said to be useful in treating barbiturate overdose, "caffeine mania," smoking, multiple sclerosis, myasthenia, head injuries, cerebral palsy, migraine, urticaria, seasickness, dysmenorrhea, ureteral colic, obesity (Figure 3.4), irritable colon, radiation sickness, Ekbom's syndrome, and other seemingly unrelated conditions—even impotence and loss of libido (Bett, 1946). It should come as no surprise that amphetamine was even recommended for use in the treatment of morphine addiction. By the time amphetamine arrived on the scene, Freud's disastrous cocaine experiments of 1885 apparently had been completely forgotten (Wax, 1997).

Moderate doses of *d*-amphetamine increase the ability to sustain attention over prolonged periods of time, especially when performing monotonous tasks. Like cocaine, *d*-amphetamine improves performance on auditory and visual reaction time tests and on the digit symbol substitution test, a measure of psychomotor skills and attention (Heishman, 1998). These actions were recognized very soon after amphetamine became commercially available, which probably explains why, during World War II, troops of both the Allied and Axis forces were supplied with amphetamines.

Soon after World War II, laws limiting amphetamine distribution were enacted, but a regulatory lapse allowed the continued sale of Smith Kline & French's nasal inhaler (Figure 3.5). Inside each inhaler were eight folded paper sections impregnated with 250 mg of amphetamine. Abusers opened the inhaler and chewed the papers. Friends mailed the strips to prison inmates, and abuse within the prison system became a problem (Monroe and Drell, 1947). In an escalating battle with would-be abusers, amphetamine manufacturers tried adding denaturants such as emetine and picric acid to the strips, but abusers found ways to extract the amphetamine, or simply put up with the transient side effects produced by the denaturants.

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For willpower alone is not enough. Your kinds of help are sorely needed. You alone can meet the patient's individual need for authoritative diagnosis and advice in the struggle against overweight. You alone can help the patient deal with underlying emotional factors and establish sensible eating habits.

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for "agitated" overeaters

BIPHETAMINE-T® *a 'strasionic' release anoretic*

BIPHETAMINE-T '20'
BIPHETAMINE-T '12½'

Single Capsule Daily Dose 10 to 14 hours before retiring

STRASENBURGH

Figure 3.4 Medical use of amphetamine. For many years, amphetamine was promoted as a treatment for obesity. When first introduced to the market, amphetamine was claimed to be something of a wonder drug. The same claims were made for amphetamine as were made for cocaine when it was first introduced. This advertisement was published in a 1961 issue of *JAMA*.

Figure 3.5 Benzedrine® Inhaler made by Smith Kline & French Laboratories. A regulatory lapse allowed continued over-the-counter sales until 1949. Each inhaler contained eight folded paper sections impregnated with 250 mg of amphetamine.

The Benzedrine® inhaler was reformulated in 1949 and renamed Benzedrex®. The new formulation contained propylhexedrine, also a potent vasoconstrictor, but with only one-twelfth the central nervous system (CNS) stimulant potency of amphetamine. Smith Kline & French's patents expired in 1953, and almost immediately, Wyeth, Rexall, Squibb, Eli Lilly, and W. S. Merrell all brought competing products to market. Inhaler abuse continued until the amphetamine inhaler was finally classified as a prescription item.

The first amphetamine-related deaths were reported within a few years of the introduction of amphetamine. The serious complications associated with amphetamine abuse are essentially the same as for cocaine—arrhythmic sudden death (Jacobs, 2006), stroke, psychosis, and rhabdomyolysis—but they seem to occur much less frequently than with MA. Most of the case reports are from the 1950s and 1960s. Mentions of toxicity were uncommon during the 1980s, when use was largely confined to the desert Southwest, but have been steadily increasing since the turn of this century.

With the introduction of smokable "ice," a pure form of (+)-methamphetamine hydrochloride, interest in MA as a drug of abuse revived, and new case reports of toxicity began to appear (Cho and Wright, 1978). MA becomes "ice" when it is crystallized out of a saturated solution. The size of the crystal depends on the volatility of the solvent. Depending on how MA is initially prepared (and a number of ways are possible), solvent is captured within the structure of the crystals. The type of solvent is a clue to which processes were used in the manufacture of the drug and may also suggest where the illicit drug was made (Skinner and Gilmore, 1964). With very volatile solvents, such as Freon®, crystallization is so rapid that only very small crystals form. With less volatile solvents, such as methanol, larger crystals are produced. No matter the size of the crystals, they can all be smoked and all produce exactly the same effects (Figure 3.6).

The first illicit "ice" laboratories were located in Japan. The Japanese have referred to this particular form of MA by a number of different names, including *kaksonjae*, *hanyak*, *batu*, and *hiropon*. The use of the name *hiropon* is doubly ironic because that was the name the Japanese used for ephedrine during the epidemic of ephedrine abuse that swept Japan just after World War II. Today, ephedrine is the universally preferred starting material for making MA. Large-scale "ice" production began in the early 1980s.

Figure 3.6 "Ice" crystals. Crystal size depends on how rapidly the solvent evaporates and has nothing to do with the effect observed. If MA were powdered and smoked, it would exert the same effect. (From the website of the DEA.)

Enforcement efforts by police convinced the illegal chemists to transfer their operations out of Japan to Korea. To this day, Korea remains the principal manufacturer of "ice." At first, the market for this form of amphetamine was confined to Taiwan, Japan, and the Philippine Islands. Japanese and Korean abusers took it intravenously, but the Filipinos began smoking it. The Filipinos were already used to smoking stimulants, having smoked *shabu* (a mixture of ephedrine and caffeine) for years. Demand within the Filipino community was also thought to be responsible for the introduction of "ice" into Hawaii (Skinner and Gilmore, 1964).

In the late 1980s, Korean chemists emigrated and established illicit laboratories in Portland, Oregon, and Los Angeles. Most of their production was shipped back to the Philippines. In 1988, sporadic seizures of "ice" took place across the United States, but no laboratories were seized in 1989. By 1990, the Drug Enforcement Agency (DEA) had seized seven laboratories in California alone. Impressive amounts of "ice" continue to be seized in China and Korea, but not in the United States. No "ice" laboratories have been raided in the United States for several years, nor do any of the thousands of clandestine "meth" laboratories raided each year appear to be producing "ice."

The UN's Synthetics Monitoring: Analyses, Reporting and Trends (SMART) program was first launched in Bangkok in 2008 and now collects data throughout the East Asia and the Pacific region. It reported that in 2012, a total of 227 million MA pills were seized in East and Southeast Asia, a 59% increase from the 142 million pills seized in 2011 and a more than sevenfold increase since 2008, when 31.1 million pills were seized. Most pills were seized in China (102.2 million), Thailand (95.3 million), Myanmar (18.2 million), and Lao PDR (10.1 million), which together account for 99% of all pills seized in 2012. The total amount of crystalline MA seized increased by 12% to 11.6 tons. Record level seizures were reported in a number of countries (UNODC, 2013a). Parallel increases also occurred in production and seizures of crystalline MA.

Amphetamine trafficking has spread well beyond its traditional regions. Increases have been reported, inter alia, from South Africa, while use of MA has increased in parts of Asia not traditionally affected by these drugs, and this trend is likely to continue. The European market for MA, which at one time was nearly nonexistent, is now much greater. MA abuse is a major problem in Germany (where amphetamine was always the drug of choice) (UNODC, 2006). Traditionally, different regions have had problems with different amphetamine-type drugs. Initially, ecstasy abuse seems to have been limited to English-speaking countries, but now has expanded throughout Europe, the Americas, the Oceania region, and many parts of East and Southeast Asia. MA has been problematic in East Asia and Southeast Asia over the last decade, as well as in North America and Oceania. Amphetamine was found primarily in Western Europe, though in recent years the Middle East has emerged as a major new market, with demand for pills called "Captagon" (a type of amphetamine), for which the UN reports increasing demand in the Middle East.

3.1.6 Illicit Manufacture

Legitimate medical indications for MA have essentially ceased to exist (save for narcolepsy), but amphetamine salts are very widely used for the treatment of ADHD, as is methylphenidate, and legal production of these two ADHD drugs has increased. Whenever supply increases, concerns regarding illegal diversion always arise, but this does not appear to be

happening, at least with these drugs, on a significant scale. The real concern is the dramatic rise in the number of illicit MA laboratories operating around the world. In 1992, the U.S. DEA reported the seizure of only 263 in 1994, but by 1999 the number had surpassed 2000 to 1627. By 2008, nearly 2000 clandestine MA laboratories were seized in the state of Iowa alone. These findings are proof that a thriving market for these products exists (Tichacek and Napolitano, 1999). Raw data from various U.S. agencies continue to suggest modest increases in MA production. The most recent U.S. report to discuss production is the "Fact Sheet" posted by the White House Drug Policy; it has not been revised since 2009 and is, no doubt, well out of date.

Mexico finally imposed restrictions on pseudoephedrine imports and ephedrine exports (the precursor chemicals used for making MA; see later) in 2007, leading to a sharp decline in Mexican MA production in 2007 and 2008. However, by the time the Mexican producers were finally reined in, production in the United States began to increase. Users and distributors compensated for the reduced foreign supply by shifting to different precursors, and production in the United States showed no decline. In fact, in the United States, the number of MA laboratory seizures in 2009 exceeded the number in 2008.

Ephedra is grown commercially in both India and Pakistan and sold to clandestine laboratories in Myanmar (which produces mainly for export) and Thailand (which produces mainly for local and regional consumption). Occasional batches of MA from both countries have been confiscated by U.S. customs, but the amount of MA being exported to the United States from Southeast Asia is not really known. Similarly, clandestine laboratories have sprung up in Poland and the Czech Republic, but data from these locations are quite limited and the true magnitude of production at these locations is also not known (Patterson, 2006).

As phenyl-2-propanone (P2P) is no longer freely available, the precursors of choice have become a mixture of methylamine, aluminum foil, mercuric chloride, diethyl ether, and isopropanol. High yields can be obtained via this synthetic route. Formulas for production using readily available materials are easily found on the Internet and the materials can be purchased legally at hardware stores. Legislation has been introduced in the U.S. Congress that would make the act of disseminating information about drug manufacture illegal, and the Patriot Act was amended in 2006 to severely limit the sales of ephedrine, pseudoephedrine, and phenylpropanolamine, but the basic materials required are used so universally it is hard to envision that there will ever be anything like total eradication.

MA made from P2P yields a racemic mixture. Because the (+) form of MA is five times as potent as the (–) isomer, the potency and yield of the final product can be variable. Not only can the potency vary, but an assortment of contaminants can also be introduced. Some of the contaminants have strong stimulant properties themselves (Soine, 1986), while others may be quite toxic, possibly even more toxic than amphetamine itself. In the United States, the P2P synthetic route has been entirely replaced by the "red phosphorus" route (Figure 3.3), and P2P toxicity is no longer an issue.

Other alternative synthetic routes use either (–)-ephedrine or (+)-pseudoephedrine, which can be converted to MA by reductive dehalogenation using red phosphorus as a catalyst. If (–)-ephedrine is used as the starting point, the process generates (+)-methamphetamine. Pseudoephedrine also yields dextromethamphetamine. Regardless of the isomer produced, contaminants will be present. As is true with the P2P route, some of these contaminants, particularly 2-(phenylmethyl) phenethylamine, may also be toxic in their own right. Unfortunately, the subject still has not been studied in any detail (Soine, 1986).

The popularity of the red phosphorus route created an unprecedented demand for ephedrine, which the Food and Drug Administration (FDA) removed from the market—not because of ephedrine's use in MA manufacture but rather because the FDA believes that ephedrine itself is dangerous. As a consequence, pseudoephedrine is now largely used instead of ephedrine, and if the latter is used as a starting material in MA synthesis, it yields pure *l*-methamphetamine. Occasionally, MA "cooks" who can no longer obtain the usual epinephrine use assorted cold remedies instead. Mixtures of diphenhydramine, chlorpheniramine, dextromethorphan, doxylamine, and even guaifenesin have all been reported. All of these chemicals, except for chlorpheniramine, react with red phosphorus and hydroiodic acid to form an assortment of by-products that may be detected in the finished product.

For example, diphenhydramine (Benadryl™) is converted to diphenylmethane, but because it does not crystallize, at least not with the techniques used to crystallize MA, it is unlikely to appear in the final product. Other by-products generated by the hydroiodic acid/red phosphorus reduction method include 1,3-dimethyl-2-phenylnaphthalene and 1-benzyl-3-methylnaphthalene (Lurie et al., 2000). Whether any of the other intermediates and by-products produced in this fashion are toxic in their own right is not known.

Now that phenylpropanol, ephedrine, and pseudoephedrine are all considered controlled substances, either by international treaties on drug precursors or by federal law, the phenylacetone synthetic route is now increasingly used. This route also has the virtue of simplicity. It yields a racemic mixture, and the effects of a synthetic mixture are not necessarily benign. The *l* form of MA had been thought to be benign and is still sold as a nasal decongestant (Vicks™); however, its presence creates a forensic challenge, because *l*-methamphetamine is a legal substance and *d*-methamphetamine is not, and both forms have slightly different pharmacokinetic profiles (Mendelson et al., 2008). A pathologist investigating a possible MA-related death has no way to tell how much of the MA concentration reported by the laboratory is active drug and how much is inert. Chiral separation can be used to separate the two forms, but the separation process is difficult and time-consuming and is not performed by most toxicology laboratories. A special request will need to be made to the laboratory if use of *l*-methamphetamine is suspected.

MA can also be made using hypophosphorous acid (HI), an industrial chemical used legally for a variety of commercial purposes. This approach works just as well as the red phosphorus route. Although HI is a List I chemical under the Controlled Substances Act, MA producers typically purchase the chemical via the Internet or from associates who also are engaged in MA production. Unlike red phosphorus, the use of HI in MA production is an extremely dangerous practice. Deadly gases can be generated and there is significant risk of fire or explosion.

Even though a comprehensive agreement on international chemical control was adopted in the 1988 UN Convention Against Illicit Traffic in Narcotic Drugs and Psychotropic Substances, HI remains readily available. The convention covers MA's precursor chemicals such as ephedrine and pseudoephedrine, but it exempts finished pharmaceutical preparations containing them. This "loophole" allows criminal organizations to circumvent the convention by purchasing uncontrolled pharmaceutical preparations on the international market, instead of the regulated bulk precursor chemicals. Furthermore, many countries have simply been reluctant to share information regarding their trade in these substances, because much of the data are commercially sensitive. Complicating matters still further, in some countries these chemicals are regulated by health, rather than law enforcement, agencies (Patterson, 2006).

3.1.7 Routes of Administration

MA can be swallowed, injected, smoked, or "snorted." Very little research has been published about abuser preferences, and not much more about pharmacokinetics, but smoking appears to be the most popular method, and there is some evidence that the smoked route is more addictive (McKetin et al., 2006). Similar studies with amphetamine have yielded comparable results, at least in terms of resultant blood levels. As with cocaine, "body packers" who have concealed packets of drug within their body may die from massive overdose. A 2014 report describes a young female prisoner found dead in her cell with a packet of MA in her vagina. Samples of subclavian blood, vitreous fluid, and urine contained massive concentrations of MA (42.6, 20.1, and 771 mg/L, respectively). Amphetamine, the active metabolite of MA, was also present in the subclavian blood, vitreous fluid, and urine at significant concentrations (1.3, 0.5, and 20.4 mg/L, respectively) (Jones et al., 2014).

The pharmacokinetics of smoked and intravenously injected MA have been compared in male volunteers acting as their own controls (Cook et al., 1993). The bioavailability via smoking is very high and is estimated to be over 90%. The actual amount is very much dependent on the technique of ingestion used. Blood (plasma) concentrations in MA abusers vary enormously depending on the route of administration, dose, and timing of sample collection and may easily exceed 0.1 mg/L, particularly with repeated use. The drug is very basic and significant amounts are excreted unchanged in the urine. This means that urinary pH can markedly affect the clearance of the drug. Amphetamine concentrations are always much lower than those of MA, reaching peak values about one-tenth of the parent drug (see Tables 3.1 and 3.2 for further details).

3.1.8 Metabolism (Figure 3.7)

MA metabolism is catalyzed by cytochrome P450 (CYP), mainly by the CYP2D and CYP3A subfamilies, leading to the production of 4-hydroxyamphetamine and amphetamine. Animal studies have shown that the administration of MA significantly stimulates the metabolic activity of CYP2D2 as well as that of CYP3A1/2. The same process very likely occurs in humans (Dostalek et al., 2005). The most recently published research shows that human immunodeficiency virus (HIV)-glycoprotein 120 and MA both cause apoptotic cell death by inducing CYP enzymes (CYP2E1), which, in turn, stimulates nitric oxide pathways (Shah et al., 2013).

Nonetheless, several different categories of genetic polymorphism appear to explain increased susceptibility to MA use disorders (i.e., abuse, dependence, and psychosis). Research is still ongoing, but three genes (*COMT*, *DRD4*, and *GABRA1*) are known to be associated with MA abuse, 10 other genes (*ARRB2*, *BDNF*, *CYP2D6*, *GLYT1*, *GSTM1*, *GSTP1*, *PDYN*, *PICK1*, *ADORA2A*, and *SLC22A3*) with MA dependence, two genes (*AKT1* and *GABRG2*) with MA abuse/dependence, and four genes (*DTNBP1*, *OPRM1*, *SNCA*, and *SOD2*) with MA-induced psychosis (Bousman et al., 2009).

The link with *ADORA2A* is particularly intriguing because this gene encodes the A2A adenosine receptor, implying that at least some component of psychosis in MA abusers has to do to with adenosine receptors. Serotonin receptors type 1A and 6 (Kishi et al., 2010a,b, 2011) and polymorphisms of *COMT* have already been implicated in the process (Suzuki et al., 2006; Bousman et al., 2010), and it is likely that other

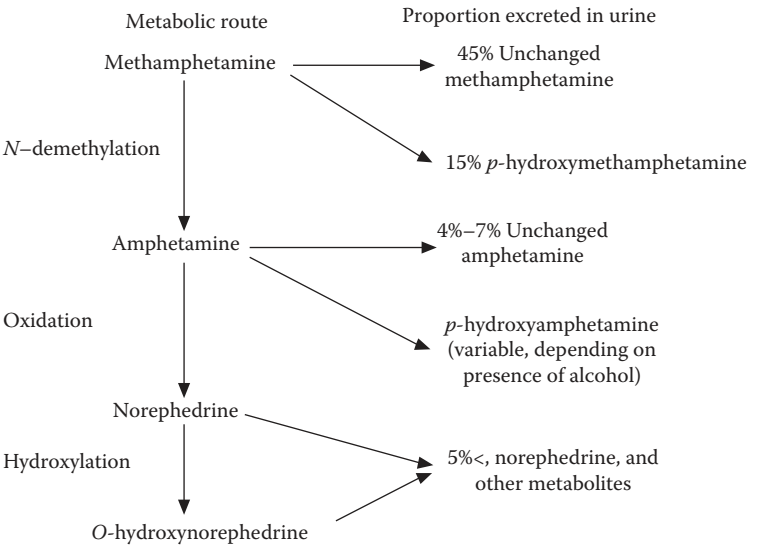


Figure 3.7 MA metabolism. MA is demethylated to produce amphetamine. Ephedrine and other analogs are not converted to amphetamine. Thus, the presence of amphetamine in a sample containing MA is proof that MA, and not some harmless analog, has been taken.

polymorphisms, some significant, some not, will be identified. Another factor that may help explain MA psychosis is the widespread uptake of this drug over cortical and subcortical brain regions as opposed to cocaine that tends to localize in the striatum (see Figure 3.8; Fowler et al., 2007).

There are important differences in the way that humans metabolize the different chiral forms of MA. Humans excrete the *l*-enantiomers of both amphetamine and MA more slowly than the *d* isomers. In one study, the apparent half-life of *d*-amphetamine was 7 ± 1 h versus 11 ± 2 h for *l*-amphetamine, but the values were closer to 5 and 6 h for *d*- and *l*-methamphetamine, respectively (Beckett and Shenoy, 1973). There is also some evidence that *d*-methamphetamine is metabolized more extensively than *l*-methamphetamine, at least in humans (Rowland and Beckett, 1966), the urinary excretion of *d*-methamphetamine being lower than *l*-methamphetamine, and the excretion time longer.

In a study to evaluate the abuse potential of *l*-methamphetamine, Mendelson et al. recruited 12 subjects who self-administered *l*-methamphetamine from a nonprescription (Vick's) inhaler at the recommended dose (16 inhalations over 6 h), and then at 2 and 4 times the recommended dosage (32 and 64 inhalations). Plasma concentrations of *l*-methamphetamine were often below the level of quantification. Physiologic changes were minimal and not dose dependent. Small decreases in stroke volume and cardiac output were observed. This suggests that mild cardiac depression had occurred (Mendelson et al., 2008), but it is far from clear that this decrease is significant, especially given that in later studies, it was found that *l*-methamphetamine had no effect on performance, either positive or negative (Dufka et al., 2009).

Even after intravenous dosing, no very great differences were observed between the behaviors of the two isomers (Mendelson et al., 2006). The area under the curve (AUC) for the *l* form was slightly greater (i.e., the amount absorbed), but the C_{max} (*d* = 60.6–92.8 ng/mL, *l* = 53.9–76.9 ng/mL), $T_{½}$ (10 vs. 14 h), and volume of distribution (3.3–4.3 vs.

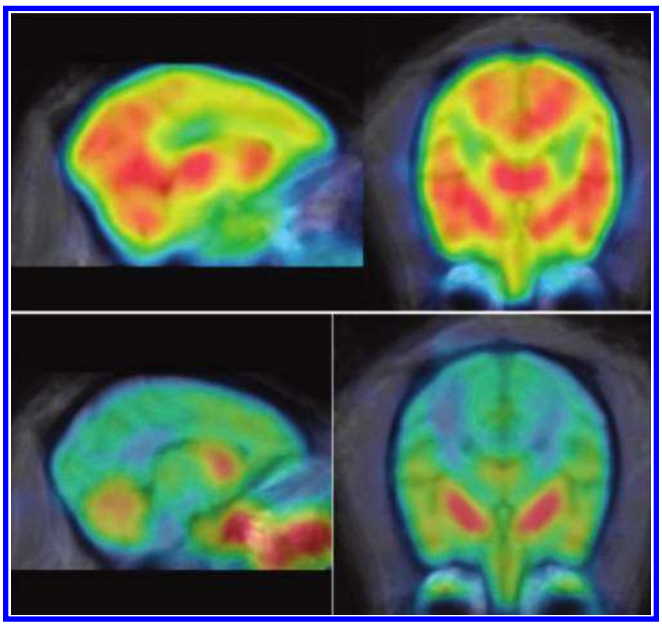


Figure 3.8 Summed brain images for ^{11}C-*d*-methamphetamine (top row, from 0 to 90 min) and ^{11}C-*l*-(—)-cocaine (bottom row, from 0 to 54 min) in the same animal. ^{11}C distribution is widespread over cortical and subcortical brain regions for ^{11}C-*d*-methamphetamine but is highly localized in striatum for ^{11}C-(—)-cocaine. (From Fowler, J.S. et al., *J. Nucl. Med.*, 48(10), 1724, 2007.)

3.7–4.6 L/kg) of both forms were similar, no matter whether the isomers were given separately or as a mixture. The clearance rates for both isomers were also similar. For amphetamine, the principal MA metabolite, the C_{max} was only a tenth of that for MA (an observation that also hold true in postmortem studies) and the half-life considerably longer (18–46 h) (Meyer et al. 1997).

Amphetamine is sometimes detected in MA from clandestine laboratories. It follows that the mere demonstration of amphetamine in hair or sweat samples does not necessarily prove that MA was ingested and demethylated to amphetamine within the body. Its presence could equally well be explained by contact (innocent or otherwise) environmental contamination, such that it might occur in a clandestine laboratory, or in the home of a drug user or drug dealer, or even following the ingestion of amphetamine. Of course this observation has no significance so far as workplace urine testing is concerned, but it could make a great deal of difference to probationers or those involved in child-custody lawsuits.

Over a period of several days, 35%–45% of a given dose of MA is excreted unchanged in the urine (Cook et al., 1993). If the urine is acidic, that amount may increase to over 75%. On the other hand, when the urine is extremely alkaline, the amount excreted unchanged in the urine may drop to as little as 2% (Beckett et al., 1965). In carbon-14 tracer studies of two volunteers, 23% was excreted in the urine within the first 24 h. Other metabolites also appear in substantial quantities, including 4-hydroxymethamphetamine, norephedrine, and 4-hydroxynorephedrine (Caldwell et al., 1972). The (+) isomer of amphetamine is metabolized more rapidly than the (–) isomer and appears in the urine sooner.

The situation has become more complex today now that most MA abusers smoke the drug. When smoked, thermal degradation of the MA occurs, and there are several decomposition products. These include *trans*-phenylpropene, allylbenzene, amphetamine, phenylacetone, and dimethylamphetamine (Sato et al., 2004). One of the main alkenylbenzene breakdown products is *trans*-phenylpropene, formed by elimination of methylamine in a Hoffman-like degradation reaction that converts an amine into an alkene. This compound is a marker for smoked MA (Shakleya et al., 2006). *Trans*-phenylpropene is structurally similar to styrene analogs, which are known to be carcinogens. They are formed by the activity of three different CYP enzymes—CYP2E1, CYP1A2, and CYP3A4 (Sanga et al., 2006). The resultant end products are cytotoxic in tissue culture. Other than the fact that the demonstration of *trans*-phenylpropene in the urine is proof of MA ingestion, the situation in humans is unclear.

No one has ever reported an increased rate of hepatic cancer in MA abusers, although hepatic steatosis in regular users is extremely common. If the patient is infected with hepatitis C, steatosis and hepatic fibrosis both seem to contribute to the occurrence of malignancy (Nieminen et al., 2008). Nearly all of the intravenous drug users in California (and very large percentages in other states and quite probably other countries as well) are infected with the hepatitis C virus (Amon et al., 2008).

Daily oral dosing with MA appears to have little effect on either metabolism or peak blood levels. No significant metabolic changes were noted in six volunteers given 10 mg of MA per day for 2 weeks, nor was there any change in blood concentrations (Cook et al., 1993).

3.1.9 Tissue Disposition

Studies using positron-emission tomography (PET) in conjunction with [^{11}C] *d*-methamphetamine have been used to measure the whole-body distribution and bioavailability of labeled MA as assessed by peak uptake (% dose/cc), rate of clearance (time to reach 50% peak clearance), and accumulation (AUC) in 19 healthy volunteers (9 Caucasians and 10 African Americans). These studies showed that MA is distributed through most organs. Highest whole organ uptake occurs in the lungs (22%), liver (23%), and brain (10%). Kidneys also show a high uptake. These findings are illustrated in Figure 3.9.

MA's clearance occurred most quickly in the heart and lungs (7–16 min) and took longest in the brain, liver, and stomach (>75 min). Clearance time was intermediate in the kidneys, spleen, and pancreas (22–50 min). For unexplained reasons, lung accumulation of [^{11}C] *d*-methamphetamine is higher in African Americans than Caucasians ($p < 0.05$) but does not differ in other organs. When tissue distributions were measured in volunteers, the heart was found to have lower MA uptake than other organs (2.6% of injected dose at 1 min after injection) and retention in the heart was very short (Volkow et al., 2010). Nonetheless, cardiomyopathy is a recognized consequence of MA abuse (see Section 3.1.10.1.3).

Only one postmortem study has ever quantified tissue amphetamine concentrations in multiple organs, and it showed essentially similar drug concentrations in all tissues measured. The study results are shown in Table 3.4. Unfortunately, drug concentrations were not measured in either the lungs or heart, so it is impossible to make comparison with the PET study described earlier (Meyer et al., 1997). Just how postmortem measurements relate to antemortem plasma concentration, if they relate at all, is a matter of dispute. A report published in 2009 described a "body stuffer" who ingested approximately 3 g of MA in order to conceal it from the police. Plasma from the hospital, drawn 12 h

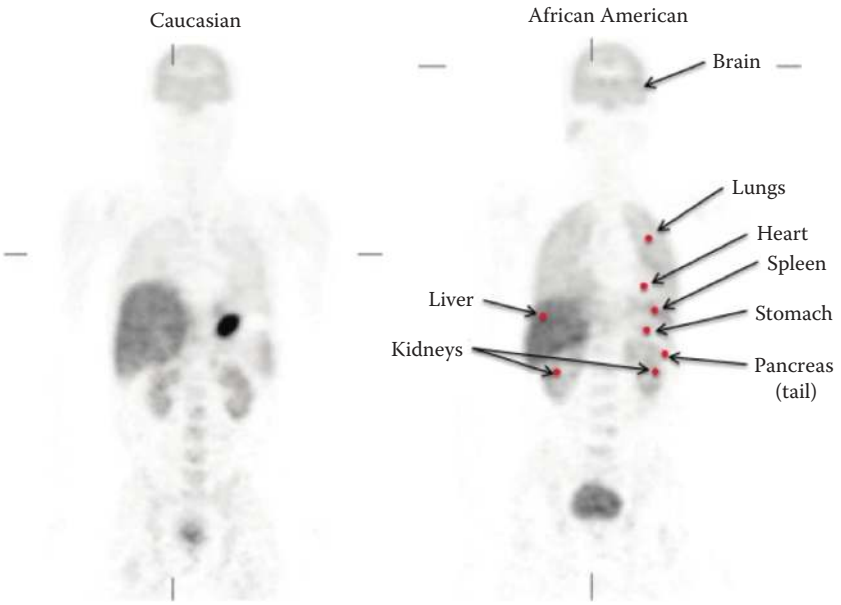


Figure 3.9 PET scans of two individuals, one Caucasian and one African, showing the distribution and organ uptake of labeled MA. The only significant difference between races was that lung MA uptake was nearly one-third higher in African Americans. (Reproduced from Volkow, N.D. et al., *PLoS One*, 5(12), e15269, 2010. With permission.)

Table 3.4 Postmortem Tissue Distribution in a Case of Amphetamine Overdose

Tissue	Total Amphetamine	R Isomer	L Isomer
Blood (μg/mL)	2.44	1.26	1.18
Urine (μg/mL)	33.4	16.7	16.7
Liver (μg/g)	11.7	6.07	5.64
Kidney	3.85	2.00	1.85
Brain (μg/g)	5.50	2.95	2.55

Source: Adapted from Meyer, E. et al., *J. Anal. Toxicol.*, 21(3), 236, 1997.

after ingestion, while the patient was still alive, had an MA concentration of 3.0 mg/L. The concentration in femoral blood that was obtained at autopsy 1 day later was 30 mg/L (Kiely et al., 2009).

3.1.10 Toxicity by Organ System

Literature on reported deaths and medical complications remains sparse. The explanation may be that the most important complications are cardiovascular and that cardiovascular damage only occurs after protracted use. Table 3.5 is based on the only large autopsy series of MA-related deaths ever published. This pattern may be different in cities other than San Francisco and may not be the same as it was when the study was first published in 1998. Liver disease, ranging from steatosis to cirrhosis, was present in nearly 40% of the cases, followed by heart and lung disease.

Table 3.5 Top 10 Abnormalities in Methamphetamine Abusers at Autopsy

Abnormality	Percent (%)
1. Fatty liver	16.2
2. Moderate coronary artery disease	10.3
3. Cirrhosis	9.0
4. Pneumonia	8.2
5. Myocardial fibrosis	6.7
6. Triaditis	6.1
7. Severe coronary artery disease	6.1
8. AIDS	5.4
9. Emphysema	5.1
10. Hepatitis	4.1

Source: From Karch, S.B. et al., *J. Forensic Sci.*, 44(2), 359, 1999. With permission.

3.1.10.1 Basis of Cardiovascular Disease

Ultimately, most of the lesions observed in the hearts of stimulant abusers are due to disordered calcium metabolism. Amphetamines increase release of dopamine (DA) and other biogenic amines from presynaptic terminals in the central and peripheral nervous systems. Amphetamines also inhibit neuronal and vesicular monoamine transporters, as well as monoamine oxidase (MAO), an ability not possessed by cocaine. Traditionally, it has been assumed that MA toxicity was a consequence of MA's indirect effects (i.e., catecholamine toxicity). Within the last few years, it has become increasingly obvious that more is involved. MA has a direct effect upon the cardiomyocytes themselves, and this effect is a result of MA's direct interaction with L-type calcium channels. To complicate issues further, newer research suggests that amphetamine exerts effects on the aorta that are utterly unrelated to catecholamine reuptake (Lamarre et al., 2013). In vitro studies show there is no question that trace amines and amphetamines therefore, exert vasoconstriction independently of adrenoceptors, neuronal transport, and 5-HT receptor activation (Broadley et al., 2013).

Calcium signaling regulates many cellular functions. Within cardiomyocytes, calcium controls excitation–contraction coupling. Most of the calcium that takes part in the process is stored in the sarcoplasmic reticulum (SR); however, some of the calcium that controls contraction comes from outside of the cell via the L-type Ca^{2+} channel (LTCC). A very small flow of calcium introduced through the LTCC induces massive release of sarcoplasmic Ca^{2+} via Ca^{2+} release channels, mainly because they open channels in the sarcoplasmic ryanodine receptor type 2 (RyR2). A flood of calcium is then released, and it is this calcium that interacts with the contractile proteins initiating systole. During diastole, Ca^{2+} is pumped back into the SR via SR Ca^{2+}.

When isolated rat cardiomyocytes (there are no human studies) are treated directly with catecholamines, the rate of contraction does not change, nor does it change when calcium-releasing RyR2 receptors are blocked. However, MA entering through L-type calcium receptors exerts a direct action, causing increased cardiomyocyte rate of contraction. If the L-type channel is blocked, then there is no effect on cardiomyocyte rate. These observations very strongly suggest that MA cardiotoxicity is, in a very important sense, calcium-overload toxicity (Sugimoto et al., 2009). There is also mounting evidence that MA acts directly to alter most of the family of currents that affect the shape of the action potential, including that of potassium ion channels (Liang et al., 2010).

3.1.10.2 Coronary Artery Disease

The hearts of MA and cocaine users demonstrate essentially the same microscopic features: hypertrophy, interstitial fibrosis, and microvascular disease. Amphetamines and cocaine promote perivascular and interstitial fibrosis, myocyte hypertrophy, and intimal and medial hyperplasia (Cohle, 2013). This pattern is indistinguishable from remodeling seen in the hearts of non-drug-using hypertensives and is, therefore, nondiagnostic if the decedent was hypertensive. Nor are these findings diagnostic for one stimulant as opposed to any other stimulant. Accelerated coronary artery disease (CAD) is to be expected in both MA and cocaine abusers, but, for reasons that are not understood, actual myocardial infarction is much more common in cocaine abusers than in MA abusers, even though the extent of disease present seems roughly parallel (Figure 3.10).

Cocaine activates myocardial calmodulin kinase II, initiating essentially the same signaling cascade that occurs in hypertensive subjects (Henning and Cuevas, 2006). This same mechanism very likely applies to MA abusers. One area of difference seems to be that stimulant abusers in general, and cocaine users in particular, are prone to the accelerated development of CAD (Karch et al., 1999). The incidence of CAD is significantly higher than that observed in cocaine abusers. The paradox here is that cocaine-related myocardial infarction is such a chronic problem it is no longer reportable; MA-related infarcts are much less common, even though clinical and autopsy experience generally suggest that MA abusers are just as likely to develop an accelerated form of the disease as cocaine abusers.

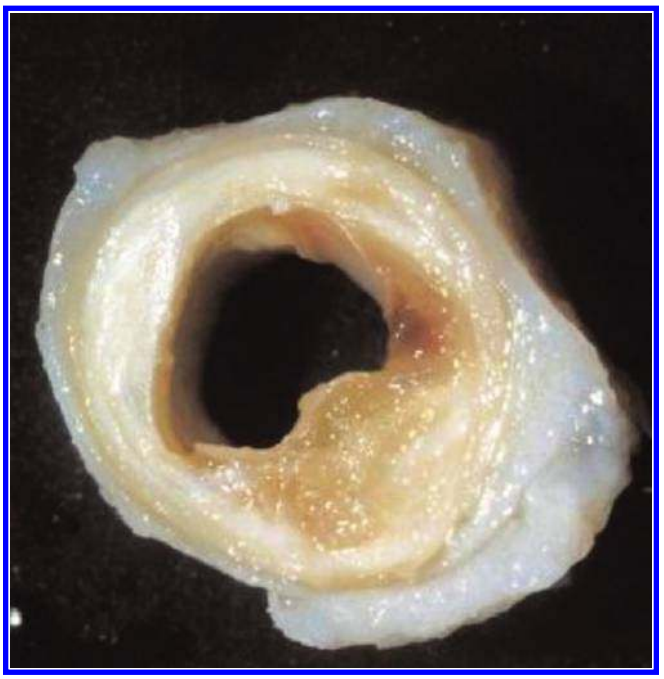


Figure 3.10 Like cocaine abusers, MA abusers experience accelerated CAD, though they seem to experience fewer cases of myocardial infarction than cocaine abusers. This section of coronary artery, involving a large intramyocardial vessel, is from an asymptomatic 28-year-old man who died from trauma.

Causation is always difficult to prove. Heart disease is strongly associated with amphetamine abusers. The use of other illicit drugs, even nonstimulants (e.g., opiates and caffeine), is also associated with increased incidence of heart disease, particularly in younger populations. Cigarette smoking (and marijuana smoking) are also strongly associated with CAD (Karch et al., 1999). At least one population study has demonstrated a strong link between amphetamine use and acute myocardial infarction (Westover et al., 2008). A number of published studies (some single case reports and other case series) have reported the same findings (Costa et al., 2001; Sztajnkrycer et al., 2002; Gandhi et al., 2005; Pilgrim et al., 2009).

Some data suggest that the amphetamines possess several properties that may make users less, rather than more, likely to experience myocardial infarction than cocaine users. MA induces the production of heat shock proteins (HSPs) (Kiyatkin, 2010) and cocaine does not. Not all the HSPs are always present, but their expression is increased when cells are exposed to many different types of stress, including heat, anoxia, and ischemia (Sanchez and Lindquist, 1990; DeMaio, 2000). HSPs are named according to their molecular weight. For example, Hsp60, Hsp70, and Hsp90 (the most studied members of the family) refer to families of HSPs on the order of 60, 70, and 90 kDa in size, respectively (Srivastava, 2004). It has been known for decades that HSP production is an adaptive myocardial response that occurs within 24 h after short episodes of cardiac ischemia and that the production of HSP proteins (it is not clear which of the proteins predominate) increases myocardial resistance to infarction (Marber, et al., 1993). Production of HSP is a logical explanation of the known ability of MA, and most other amphetamines, to cause hyperthermia (Kiyatkin, 2010).

Another factor in amphetamine-related CAD might be the degree of vasoconstriction produced by different drugs. Given the presence of only modest to moderate CAD, intense vasoconstriction (coronary artery spasm) can convert an asymptomatic into a symptomatic lesion (Figure 3.11). Cocaine-induced vasoconstriction has been demonstrated in humans under controlled conditions, but is not very great, only on the order of 15% (Lange

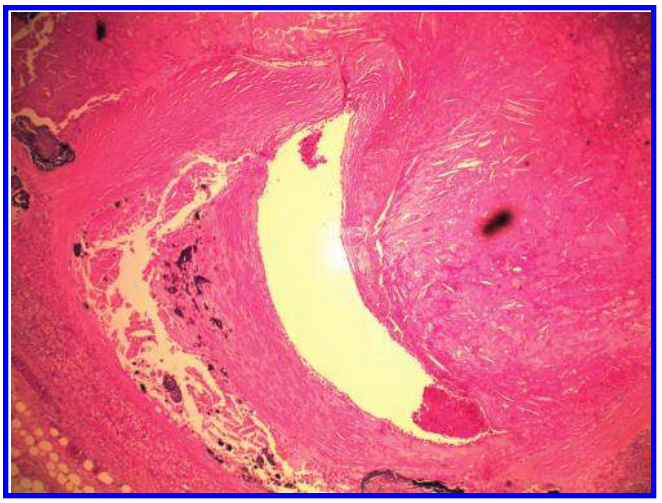


Figure 3.11 A 90% occlusion of the LAD in a 30-year-old chronic MA abuser who died during a police altercation. The dark material is calcium deposits. The white sharp-edged shapes represent cholesterol deposits that were dissolved out during slide preparation. (From the author's collection.)

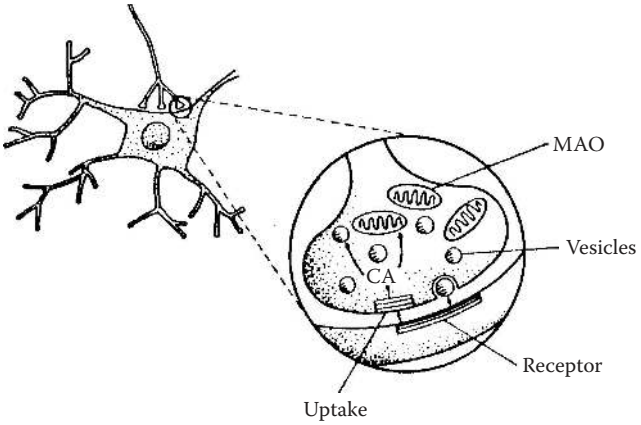


Figure 3.12 Effects of amphetamine on nerve endings. The effects of amphetamine on catecholamine metabolism are more complex than those of cocaine. In addition to blocking reuptake, amphetamine also causes increased release of neurotransmitters. (Courtesy of Dr. Arthur K. Cho, Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA.)

et al., 1989). Only two studies on MA-induced vasoconstriction, one in the colon (Johnson et al., 1991) and one in the brain (Fang et al., 2006), have been reported, and then only in experimental animals. Measurable effects were observed but were unimpressive.

Vasoconstriction, to the degree it occurs, must be at least partially a consequence of catecholamine stimulation. Both cocaine and the amphetamines cause NE to accumulate in the synaptic cleft, from which it overflows into the circulation. Amphetamines exert additional effects over and above those produced by cocaine. Amphetamine is transported into the presynaptic terminal, where it inhibits MAO and prevents further storage of catecholamines within the nerve ending (Figure 3.12). Taken together, the result is increased sympathetic stimulation and increased circulating levels of catecholamines in the periphery (Fukunaga et al., 1987).

3.1.10.3 Tako-Tsubo Disease and Cardiomyopathy

This syndrome, consisting of transient left ventricular dysfunction, is characterized by "apical ballooning." It was first reported from Japan where it is referred to as *tako-tsubo cardiomyopathy* (TTC) simply because the angiographic appearance of the heart resembles that of a Japanese octopus trap (Asano et al., 2001) (Figures 3.13 and 3.14). Though first recognized in Japan it seems to occur worldwide (Ieva et al., 2009).

TTC is a reversible cardiomyopathy with a clinical presentation indistinguishable from myocardial infarction. In fact, it is estimated to occur in 1%–2% of patients presenting with acute myocardial infarction. Chest pain and dyspnea are the typical presenting symptoms, while transient ST segment elevation on electrocardiogram (EKG) and a small rise in cardiac biomarkers are common. Patients with this syndrome often present with a bulging outward of the left ventricular apex with, at the same time, hypercontraction of the base of the left ventricle. TTC is frequently precipitated by a stressful event, which is why some refer to it as "stress cardiomyopathy" or even "broken heart syndrome." The occurrence of this disorder has nothing to do with ischemia (angiograms are always normal, but if they are not, the problem is, by definition, not tako-tsubo). When tako-tsubo does occur, acute heart failure, lethal ventricular arrhythmias, and even ventricular rupture all may

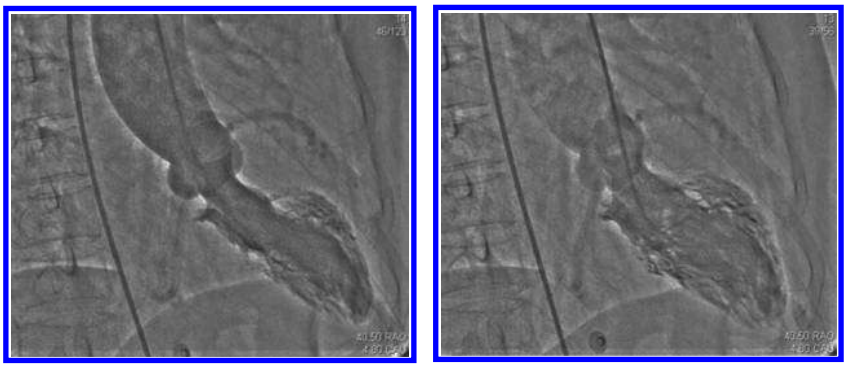


Figure 3.13 Tako-tsubo cardiomyopathy is illustrated in this left ventriculogram. Hyperkinesis of the heart's basal segments with apical akinesia is evident. Tako-tsubo derives its name from the shape of a Japanese crab pot, illustrated in Figure 3.14. (Reproduced from Chia, P.L. and Foo, D., *Cardiol. J.*, 18(5), 564, 2011.)

Figure 3.14 A Japanese crab fishing pot. Tako-tsubo was named because the original fishing trap is so similar in appearance to the ventriculogram shown in Figure 3.13.

follow (Akashi et al., 2010; Mariscalco et al., 2010; Parodi et al., 2010; Brunetti et al., 2012; Copetti et al., 2012; Kurisu and Kihara, 2012).

Tako-tsubo occurs mainly in postmenopausal women, but younger women are not spared. If no other epicardial lesions are present, the characteristic wall motion abnormalities can involve other territories besides those subserved by a single epicardial vessel. In younger women, it is more likely to involve the base of the heart than the apex. A connection to catecholamine excess seems obvious, given that several cases of basal ballooning have been reported in conjunction with pheochromocytoma (Lassnig et al., 2009; Di Palma et al., 2010), and others have linked its occurrence with MA abuse. The first report of MA-induced tako-tsubo was published in 2007 (Reuss et al., 2007) and that was followed

by a number of others (Srikanth et al., 2008; Hurst et al., 2010). The same disorder has also been observed in cocaine users, confirming the notion that the underlying disorder is catecholamine related (Arora et al., 2006).

A unifying mechanistic explanation for this type of acute but rapidly reversible contractile dysfunction appears to be the distribution of β receptors and their activation, which leads to a transient disorder of cardiac function (Ueyama et al., 2002; Wittstein et al., 2005; Sharkey et al., 2009). Chronic β-receptor stimulation, as would occur in amphetamine and cocaine users, upregulates inducible nitric oxidase synthetase. The result is increased myocardial nitric oxide production. The presence of reactive nitrogen species/peroxynitrite within the heart results in myocardial apoptosis and cell death (Hu et al., 2006). This mechanism could account for some reported cases of ventricular rupture during tako-tsubo (Kurisu and Kihara, 2012).

One case report described autopsy findings in a 45-year-old woman who abused oral amphetamine and died of heart failure. Her heart was enlarged (530 g) but the coronary arteries were widely patent, as in all episodes of tako-tsubo. Widespread interstitial edema, with scattered lymphocytic and histiocytic infiltrates, was also evident, along with degeneration of individual fibers and patchy myocardial fibrosis. Histologic alterations are almost always present if the affected person dies (which is, granted, uncommon). A 2014 report described the death in custody of a young man ("midtwenties"). He died suddenly after displaying symptoms consistent with excited delirium (Hisahi). He had just taken an unspecified amount of a cathinone derivative, α-pyrrolidinovalerophenone. Apparently an electrocardiogram was never obtained. However, like the heart of most patients who suffer from excited delirium, multiple abnormalities were evident including concentric hypertrophy, fibrosis, and fatty replacement (Nagi et al., 2014). Why sustained restraint is considered part of the diagnosis is unclear. If it is suspected that the death was tako-tsubo related, it is fairly easy to visualize myocardial β_1 receptors using confocal microscopy (Turillazzi et al., 2008) and also to demonstrate nuclear apoptosis by using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) immunostaining (D'Errico et al., 2010). Unfortunately, the chances of finding a confocal microscope in the medical examiner's office are remote.

Tako-tsubo is not the only type of cardiomyopathy to affect MA abusers. Myocardial remodeling (hypertrophy and fibrosis) is demonstrable in almost every case, and reports of MA users with heart failure are relatively common (Sadeghi et al., 2012; Won et al., 2013). However, as illustrated in Figure 3.15, some individuals develop full-blown dilated floppy cardiomyopathy with vacuolization (autophagy) and bizarrely shaped cell nuclei (Karch, 2011). The mechanism for these changes in stimulant abusers has never been investigated, but cases like these appear to be becoming more common.

Without specific tests to rule out cardiomyopathy secondary to other causes, such as viral infection or drug toxicity, there is no way to look at the histology and make a specific diagnosis except to say that evidence of cardiomyopathy is apparent (Figure 3.16).

3.1.10.4 Coronary and Aortic Dissection

Coronary artery dissection (Figures 3.17 and 3.18a) is an event usually confined to peripartum women, but it is being reported with increasing frequency in cocaine (Ijsselmuiden and Verheye, 2009) and MA abusers (Kanwar and Gill, 2010). Aortic dissection, separate from coronary dissection, is now a well-recognized complication of MA abuse (Davis and Swalwell, 1994; Swalwell and Davis, 1999; Anzalone et al., 2002; Gotway et al., 2002; Wijetunga et al., 2003; Fikar, 2008). Even dissection of the femoral artery has been reported (Figure 3.18b; Karch

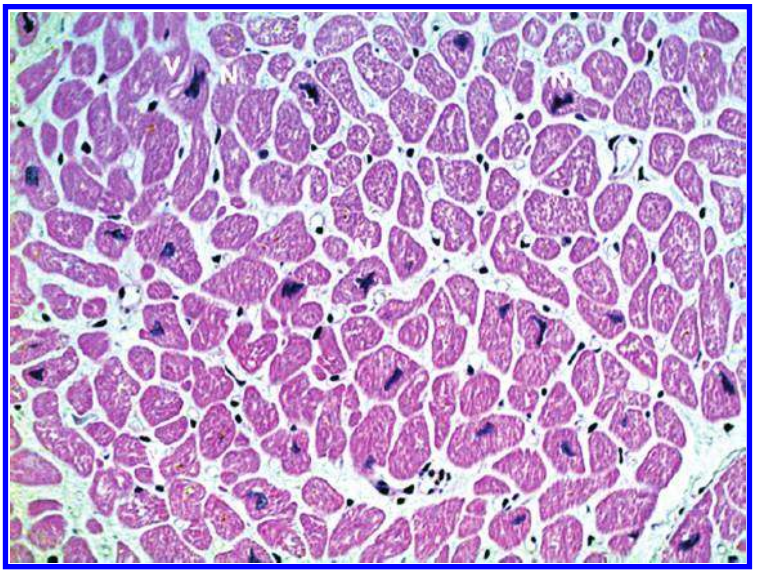


Figure 3.15 Cardiomyopathy. Heart from a chronic MA abuser, in heart failure, who sustained a cardiac arrest. Note the extreme degree of nuclear atypism and vacuolization. Edema of the myocardium is characteristic of this condition, which explains why the enlarged myocytes are abnormally separated. (From the author's collection.)

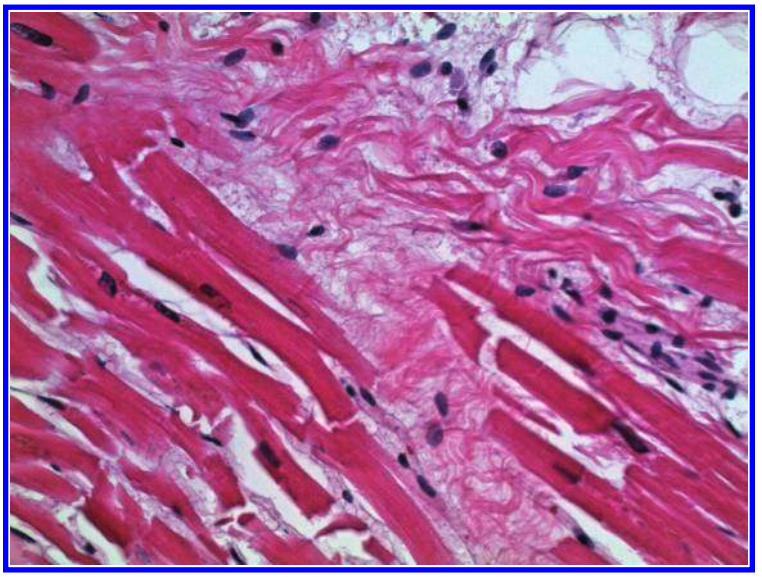


Figure 3.16 Fibrosis, interstitial and perivascular, along with myocyte hypertrophy, is part of the remodeling process and almost always evident in chronic stimulant abusers. These changes are thought to be the result of an underlying inflammatory process.

et al., 1999). An area of aortic dissection from a chronic MA smoker is shown in Figure 3.16. Some recent evidence points to a role for an abnormality of cholesterol carried within HDL particles (HDL-C), in both men and women even if they are not cocaine abusers (Abdel-Maksoud, 2012).

Not only is the mechanism of acute dissection in MA abusers not known, the few autopsies that have been performed are unrevealing (Karch et al., 1999). One common factor in

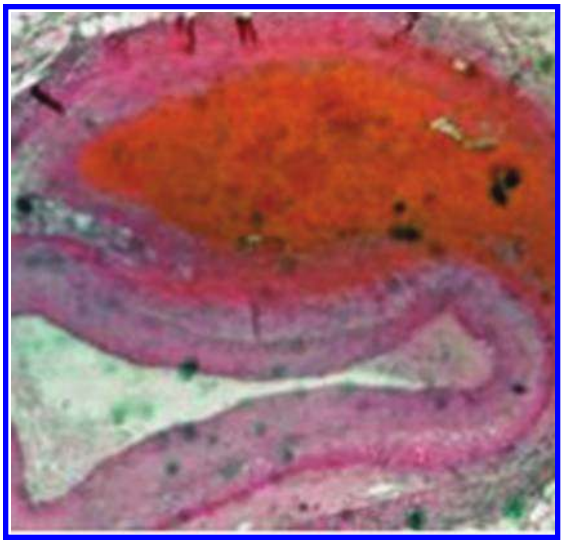


Figure 3.17 Coronary artery dissection was thought to be mainly a disease of pregnancy, but in the last decade, instances of coronary artery dissection in cocaine and MA users have been reported.

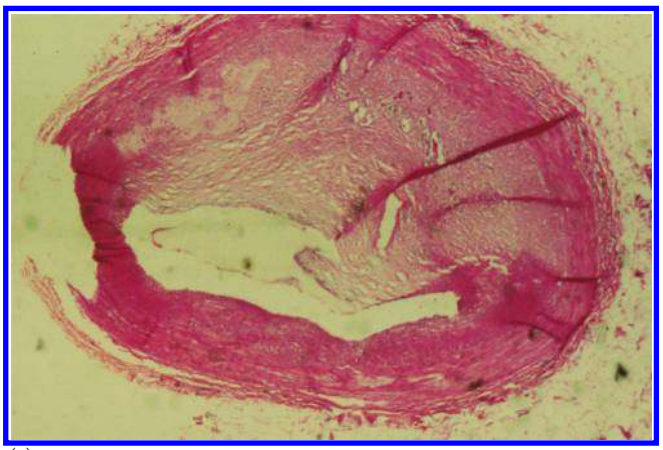
all these cases seems to be hypertension (Jonker et al., 2009), but here too, it can be anticipated that elevated plasma and tissue concentrations of catecholamines would not only raise blood pressure but also lead to free radical generation. Metabolites of these agents, called *o*-quinones, contribute to redox cycling, toxicity, and apoptosis. Cyclized *o*-quinones, including aminochrome, dopachrome, adrenochrome, and noradrenochrome (formed from dopamine, dopa, adrenaline, and noradrenaline, respectively), are all highly reactive compounds. They are capable of producing damage to the endothelium of large and small vessels, not to mention the destruction of neurons (Baez et al., 1997). Another line of evidence suggests that NADP oxygenase 1 (NOX1) is involved in the mechanisms of angiotensin II–dependent aortic dissection. It is speculated that NOX1-dependent suppression of tissue inhibitor of metalloproteinase 1 expression (Lee and Yang, 2012) could lead to tissue damage through an altered protease/inhibitor balance, thereby favoring the occurrence of dissection.

3.1.10.5 Myocardial Remodeling

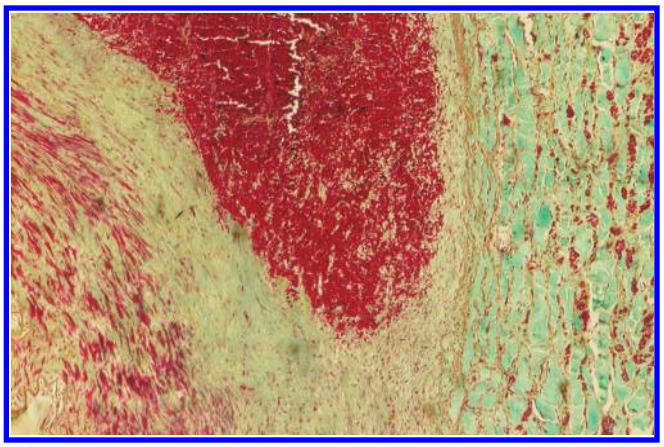
"Myocardial remodeling" is a collective term used to denote specific alterations in the dimensions, mass, and shape of the heart (also called cardiac or ventricular remodeling) in response to hemodynamic load and/or cardiac injury occurring in conjunction with neurohumoral activation. Remodeling may be either physiologic or pathologic (Burchfield, 2013). When remodeling occurs after myocardial infarction, it is classified as "adaptive." If the same changes occur because of stimulant drug abuse, they are clearly "maladaptive." Most of these changes involve muscle, but stimulant abuse should strongly be suspected when there is fibromuscular dysplasia of the intramyocardial arteries (Figures 3.19 through 3.23).

3.1.10.6 Pulmonary Disease

The effects of amphetamine on pulmonary pathology are still largely unstudied. There are no long-term human studies evaluating the effect of MA on the lungs (as there are for



(a)



(b)

Figure 3.18 (a) Cross section of the LAD in a gunshot wound victim, where MA abuse was unrelated to the cause of death. Extensive CAD is common among MA abusers and occurs at a much younger age than CAD in the general population. (b) Dissecting femoral artery aneurysm in an MA abuser. Aortic dissection is a recognized complication of MA abuse, although the mechanism is not known. Special staining procedures are unrevealing, and none of the reported cases has involved individuals with Marfan's syndrome. (From the office of the San Francisco Medical Examiner.)

marijuana and cigarette smoking). In the only large autopsy series of MA-related deaths ever to be published, pulmonary edema was present in over 70%, pneumonia in 8%, and emphysema in 5%. Birefringent crystals were noted in 11% (Karch et al., 1999).

When drug tablets are crushed and injected intravenously, the insoluble fillers (microcrystalline cellulose, corn starch, or cotton fibers) contained in the tablet become trapped within the pulmonary microvasculature, where they are easily visualized as birefringent crystals. If enough crystals are deposited, the smaller vessels become thrombosed and foreign body granulomas form. Eventually, some of the foreign material will work its way into the perivascular spaces, leading to further granuloma formation and more fibrosis (Tomashefski and Hirsch, 1980; Tomashefski et al., 1981; Tomashefski and Felo, 2004). Typical granulomas are illustrated in Chapter 5. Any pill that is ground or injected, no

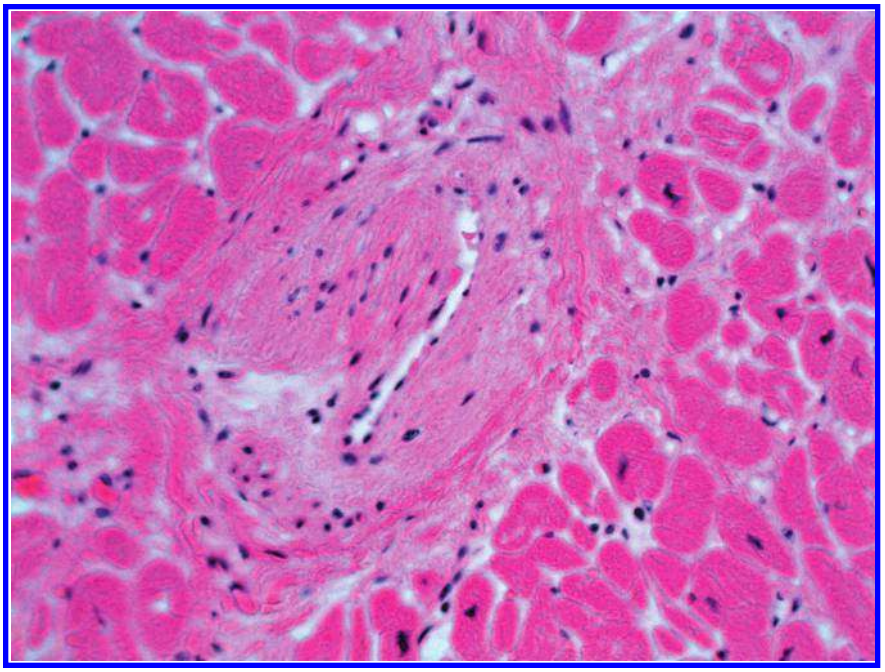


Figure 3.19 Fibromuscular dysplasia of a small intramyocardial artery. The lumen of this small intramyocardial artery is nearly obliterated by proliferating fibromuscular cells. The patient was a chronic MA abuser who died of heart failure. These vessels are often surrounded by perivascular fibrosis.

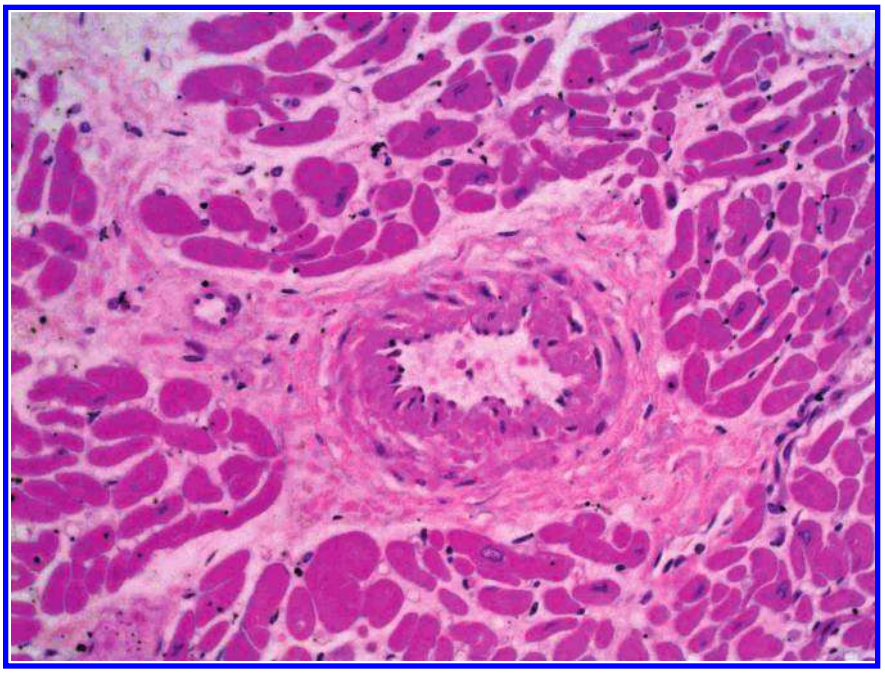


Figure 3.20 As in cardiomyopathy, evidence of both interstitial and perivascular fibrosis can usually be found if sought.

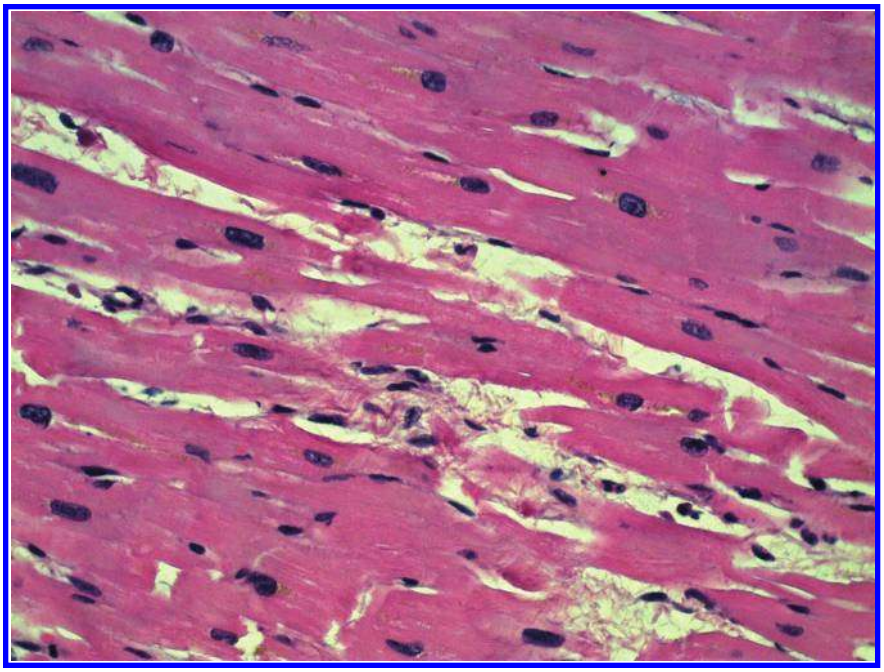


Figure 3.21 Interstitial fibrosis is part of the remodeling process. This micrograph shows disintegrating myocytes, partly digested, in the process of being replaced by new fibrous tissue within the interstitium.

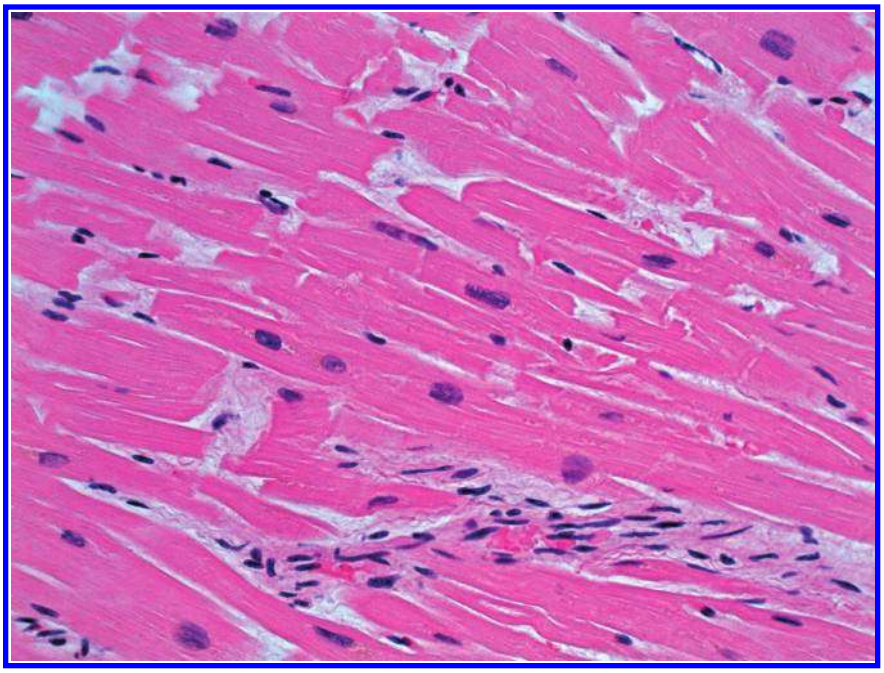


Figure 3.22 Interstitial fibrosis. Damaged myocytes are being digested and replaced with fibrous tissue. In this slide, the typical "boxcar" nuclei associated with hypertrophy can also be visualized.

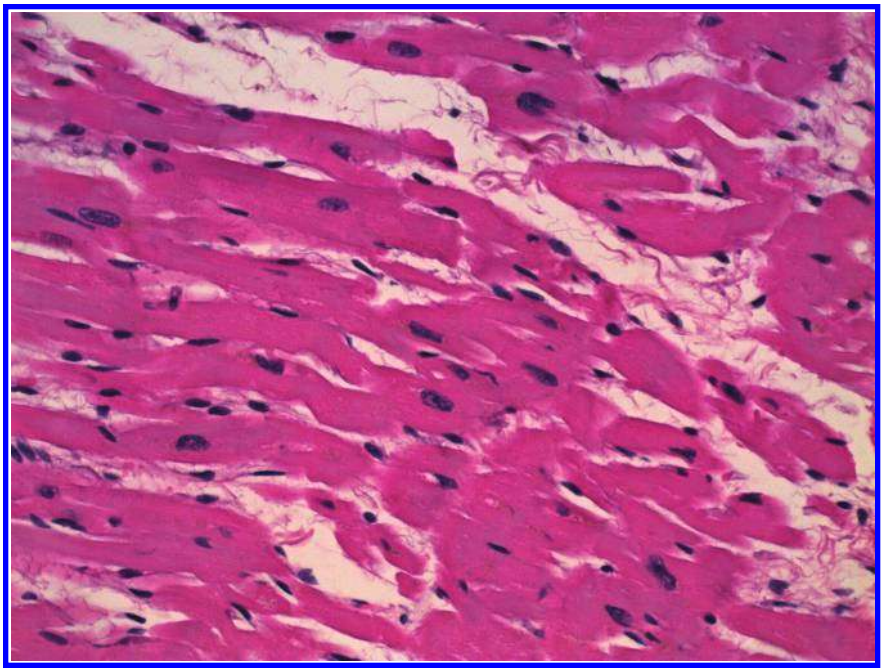


Figure 3.23 Note "cigar-shaped" myocyte nuclei, indicating active cardiomyocyte hypertrophy. In the lower right-hand corner, dissolving cardiomyocytes in the process of being replaced by fibromyocytes can be seen. These are a type of lymphocyte that can differentiate into fibroblasts. All of these changes are part of the remodeling process.

matter whether it is a stimulant or opiate, will leave detectable foreign bodies; talc is the agent most often encountered.

Repeated amphetamine injections also result in a net reduction of the capacity of the pulmonary vascular bed, leading to an increase in pulmonary vascular resistance. At autopsy, organizing and recanalizing thrombi will be evident, along with easily identifiable birefringent material (Rajs et al., 1984; Kringsholm and Christoffersen, 1987). Histologically, this type of pulmonary hypertension can be distinguished from the much rarer primary variety of idiopathic pulmonary hypertension by the presence of plexiform lesions, seen at branching points of the obstructed small arteries (Pietra, 1991). This complication seems to be associated more with some abused drugs than with others. It is now known, for example, that the levamisole used to adulterate cocaine, and even occasionally heroin (Schneider and Meys, 2011), is metabolized to form aminorex, a drug known to cause pulmonary arterial hypertension (PAH) (World Health Category, I) (Bertol et al., 2011), but, in general, heroin abusers are much more prone to thromboembolic arteriopathy than stimulant abusers.

Elevations in plasma 5-HT (serotonin) have been implicated in the pathogenesis of the cardiac and pulmonary disorders associated with MA abuse. Under normal circumstances, plasma 5-HT concentrations are kept at low levels by transporter-mediated uptake of 5-HT into platelets and also by metabolism to form 5-hydroxyindoleacetic acid. Many abused drugs, including the amphetamines, target 5-HT transporters and, indirectly, increase the amount of circulating 5-HT. Studies of animals have shown that drugs such as amphetamine and MA raise 5-HT levels sufficiently to initiate mitogenesis in pulmonary artery smooth muscle cells, setting off the process of pulmonary hypertension (Shimoda and

Laurie, 2013; Thomas et al., 2013). Additional studies are needed to determine the effects of chronic administration of amphetamines on circulating 5-HT, but it seems likely that the elevated 5-HT concentrations may also account for the smooth muscle hypertrophy that is always evident in the hearts of chronic stimulant abusers (Shannon and Chaudhry, 2006).

Another possible mechanism for lung injury in MA abusers has to do with free radical formation. In experimental studies, when mice were exposed to MA vapor, a 53% increase in total protein was observed in bronchoalveolar lavage; increases of 20% occurred in albumin levels, and, at the same time, a 2.5-fold increase in lactate dehydrogenase levels was observed. Concurrently, there was a more than 25% reduction in the number of cells found in the fluid, suggesting significantly increased free radical generation. The results of this study also suggest that, at least in experimental mice, MA abuse is associated with elevated free radical formation and significant lung injury (Wells et al., 2008).

Finally, there is emerging evidence, or at least a strong suspicion, that MA itself, regardless of any other contaminants or adulterants, could play a role in the development of PAH. This suspicion arises largely because of the structural similarity between MA and fenfluramine and aminorex—agents known to cause idiopathic pulmonary hypertension. In 2006, a retrospective study of 340 patients with idiopathic pulmonary hypertension was performed. The researchers found there was a history of stimulant abuse in 28.9% of patients, compared with only 3.8% of patients with pulmonary hypertension who actually had a known risk factor for that disease, and 4.3% of patients with chronic thrombotic embolism (including those who inject crushed pills not meant for injection) (Chin et al., 2006). After adjustment for differences in age, patients with idiopathic PAH were 10.14 times (95% confidence interval, 3.39–30.3; $p < .0001$) more likely to have used stimulants than patients with PAH and known risk factors and 7.63 times (95% confidence interval, 2.99–19.5; $p < .0001$) more likely to have used stimulants than patients with chronic thromboembolic pulmonary hypertension.

More and more young people are injecting crushed tablets and the findings of this study are disturbing. It will be essential to determine whether the recreational use of other illicit serotonin substrates like MDMA (ecstasy) increases the risk of idiopathic pulmonary arterial hypertension (IPAH) (Rothman and Baumann, 2007). At a minimum, the finding of IPAH at autopsy should prompt a search for evidence of past drug use, perhaps including hair testing. Another recent development of note is that concentrations of brain natriuretic peptide and atrial natriuretic peptide in the myocardium of MA abusers far exceed those of normal controls and may play a role in promoting the hyperthermia often seen in MA abusers (Chen et al., 2012).

3.1.10.7 Central Nervous System

With the exception of ADHD, the only legitimate use for any of the drugs in this group is appetite suppression, and there is increasing doubt about their effectiveness even for that application. *d*-Amphetamine, phentermine, and aminorex (withdrawn from the market many years ago because of the obvious link with IPAH) are anorectic only by virtue of their ability to cause the release and/or prevent the reuptake of neurotransmitters. In drug abusers (as opposed to individuals taking pharmacologic doses of MA), the most obvious manifestations of toxicity are psychosis and stroke. Large doses of MA (10–15 mg/kg) given to experimental animals quickly alter the brain's serotonergic and dopaminergic systems. Activity of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of DA and NE, decreases in a dose-related fashion after exposure to MA, and so does the content of DA and homovanillic acid within the brain (Koda and Gibb, 1973).

Countless morphologic and volumetric scanning studies have been published, and there does seem to be a trend in the sorts of anatomic changes produced by amphetamine. Certain areas of the brain seem to be shrunken, but not greatly (approximately 10% on average), and this finding is consistent. Almost all studies performed to date indicate that the volume in all or parts of the frontal cortex is diminished. Others have shown prominent diminutions within the ventromedial prefrontal cortex (including the medial portion of the orbital frontal cortex), as well as volume decreases in the insula. Surprisingly, enlarged striatal volume has been repeatedly observed. Reports on volume differences in the hippocampus and amygdala have been equivocal. Based on the evidence now in hand, it is almost impossible to say whether amphetamine and cocaine exert the same or different effects. Evidence supporting differential interaction of brain structures with cocaine versus ATS is scant. However, the volume of all or portions of the temporal cortex appears lower in a majority of studies on cocaine abusers but not amphetamine-type stimulant abusers. Future research should include longitudinal designs on larger sample sizes and examine other stages of exposure to psychostimulants (Mackey and Paulus, 2013).

The pattern of injury produced by chronic amphetamine ingestion (excluding the hallucinogenic amphetamines) is similar to the pattern of injury seen with MA, though generally less severe (Fonseca and Ferro, 2013). MA is a neurotoxin and it damages the neuronal DA system in a highly selective pattern. The striatum is the region where long-term DA depletion and microglial activation are maximal. Endogenous DA has also been implicated as a critical component of MA-induced neurotoxicity, probably because it acts as a substrate for nonenzymatic oxidation by reactive oxygen species (ROS).

The striatum is also extensively innervated by 5-HT nerve endings, and these nerve endings undergo much the same process as do damaged dopaminergic neurons, resulting in an increased release of 5-HT, loss of tryptophan hydroxylase function, and loss of the 5-HT transporter, as well as long-term depletion of 5-HT stores. In some situations 5-HT can also be modified by ROS to form different but still highly reactive species capable of damaging neurons. It is not known with certainty whether MA leads to the production of this second type of reactive species (Thomas et al., 2010).

Except for the fact that those with preexisting malformations are obviously at increased risk for hemorrhagic stroke, amphetamine-related hemorrhages are more often intracerebral, or simultaneously intracerebral and subarachnoid, than pure subarachnoid. Hemorrhage is most often confined to the frontal lobes, though it occasionally involves the basal ganglia. This distribution is in contrast with the pattern seen in hypertensive hemorrhages where the basal ganglia and hypothalamus are usually involved. The pattern is, however, almost exactly the same as that seen in cocaine abuse, in that the frontal lobes are most often involved. Rarely, the etiology of a bleed may be embolic (Imanishi et al., 1997). The evidence for vasculitis is slim but probably real (Ho et al., 2009; Kahn et al., 2012). It has been shown that amphetamines can induce inflammatory genes found in human brain endothelium.

Whether or not neurotoxicity occurs depends on the dose, dosing interval, route of administration, and temperature. The availability and proper function of dopamine transporter (DAT) and 5-HT transporter is a requirement for the expression of amphetamine-related neurotoxicity. Still, the mechanism by which amphetamine leads to selective damage to cells that produce DA and 5-HT is not yet known nor are the mechanisms, time course, and features of recovery from the damage, which does seem to occur, at least partly.

One reality that makes experimental work with amphetamines difficult is the very wide difference in species response. Another is the presence of multiple confounding diseases, including both HIV and hepatitis C, either of which can cause neuroanatomic alterations (Ricaurte et al., 2005). Finally, it is becoming clear that the neurobiology of monoaminergic neurons plays a key role in neurodegenerative diseases such as Parkinson's disease (Yuan et al., 2006). And there is emerging evidence for a connection. Biochemical and neuroimaging studies in human MA users have shown decreased levels of DA and DAT along with obvious microglial activation in the striatum (and other areas of the brain). These changes are identical to the changes seen in patients suffering parkinsonism. Furthermore, recent epidemiologic studies have shown that MA users are almost twice as likely as nonusers to develop parkinsonism (Buttner, 2011; Granado et al., 2013).

MA causes a type of psychosis that may remain for months after the drug has been discontinued (Young and Scoville, 1938). Amphetamine-related psychotic reactions were recognized soon after the drug was first introduced; the first paper on the subject appeared in 1938 (Iwanami et al., 1994). By 1958 a paper reviewing 36 cases from the world's literature was published. The author concluded that there were striking similarities between the symptoms of amphetamine-induced psychosis and schizophrenia (Connell, 1958). The similarities were so striking that at one time, large numbers of amphetamine abusers were admitted to mental hospitals with the mistaken diagnosis of schizophrenia!

Cultural determinants have some bearing on the psychiatric manifestations of amphetamine abuse; MA psychosis is responsible for many more psychiatric hospitalizations in Japan than in the United States (Iwanami et al., 1994). It has been suggested that patients with less catechol-O-methyltransferase activity are more likely both to experience MA psychosis and to suffer from relapse (Suzuki et al., 2006; Bousman et al., 2009); however, other workers have failed to confirm this connection.

Amphetamine-related vasculitis was first described by Citron et al. in 1970 (Citron et al., 1970), and there have been intermittent reports ever since (Cohle, 2013). Additional cases continue to be reported, but none of these new reports includes the histopathologic findings, so there is no way to determine whether vasculitis or vasospasm is being demonstrated by noninvasive studies. In the cases first described by Citron et al., the histologic appearance was identical to that seen in polyarteritis nodosa, with fibrinoid necrosis of the intima and media, and mixed cellular infiltrates. With longer periods of survival, intimal proliferation occurs leading to marked luminal narrowing, especially at the bifurcation of vessels. Characteristically, giant cells were absent in Citron's report and the veins were spared. Some reports described involvement of just the smaller vessels (Stafford et al., 1975; Bostwick, 1981; Shibata et al., 1991). A report published in 2004 described the autopsy findings in an MA abuser who died of a ruptured aneurysm; there was no evidence of vasculitis (McGee et al., 2004). If MA vasculitis does occur, it is probably the result of an adulterant added to the drug rather than the drug itself.

One of the contaminants most likely to be found in MA, one which would certainly be toxic to blood vessels, is hydrochloric acid. In the summer of 2005, the German government noted that its €50 notes were disintegrating. Even newly minted bills displayed a frayed moth-eaten appearance. MA has now surpassed amphetamine in popularity among Germans and it transpires that the €50 note is the perfect size for snorting MA. Users crush crystal MA on the bank notes, then roll them up, and insert the bill into a nostril—exactly the same way that most Americans took cocaine before crack arrived on the scene.

3.1.10.8 Genitourinary Tract

Except for episodes of rhabdomyolysis, MA-related kidney damage is almost always secondary to a disease somewhere else in the body. Renal complications of amphetamine and MA seem to result from some direct toxic action of MA on renal tubules. When rats are repeatedly injected with MA, ubiquitin (a protein that regulates gene turnover) immunoreactivity increases, but only in the renal tubules. The same animals also manifest an increase in creatinine, but potassium, calcium, and phosphorus decrease. Most importantly, creatine phosphokinase (CPK) in these rats increased significantly ($p < .01$), suggesting that MA-induced rhabdomyolysis may lead to damage of the renal tubule. The connection may be of forensic value in the investigation of MA-related deaths, though few medical examiner offices have the facilities to do the test for ubiquitin (Tokunaga et al., 2006). When renal disease is detected, most often it consists of nephrosclerotic lesions involving the glomeruli, the same change that is often seen in hypertensives and cocaine abusers alike. In fact, the histologic appearance of the two conditions is indistinguishable (Luke, 1999). A typical example of glomerulosclerosis in an MA user is shown in Figure 3.24.

The first report linking amphetamine ingestion and reversible renal failure was published in 1970 (Ginsberg et al., 1970), but few additional mentions have appeared since then. Most recently, reported cases have involved the use of "designer" amphetamines, particularly MDMA (also known as ecstasy) (Sultana and Bryune, 1996) and PMA (Byard et al., 1999) (see Section 4.5).

MA abuse is a relatively common cause of rhabdomyolysis (Figure 3.25) and must always be considered in the differential diagnosis of that disorder. Occasionally, it is seen

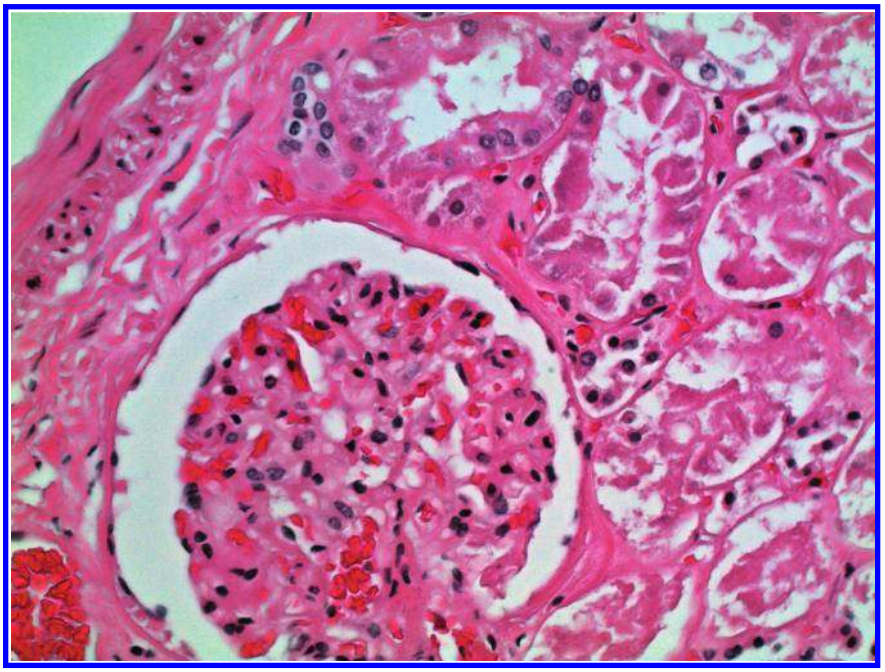


Figure 3.24 Glomerulosclerosis is a very common finding in stimulant abusers, but the presence of the lesion does not indicate the use of any particular drug. (Micrograph from author's collection.)

Figure 3.25 An individual with rhabdomyolysis and compartment syndrome. Note bulging muscle and discoloration from muscle cell breakdown. (Courtesy of ASCP.)

as a complication of "body packing" (Hendrickson et al., 2006), but more often, it occurs in "ravers" and MDMA users.

In 2000, a retrospective review of 367 emergency room patients with rhabdomyolysis found that nearly half were MA users (Richards, 2000). That they developed rhabdomyolysis was probably a result of concomitant hyperthermia. Even so, the most common manifestations of intoxication were restlessness and agitation, but only rarely rhabdomyolysis (Halpern et al., 2010).

Hyperthermia in amphetamine users can occur for a number of reasons. Increased motor activity, even without seizures, raises body temperature, especially when heat loss from the skin is inhibited because of catecholamine-induced vasoconstriction. Altered thermoregulation may also be the result of the direct actions of amphetamine on the hypothalamic temperature centers. However, a number of other unrelated insults can also lead to rhabdomyolysis: alcoholism, drug toxicity, hypokalemia, muscle ischemia, hypotension, and prolonged immobilization have all been implicated as possible factors (Scandling and Spital, 1982; Terada et al., 1988). Rhabdomyolysis may even result from heritable muscle enzyme deficiencies, not to mention many structural muscle diseases, exercise, and even general anesthesia. However, there is no evidence that the occurrence of hyperthermia in amphetamine abusers has any genetic link (Matsusue et al., 2011).

When rhabdomyolysis does occur, myoglobin, potassium, and phosphorus are released into the plasma. The presence of these substances in the plasma sets into motion a series of metabolic derangements and fluid shifts. The resultant damage to the kidneys can be indirect, resulting from hypotension and renal ischemia, or direct, as when myoglobin or its decomposition products cause tubular obstruction. Much of the damage may be mediated by free radical formation (Odeh, 1991).

Another possible explanation for MA-related rhabdomyolysis is that amphetamines themselves are myotoxic. After use of amphetamine, prolonged elevations in CPK can occur, even in patients who do not go on to develop full-blown rhabdomyolysis (Williams and Unwin, 1997). In one retrospective review of individuals with MA-related rhabdomyolysis, mean concentrations of CPK in the MA users were much higher than in individuals with rhabdomyolysis from other causes (12,439 vs. 5,678 U/L; $p = .02$) (Richards, 2000).

How any amphetamine interacts with the ryanodine receptors within muscles to release calcium from the endoplasmic reticulum has never been established, but it is now known that MA interferes with L-type calcium channel function, leading to increased rate and force of myocyte contraction (Liang et al., 2010). If sufficient calcium were to enter the cytosol, it would lead to additional release of calcium stores from within the endoplasmic reticulum and, perhaps, destroy the cell.

3.1.10.9 Oral Complications

Chronic MA abuse is associated with severe oral health effects. Rampant caries are the most notable manifestation (Ravenel et al., 2012). The term "meth mouth" is often used to describe the disease process. The cause of these changes is not entirely clear, but it may be that a craving for sugar-rich carbonated beverages, a common feature of MA abuse, is to blame. Prolonged exposure to these drinks promotes the formation of dental caries.

The possibility also exists that chronic MA use may result in decreased salivary flow rate, causing central inhibition of the salivatory nuclei via stimulation of α_2 receptors in the brain. A decreased salivary flow rate, either secondary to a central inhibitory action of MA or generalized dehydration, would contribute to the increased occurrence of dental caries in abusers (Robinson et al., 2005; Saini et al., 2005). A chronic lack of saliva leads to decreased cleansing of plaque from the teeth. Also, abusers often report the subjective perception of xerostomia, which cannot be explained by the direct peripheral action of MA on the secretory acini. This is especially true for MDMA users who, in addition to other symptoms of MA abuse, also have a tendency toward bruxism, which can lead to fracture of teeth already weakened by prolonged bathing in the acid solution of MA that drips from the sinuses into the mouth. After the enamel has been broken off, the softer underlying dentine is more prone to decay and less resistant to fracture.

Because MA abusers are generally distracted, they may not even notice the changes that are occurring. Without proper oral hygiene, gingivitis is almost certain to develop. The areas most commonly affected are the buccal surfaces of the teeth and the interproximal areas of the anterior teeth. Until proven otherwise, it would seem that the dental changes result from a combination of trauma, neglect, and poor diet (Laslett and Crofts, 2007; Hamamoto and Rhodus, 2009).

3.1.10.10 Gastrointestinal Tract

Chronic MA abuse is associated with liver damage. In one very large autopsy series, hepatic steatosis was evident in 15.4% of all cases, cirrhosis in nearly 9%, cellular infiltrates of the portal triads ("triaditis") in 6%, and hepatitis in 5% (Karch et al., 1999); these disorders are illustrated in Chapter 1 and are identical in appearance to the same lesions seen in cocaine abusers.

Hepatitis and infiltration of the portal triads are both common findings in intravenous drug users, and their occurrence in MA drug users probably is coincidental, having nothing to do with the basic pharmacologic properties of MA, but rather occurring as a consequence of polydrug abuse, exposing them to both hepatitis B and C, as well as HIV. A very high percentage of heroin users in California are HIV positive, but rates are much higher in other countries. In 2012, Garfein et al. found that 95% of intravenous drug users in Tijuana, Mexico, were infected, but that in San Diego, just across the border, the rate of infection of those less than 40 years of age was only 30% (Garfein et al., 2013). Infection

with these viruses likely is the explanation of the infiltrates seen in the portal triads of many MA users. It also probably explains why the immunocompromised (i.e., HIV infected) are likely to develop hepatic fibrosis at such a high rate (Fierer et al., 2008). Hepatic fibrosis in chronic MA abusers is probably the result of some other disease, not a direct effect of any amphetamine.

3.1.10.11 Attention Deficit/Hyperactivity Disorder

Approximately 1.5 million adults and 2.5 million children in the United States take ADHD medications, either proprietary mixtures of amphetamine salts (Adderall™) or methylphenidate (Ritalin). The quantity is sufficiently large that, inevitably, some of the drug will be diverted to the black market, and some have suggested that this may lead to excess morbidity and mortality. The U.S. FDA has added to these concerns by requiring the manufacturers of Adderall and Ritalin to add "black box warnings" to their package inserts warning that use may be associated with sudden cardiac death (SCD).

ADHD is not a single pathophysiologic entity. Rather, it appears to be the result of multiple genetic and epigenetic factors, each having a small individual effect that combined lead to behavioral abnormalities. Structural imaging studies show that brains of children with ADHD are significantly smaller than those of unaffected controls. The prefrontal cortex, basal ganglia, and cerebellum are differentially affected, and other evidence exists that shows that there is reduced connectivity in some white matter tracts. DA dysregulation plays an established role in the neurobiology of ADHD, and stimulants remain the most effective drug treatment available for that disorder. Methylphenidate remains the preferred treatment, though large numbers of physicians around the world use amphetamine salts (Adderall in the United States, others elsewhere in the world) (Curatolo et al., 2010).

There is only epidemiologic evidence to support the claim that medical or illicit amphetamine or methylphenidate use is associated with SCD, but most think the evidence insufficient to establish a nexus. The regulatory agencies have their doubts about the dangers of these drugs. Amidst mounting concern, the Canadian government removed them from the market in 2005, only to return them a few months later, after a more thorough review of the evidence. The same considerations apply to diversion. It undoubtedly occurs, but there are no reliable data on the extent of the problem.

In the largest case-control study yet performed, mortality data from 1985 to 1996 state vital statistics were used to identify 564 cases of sudden death occurring in the United States among those young people aged 7–19 years. The decedents were compared with a matched group of 564 young people who died as passengers in motor vehicle traffic accidents. In 1.8% of the cases, it was determined that the decedents were taking stimulants, specifically methylphenidate; in contrast, use of stimulants was found in only two subjects in the motor vehicle accident comparison group (0.4%), with only one involving methylphenidate use, suggesting a very significant association between stimulant use and sudden unexplained death in young people (odds ratio [OR] 7.4, 95% CI = 1.4–74.9) (Gould et al., 2009). Other epidemiologic studies tend to support this conclusion. The American Psychiatric Association did a prospective, case-matched study and concluded "Although initiation of methylphenidate was associated with a 1.8-fold increase in risk of sudden death or ventricular arrhythmia, the lack of a dose-response relationship suggests that this association may not be a causal one" (Schelleman et al., 2012). There is one case report of acute myocardial infarction in a young man taking AdderallXR, but the urine-screening

test showed MA and was, in any event, unconfirmed. Since there was no autopsy, there is no way to determine postmortem what the actual cause of death was (Jiao et al., 2009). There is clear evidence that Adderall and methylphenidate do exert a measurable effect on pulse and blood pressure, but neither drug causes cardiotoxicity per se (Martinez-Raga et al., 2012). Most of the other adverse event reports that have been submitted suffer from the same lack of detail.

3.1.10.12 Amphetamine Concentrations in the Living

The controlled administration of MA has been well studied, but there are little data on plasma concentrations actually achieved after binge use, or on what concentrations should be expected when abusers with clinically apparent toxicity present to emergency departments. One report describes seven patients with evidence of amphetamine toxicity: plasma concentrations were found to range from 105 to 560 ng/mL (Lebish et al., 1970). Blood concentrations can easily exceed 1000 ng/mL (1 mg/L) but only in extreme use situations, where subjects have a high tolerance to the drug. It is important to accept the total absence of any significant correlation between blood concentrations of any stimulant with dose consumed or with the effect produced in the living or in the dead.

The appearance of MA in tissues, including saliva, is unpredictable. In one study of 25 MA abusers, MA was found in the hair of 73%, the nails of 65%, and the sweat of 50%, but in the saliva of only 16% of the participants (Suzuki et al., 1989). Pubic hair can be used as an alternative to scalp hair to prove previous drug use, but it should be avoided when attempting to reconstruct the drug use history, because pubic hair becomes contaminated by urine. Concentrations measured in pubic hair usually exceed those measured in head hair. Accordingly, a high concentration measured in pubic hair is not an indicator of heavier drug use (Lee et al., 2011).

MA measurement in the living is fairly standard, but detection of other amphetamines is problematic. Under federally regulated workplace testing programs, a urine test is not considered to be positive for MA unless immunologic screening tests demonstrate that the specimen contains amphetamine or MA in concentrations of 1000 ng/mL or more, and subsequent gas chromatography–mass spectrometry (GC–MS) analysis confirms a concentration of at least 500 ng/mL. First-generation amphetamine screening tests often cross-reacted with compounds such as ephedrine and phenylpropanolamine, but the problem was largely eliminated when these products were removed from the market. The degree of cross-reactivity with other *N*-methylated amphetamines (e.g., MDMA) is high. In forensic situations, laboratories often adopt much lower detection limits, allowing detection of drug use in blood and urine for much longer than in situations where cutoffs are applied for urine testing in workplace programs.

3.1.10.13 Impairment

There is no evidence that low oral doses of amphetamine and MA can significantly impair psychomotor performance. In fact, there is a good deal of evidence suggesting quite the opposite (Newhouse et al., 1989; Koelega, 1993; Mills et al., 2001; Caldwell et al., 2003; Kelly et al., 2004; Van Dongen et al., 2006). However, it is generally accepted that the intake of higher doses of ATS may impair traffic-related skills, particularly cognitive function. Tunnel vision and effects on divided attention skills are most common.

An analysis of data derived from 878 Norwegian amphetamine abusers arrested for "driving under the influence" (DUI) illustrates the difficulties. A police physician examined all of the drivers. The examinations disclosed that 27% of the drivers were not thought to be impaired at all, while 73% were, and in the group that were thought to be impaired, there was a positive relationship between blood amphetamines concentrations and impairment. However, the relationship had limits and only applied when blood amphetamines concentrations were in the range of 0.27–0.53 mg/L. At the same blood concentrations, the diagnosis of impairment was made more often in younger drivers than old (Gustavsen et al., 2006). In another very large study of amphetamine abusers arrested for DUI, the median concentration of amphetamine in males was 740 and 880 ng/mL in women (Jones and Holmgren, 2005). These concentrations are likely to be lower than in postmortem cases where redistribution leads to artifactual elevation.

3.1.11 Postmortem Toxicology

3.1.11.1 Preanalytic Considerations

3.1.11.1.1 False Positives The tests most commonly used to screen urine for drugs of abuse are immunoassays and they may yield false positives for amphetamine and MA (Brahm et al., 2010). In addition, it is possible to have a true-positive result that has nothing to do with drug abuse. MA might actually be present because of the conversion of certain drugs, such as selegiline (Eldepryl, Deprenyl), to amphetamines. Selegiline is a MAO inhibitor used in the treatment of parkinsonism. It is a derivative of phenethylamine, and two of its principal metabolites are amphetamine and MA. Both may accumulate in substantial amounts in patients receiving antiparkinson therapy. In such cases, the clinical history will be necessary to make the correct diagnosis. Other drugs that can be converted to MA include benzphetamine, clobenzorex, deprenyl, dimethylamphetamine, ethylamphetamine, famprofazone, fencamine, fenethylline, fenproporex, furfenorex, mefenorex, mesocarb, and prenylamine (Musshoff, 2000); however, many of these form the less active *l* isomer and can be distinguished from the active *d* isomer through chiral analysis.

3.1.11.1.2 Tolerance In most MA-related deaths, postmortem blood concentrations fall between 0.5 and 2 mg/L. However, under no circumstances can MA concentrations, by themselves, be used to determine the cause of death. Long-term MA abuse sets in train a complicated series of interactions affecting both physiologic and behavioral responses. As is true for cocaine, death from MA may be associated with very low or very high postmortem blood concentrations (Karch and Billingham, 1988; Jenkins et al., 2002; Pilgrim et al., 2009). Tolerance to stimulant drugs cannot be assessed at autopsy, so it is not possible to attribute any particular significance to an isolated high-concentration measurement (unless, of course, no MA is detected in the hair, which would prove that the decedent was drug naïve and, therefore, not tolerant). Of all the amphetamine-type drugs, this caveat is especially true for MA, where the measured blood concentration is also highly dependent on where in the body the sample is obtained. Low concentrations are equally difficult to interpret. If significant heart disease is present (left ventricular remodeling), and there is a documented history of long-term MA abuse, and hair testing for MA is strongly positive (in one recent study, the average hair concentration found in

regular MA abusers was 10 ± 9 ng/mg [range 1.5–30 ng/mg], while amphetamine concentrations averaged 2.2 ± 2.8 ng/mg [range 0.4–13 ng/mg] [Baeck et al., 2011]), death can reasonably be attributed to MA even when MA blood drug concentrations are low or nonexistent; the MA has changed cardiac structures sufficiently to allow the occurrence of lethal arrhythmias.

3.1.11.1.3 Testing Matrices MA appears in both saliva and breath, though neither has any particular relevance to postmortem issues. However, measurement of brain drug concentrations could be an extremely valuable tool. There is substantial experimental evidence that brain concentration measurements provide a more accurate portrayal of the situation at the time of death than is suggested by isolated blood drug measurements (Spiehler and Reed, 1985). MA appears to be homogenously distributed in postmortem brain (Kalasinsky et al., 2001), so that if brain were the matrix of choice, it might indeed be possible to set meaningful ranges for toxicity and not be overly concerned about issues related to postmortem redistribution.

3.1.11.1.4 Methamphetamine Stability and Measurement There is no data on MA stability in humans. The results of animal studies suggest that, over the long term, amphetamines are stable in most tissues, no matter the degree of environmental exposure. Nagata et al. (1990) found that concentrations of both MA and amphetamine in whole blood, liver, and skeletal muscle were almost unchanged after 2 years of storage in sealed tubes. Concentrations only decreased by half in samples of bone exposed to the air over a 2-year period. The amphetamine content of marrow submerged in tap water for 2 years was determined to have barely decreased from baseline (Nagata et al., 1990). Levels in blood and urine do not decrease significantly with storage (Jimenez et al., 2006), but after exposure to formalin, MA is converted into its *N*-methyl derivative. The rate of conversion is pH dependent and also depends upon the formalin concentration; the greatest conversion occurs under alkaline conditions, and the higher the formalin concentration, the greater the rate and degree of conversion. Conversion to the *N*-methyl derivative begins within 30 min of exposure to formalin and, under certain conditions, the conversion may be complete (Tirumalai et al., 2005). Other studies have shown that after the formalin fixation, MA concentrations in fixed organ tissues decrease by 1.3%–3.1% per day, partly because the drug elutes into formalin fixative (Takayasu et al., 1994).

There is also the problem of Vicks® decongestant inhaler, which contains the levorotatory isomer of MA (Poklis et al., 1993). This isomer has only modest CNS and cardiovascular activity (Li et al., 2010), but unless special precautions are taken, it may give false-positive test results for MA during both screening and confirmatory testing, particularly when concentrations are relatively high. Few laboratories are equipped to do the testing required to discriminate between the *d* and *l* forms of MA (chiral analysis). Immunoassay-based screening for amphetamines as a group still has a variably positive predictive value for amphetamine abuse, which makes confirmatory testing a necessity. Unfortunately, in some jurisdictions, not all urine screening is done under rules requiring confirmatory testing using mass spectrometry (MS). As a consequence, false positives are still reported with some frequency. There is, however, no substitute for confirmation using alternative testing modalities.

3.1.11.1.5 Site Dependence Concentrations of MA measured at autopsy are site dependent, and concentrations in left-heart blood may be many times higher than concentrations measured in right-heart blood (Miyazaki et al., 1993; Moriya and Hashimoto, 1999). Concentrations in samples collected from other sites may also differ. The difference is at

least partly explained by the diffusion of MA out of lung tissue into the pulmonary circulation, which occurs more rapidly than diffusion from the liver into the vena cava. As a consequence, MA concentrations in the blood from the pulmonary artery and left ventricle may be many times higher than in the blood collected from the right side of the heart (Nagata et al., 1990; Moriya and Hashimoto, 1999). Results may also be altered by the size of the specimen collected. If large quantities of blood (approximately 50 mL) are drawn from the inferior vena cava, the values measured may be more a reflection of hepatic drug concentration than of the amount of drug circulating in the blood at the time of death. Femoral blood is the recommended site of collection, wherever possible, since this reduces artifactual changes.

3.1.11.2 Recommended Analytic Methods

Numerous measurement methods are available. The most commonly used screening test is the immunoassay for blood, oral fluid, hair, and urine. Typically there are separate immunoassays for amphetamine and for the MA-like compounds. The latter includes most commonly MA and MDMA since both have an *N*-methyl moiety. A different antibody to show adequate sensitivity and specificity for the other amphetamine-like compounds is required.

Radioimmunoassay (RIA), once the most common type of immunoassay, can accurately detect concentrations as low as 25–50 ng/mL for MA (Collison et al., 1998). Enzyme-linked immunosorbent assay (ELISA) methods applied to blood and oral fluid are now more common than RIA, as they do not involve the use of radioisotopes and have similar detection limits (Collison et al., 1998; Kupiec et al., 2002; Laloup et al., 2005). Urine testing for these amphetamines is affected by the same issues, although analysis via this route is easier, mainly because detection limits and cutoffs required are higher (250–1000 ng/mL) than for blood (Verstraete and Heyden, 2005). The use of monoclonal antibodies has reduced cross-reactivity with ephedrine and pseudoephedrine and related sympathomimetics. As with all immunoassays, confirmation testing is always required, preferably utilizing an MS method.

Hair testing for the amphetamines requires much lower detection limits ranging from 0.1 to 0.3 ng/mg and can be adequately achieved using ELISA (Musshoff et al., 2012). As with other drugs, chromatographic screening can also be performed, with liquid chromatography–tandem mass spectrometry (LC–MS–MS) being the most common method. This approach eliminates the need for derivatization when using GC–MS (Staack and Maurer, 2005; Wohlfarth et al., 2010). Drug is extracted using mixed-mode solid-phase or solvent extraction and enables the screening of a larger range of drugs and drug metabolites than is possible for a single immunoassay test.

Confirmation testing must use MS and is usually performed following an immunoassay screening test for this class of drug. The mass spectrum of amphetamine and MA, particularly after electron impact ionization, does not provide useful characteristic ions for unambiguous identification; hence, derivatization is required for these drugs when using GC. There are numerous GC–MS methods including derivatization by extractive acylation with pentafluoropropionic anhydride (Marais et al., 2011) and derivatization with heptafluorobutyric anhydride (Dallakian et al., 1996; Stout et al., 2002; Gunn et al., 2010), or even with propylchloroformate (Meatherall, 1995). When MA is targeted, some amphetamine is usually expected as a minor metabolite to assist in the confirmation process. LC–MS/MS methods are also available in all tissues to confirm these amphetamines and other drugs (Fernandez Mdel et al., 2009; Bjork et al., 2010).

When identification of the stereoisomer is required, chiral analysis can be performed on GC–MS using one of a number of reagents including *R*-(−)-α-methoxy-α-trifluoromethylphenylacetylchloride (Walther-Larsen and Rasmussen, 2006) and trifluoroacetyl-*l*-prolyl chloride (Hensley and Cody, 1999). Detection limits in oral fluid, urine, and blood below 10 ng/mL are possible.

3.1.11.3 Interpretation of Infant Postmortem Blood Concentrations

The American Academy of Pediatrics considers breastfeeding to be contraindicated if the mother is using amphetamine(s) (American Academy of Pediatrics Committee on Drugs, 2001). However, at the time the recommendation was issued, there was only one published case report suggesting that amphetamine accumulates in human breast milk. It described a woman who was tested at 10 days and again at 42 days after delivery; amphetamine concentrations were much higher in her milk than in her plasma (Steiner et al., 1984). These measurements were made at a time when it was not appreciated that milk expressed early has a much lower concentration of fat than milk expressed later from the same breast and therefore, would be likely to have a higher amphetamine concentration (Suri et al., 2002).

There is, however, no doubt that amphetamine is transferred to infants by breast milk. A paper published in 2006 described the findings in four maternal–infant pairs. The median maternal dexamphetamine dose was 18 mg/day (range 15–45 mg/day). Median (interquartile range) descriptors were 3.3 (2.2–4.8) for milk/plasma ratio, 21 μg/kg on day 1 (range 11–39 μg/kg) for absolute infant dose, and 5.7% (4%–10.6%) for relative infant dose. No adverse effects were seen in any of the infants. In one, amphetamine was present in milk (limit of detection 1 μg/L) at a concentration of 18 μg/L. In the other two pairs, the milk concentration was 2 μg/L. Thus, the relative infant dose of amphetamine was <10% and within a range that is generally accepted as being "safe" in the short term (Ilett et al., 2002).

Although MA is certainly transferred by breast milk, the significance of this transference remains debatable. There are a number of other case reports describing women who took MA, either for narcolepsy or because they were abusers, throughout pregnancy and even while nursing, with no detectable ill effects in their children (Milkovich and van den Berg, 1974; Eriksson et al., 1978; Billing et al., 1980; Little et al., 1988; Joffe and Kasnic, 1994). Nonetheless, at least two MA-using mothers in California have been convicted of child endangerment for administering drugs by breastfeeding (Ariagno et al., 1995), and many more are on trial as this book goes to press. Such legislation is not uncommon in the United States.

In 2009, Australian investigators studied two women who had regularly used MA intravenously throughout their pregnancies and who, on their return home from hospital, began a 24 h milk collection with the first sample collected just before drug use and subsequent samples every 2–6 h afterward for 24 h. The average MA milk concentration in both cases is shown in Figure 3.26 (Bartu et al., 2009). The researchers estimated that combined absolute infant doses for MA plus amphetamine (as MA equivalents) in the 24 h after injecting would be 6.1 μg/kg/day in one woman and 8.9 μg/kg/day in the other. Blood concentrations in the infants were never measured (Bartu et al., 2009). Interestingly, the infants showed no developmental differences from controls.

A handful of anecdotal reports have described blood levels in dead infants. One case involved a 24-year-old chronic amphetamine abuser who delivered a premature, low-Apgar-score, 2 lb, 7.5 ounce child who expired at 4 h. Autopsy findings were consistent

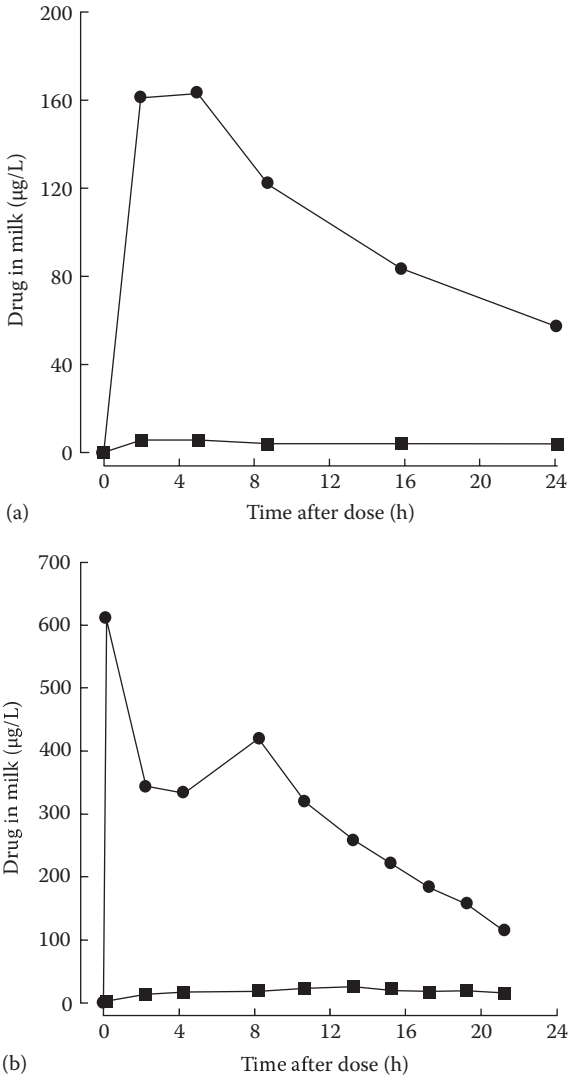


Figure 3.26 Transfer of methylamphetamine and amphetamine into breast milk following recreational use of methylamphetamine. Drug concentration in milk versus time for case 1 (a) and case 2 (b) following self-administration of one "point" of methylamphetamine at zero time. Methylamphetamine (●); amphetamine (■). (Adapted from Bartu, A. et al., *Br. J. Clin. Pharmacol.*, 67(4), 455, 2009.)

with intrauterine anoxia. MA concentration was highest in the lungs and lowest in the liver. The concentration in the lungs was nearly three times that in the blood (Garriott and Spruill, 1973). In a second case, an MA-injecting mother injected amphetamine a few hours prior to giving birth to twins. The highest amphetamine concentrations were in the kidney and liver, and the lowest concentrations were in the blood and brain, with concentrations ranging from 4.53 to 11.0 mg/kg. Amphetamine concentrations were less than 15% of the MA concentrations, which ranged from 0.18 to 1.40 mg/kg (Stek et al., 1993).

As the ability to detect nanogram quantities of drugs improves, drugs are being detected in more and more sick children. In a 2009 study, hair testing was used to investigate the prevalence of unsuspected exposure to cocaine in a group of preschool children

presenting to an urban pediatric emergency room. None of the children had signs or symptoms suggestive of drug exposure. Hair samples were obtained from 90 children between ages 18 months and 5 years. In 85 cases, hair samples from the accompanying parent were also provided. Hair samples from 21 children (23.3%) were positive for cocaine (concentration range 0.3–5.96 ng/mg of hair) with 1 sample also positive for MDMA and another for opiates. In 88% of the positive cases, cocaine was also found in the hair of the accompanying parent (15 of 17 matched parent–child hair samples) (Joya et al., 2009).

Based on those findings, one would automatically assume that any other drug in the environment, including alcohol (Kulaga et al., 2010), could become deposited in/on the child's hair, as in fact it does. Nearly half the children removed from houses where illicit MA is being manufactured have hair that tests positive for MA or one of its precursors (Bousman et al., 2010). Given this reality, it must be emphasized (especially to prosecutors) that the mere presence of these drugs is not sufficient reason to implicate them as a cause of illness or death; it just proves that the drug is present. The issues of endangerment can and probably should be raised, but it is not clear that any conscious act of the parent causes contamination of the environment, although under strict interpretation of the law, even inadvertent exposure is endangerment.

In order to prove that death or illness was the result of drug toxicity, there should be some plausible explanation of the mechanism of toxicity and, preferably, anatomic or histologic evidence of toxicity. In the case of MA and cocaine, the presence of infarction, cardiac enlargement, myocardial fibrosis, or other evidence of left ventricular remodeling might provide such evidence. At the same time, other alternative causes of death must be proven absent. For example, a child with a modest blood concentration of MA might well die of a heritable long QT syndrome (LQTS) or some other genetic disorder that has nothing to do with the presence of trace amounts of any particular drug (Sarkozy and Brugada, 2005). Congenital LQTS comprises a distinct group of cardiac channelopathies characterized by QT prolongation on a 12-lead surface EKG. These mutations carry with them an increased risk for syncope, seizures, and SCD, especially in the setting of a structurally normal heart and otherwise healthy individual. Evidence suggests that the incidence of LQTS may be as high as 1 in 2500 persons (Kapplinger et al., 2009), but these syndromes cannot be detected at autopsy without DNA sequencing.

The situation is no different with other stimulant drugs; the simple detection of some modest concentration (5–10 μg/g) of cocaine or MA in a deceased child does not provide a cause of death. Unless and until testing for channelopathies has been performed, the autopsy is incomplete and the cause of death should be considered undetermined. At a minimum, the autopsy itself must be meticulous and complete, and the state's mandated sudden infant death syndrome protocol completed; but even then a meticulous autopsy is not sufficient to rule out a genetic defect. This situation is likely to change in the very near future. In November of 2012, the FDA licensed for sale (and clinical use) a third generation of DNA sequencer. The device can be used to determine the precise order of nucleotides within a DNA molecule, which will ultimately produce a protein. There is no reason to suppose that some variant of this same technology cannot, in the very near future, be used to discover the abnormal sequences responsible for LQTS channelopathies (Makita, 2013).

3.1.11.4 Interpretation of Adult Postmortem Blood Concentrations

Postmortem MA measurements have been reported in two large autopsy series (Logan et al., 1998; Karch et al., 1999) and an analysis of postmortem redistribution of MA addressed in

one other (Barnhart et al., 1999). Together, these studies show that the value of postmortem MA measurement is questionable, because tolerance cannot be directly assessed after death and because postmortem redistribution occurs (Moriya and Hashimoto, 1999). Many, if not most, MA-using decedents are polydrug users (Karch et al., 1999), and because MA concentrates in heart muscle, from which it is slowly released after death (Barnhart et al., 1999; Volkow et al., 2010), blood taken from the heart is bound to have a higher concentration than blood sampled elsewhere in the body. Measurements made on left-heart blood inevitably show spurious elevations in MA concentration, but equally spurious elevations can also be seen in peripheral blood samples. In a large series of cases where postmortem femoral blood drug concentrations were compared to those in blood obtained at autopsy several days later, the concentrations of many drugs, including MA, were substantially increased (Gerostamoulos et al., 2010).

In one of the large autopsy series, drug concentrations in a group of 132 decedents, where MA toxicity was clearly not the cause of death (trauma victims), were compared with concentrations measured in 232 decedents where MA was deemed the cause of death (Karch et al., 1999). Seventy-five percent of each group had blood MA concentrations of less than 1.32 mg/L. The mean blood MA concentration was 1.84 mg/L in the trauma group and 2.11 mg/L in the MA-induced group (not a statistically significant difference).

Blood amphetamine concentrations in the same two groups were 0.24 mg/L in the incidental finding group and 0.16 mg/L in those dying of drug toxicity. Again, the concentrations were not significantly different. Measurement of urinary MA and amphetamine concentrations revealed similar overlap; no difference was observed between cases in which MA was the cause of death and cases where it was not (38.6 vs. 27.5 for MA and 8.0 vs. 4.5 mg/L for amphetamine). Ethanol was present in one quarter of all the cases, and cocaine or cocaine metabolite in 24% of the decedents. However, the drug most often used in combination with MA was morphine, which was present in nearly one-third of the cases (Karch et al., 1999). Comparable results were also reported from a second autopsy series in Seattle (Logan et al., 1998). Similar conclusions were obtained from an Australian study of deaths attributed to MA (Pilgrim et al., 2009).

3.2 Methylphenidate

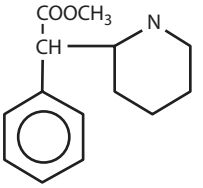
3.2.1 Physiochemical Properties and Pharmacokinetics

A summary of the principal properties of the drug and its pharmacokinetics is shown in Table 3.6.

3.2.2 Incidence and Epidemiology

Methylphenidate (Ritalin) is used extensively for the treatment of ADHD (Robison et al., 1999) and is also indicated for the treatment of narcolepsy. Because the drug is so widely available, diversion and illicit sales do occur, mostly to teenagers and young people, but it is very difficult to gauge the extent of the problem. Based on the findings of the last iteration of the DAWN report, from which national trends cannot be estimated, it would seem that the amount of drug diverted to the black market could not be very great. Twelve methylphenidate deaths were reported in the medical examiner's component of the 1999 DAWN

Table 3.6 Physiochemical Properties and Pharmacokinetics of Methylphenidate

Chemical name	*d*,*l*-threo-α-phenyl-2-piperidine acetic acid methyl ester
Physiochemical properties, structure, and form	Available as hydrochloride salt, soluble in water CAS 113-45-1 MW 233.3 p*K*a 8.8 
Brand names	Ritalin (Ritalina, Rilatine, Ritalin LA [long acting]), Attenta, Concerta (a timed-release capsule), OROS MPH, MTS, Metadate, Methylin, and Rubifen. Methylphenidate administered via a transdermal patch (under the brand name Daytrana)
Pharmacokinetic parameters	Bioavailability: Oral—*d* form, 22 ± 8; *l* form, 5 ± 3 (Aoyama et al., 1994) C_{max} (oral) oral, 10 mg, 7.8 (Pierce et al., 2010); *d* form, 18.1 ng/mL (Aoyama et al. 1994); *l* form, 3.0 ± 0.94 ng/mL (Aoyama et al., 1994). 15 mg, 4.7 ng/mL racemic (Modi et al., 2000), 0.3 mg/kg = 15.7 (Zhu et al., 2007), extended release in adults, 13.6 ng/mL (Childress and Berry, 2010) Transdermal: 10 mg, 4.2–9.3 ng/mL for single dose, depending on age (Pierce et al., 2010) T_{max} oral: 2–13 h (Aoyama et al., 1994; Modi et al., 2000; Childress and Berry, 2010) Transdermal: 8–12 h depending on age (Pierce et al., 2010) Protein binding: ~15%–30% V_d 11–33 L/kg
Common blood concentrations in users	Up to about 0.1 mg/L
Blood terminal elimination half-life	2–4 h. Likely to reduce with advanced age, and liver and kidney disease, and is affected by urine pH
Metabolism	Ritalinic acid, *p*-hydroxymethylphenidate, and 6-oxoritalinic acid
Urinary excretion	Methylphenidate: <1% Ritalinic acid: 60%–80% 6-oxoritalinic acid: 5%–12%
Postmortem artifacts	Not clear, likely to at least undergo moderate changes.
Interactions	Interactions with other drugs are unlikely, as methylphenidate is not metabolized by the P450 system, rather by a carboxylesterase. Most is excreted unchanged as ritalinic acid (α-phenyl-2-piperidine acetic acid).
Published reviews and key papers	Challman and Lipsky (2000), Klein-Schwartz (2003), Lyseng-Williamson and Keating (2002), Pierce et al. (2010)

report (0.01% of all drug-related deaths), but no mention was found in the emergency department component of the same DAWN report or in the National Household Survey (Green et al., 2000). Worse, there was no coordinated government effort to gauge the magnitude of the problem until 2001, and even then it was inadequate. Virtually all the available information is based upon national surveys that, more often than not, lump illicitly

produced or "street" versions of stimulant drugs with controlled prescription medications. For instance, prescription stimulants such as MA, Desoxyn, "uppers," "speed," Ritalin, or methylphenidate all fall under the same category, and there is no method available that would allow one to parse out individual components (Dun and Boyden, 2008).

The American Association of Poison Control Centers' National Poison Data System showed a 50% increase in prescriptions for methylphenidate to teenagers in the 8 years from 1997 to 2005. By contrast, a 2004 report issued by the U.S. National Drug Intelligence Center, updated by the government in 2010, indicates that rates of methylphenidate abuse are declining. NDIC data show an overall decrease from 2001 to 2003 among eighth (2.9%–2.6%), tenth (4.8%–4.1%), and twelfth (5.1%–4.0%) graders. Data from the 2004 Monitoring the Future survey indicated that the rate of past-year abuse of Ritalin among young adults was 2.9% in both 2002 and 2003 (National Drug Intelligence Center, 2004). The 2010 version of the same report does not even mention methylphenidate or methylphenidate diversions or associated deaths.

A retrospective study of reports to the Swiss Toxicological Information Center over a 13-year period showed an increase in reported cases of toxicity associated with methylphenidate. The finding was consistent with the increase in nonmedical use of this substance. In contrast to cocaine and amphetamine, most cases of methylphenidate overdose exhibited only mild or moderate intoxication (Bruggiser, 2010). These data suggest that methylphenidate will be used and abused for at least some years to come, and occasionally the drug will be present in medical examiner cases. Whether it has anything to do with the cause of death is another matter, but its presence, at least, must be noted.

3.2.3 Names and Drug Constants

Methylphenidate ([*d*,*l*]-threo-α-phenyl-2-piperidine acetic acid methyl ester) has two chiral centers, but the drug used in therapy comprises only the threo pair of enantiomers. *d*-Threomethylphenidate is more potent than the *l*-enantiomers. Methylphenidate is administered as a racemic mixture that undergoes stereoselective clearance (Kimko et al., 1999) and the different isomers have profoundly different tissue distributions (Thai et al., 1999). In the United States, methylphenidate is sold only under the brand name Ritalin, either as immediate or timed-release tablets.

3.2.4 Routes of Administration

Methylphenidate is rapidly absorbed after oral administration, reaching peak levels between 1 and 3 h after ingestion (Wargin et al., 1983; Volkow et al., 1995); however, timed-release formulations exhibit peak blood concentrations approximately 4 h after ingestion. There is no significant difference in the absorption profile when the drug is taken before or after a meal. Abusers inject methylphenidate (Levine et al., 1986; Gautschi and Zellweger, 2006) or occasionally snort it (Jaffe, 1991). Transnasal bioavailability is not known, but plasma concentrations after both oral and transcutaneous application have been studied. After patch application, no methylphenidate is detected in plasma for at least 3 h (range 1–6 h), and concentrations of the *d* isomer (which is the more active form) are not seen until 7–9 h have elapsed. In children, at 9 h, plasma concentrations of methylphenidate are comparable to those seen after taking the oral, timed-release form (Pierce et al., 2010). The drug is far more toxic if administered intravenously (Bruggiser et al., 2010).

3.2.5 Mode of Action

The reinforcing effects of methylphenidate are a consequence of its ability to block DATs. Even though cocaine and methylphenidate have similar in vitro affinities for the DAT, abuse of methylphenidate is extremely uncommon, at least when compared to cocaine. It has been suggested that the difference has to do with the persistence of methylphenidate within the striatum. Unlike cocaine, which is washed out in a matter of minutes, methylphenidate remains localized in the striatum for several hours (Volkow et al., 1999). Similarly, even though cocaine and methylphenidate cause comparable increases in heart rate and blood pressure, these increases persist for much longer with methylphenidate than cocaine (Volkow et al., 1999).

Even though methylphenidate binds to and blocks DAT and NE transporters, it differs from cocaine and amphetamine in that it has a very low affinity for the 5-HT transporter. When methylphenidate is administered, extracellular levels of DA in the striatum, nucleus accumbens, and prefrontal cortex all increase. Methylphenidate also raises concentrations of NE levels in many parts of the brain, including the prefrontal cortex and hippocampus. However, unlike other psychostimulants, which have a high affinity for the 5-HT transporter, methylphenidate produces no 5-HT overflow in the striatum and nucleus accumbens, which may explain its extremely low potential for significant abuse. Even though self-administration studies in animal models suggest that exposure to methylphenidate, particularly at a young age, might increase the likelihood of subsequent substance abuse in humans, there is no clinical evidence that any such process occurs (Yano and Steiner, 2007).

3.2.6 Pharmacokinetics

Methylphenidate has a much shorter half-life (about 4 h) than other amphetamines. First-pass hydrolysis to ritalinic acid (RA) occurs in the intestine, and 80% is excreted in the urine as RA. Peak plasma levels can range up to at least 20 ng/mL depending on dose, frequency of administration, and formulation. While methylphenidate and RA peak at about the same time, concentrations of RA are much higher than those of the parent drug. In vitro studies indicate that methylphenidate metabolism is not CYP dependent (DeVane et al., 2000), which suggests that there should be minimal intraindividual variation in rates of metabolism, though this has not been proven. At least one male volunteer treated with a test dose of methylphenidate exhibited a methylphenidate hydrolysis defect producing very high concentrations of methylphenidate and very low RA concentrations under all study conditions (Koehm et al., 2010). A meta-analysis published in 2012 was able to confirm any link between the use of methylphenidate and SCD (Martinez-Raga et al., 2012).

When methylphenidate and ethanol are coingested, a new, active metabolite, ethylphenidate (RA ethyl ester), is formed by a mechanism analogous to that responsible for production of cocaethylene: hepatic-carboxylesterase-dependent transesterification. Only very small amounts of ethylphenidate are produced after clinically relevant doses of alcohol are consumed, and unlike cocaethylene, which has a much longer apparent half-life than cocaine, the half-life of ethylphenidate is much shorter than that of the parent compound (Markowitz et al., 1999). New evidence suggests that when ethanol and methylphenidate are consumed simultaneously, absorption is substantially increased (Patrick et al., 2013).

Pharmacokinetic studies of male volunteers suggest that methylphenidate concentrations are not markedly affected by ethanol, but RA concentrations were lower, especially if ethanol was ingested first. Ethylphenidate concentrations are always low when compared with those of methylphenidate (about 10%). This suggests that concurrent ethanol use does not impair methylphenidate's therapeutic efficacy (Koehm et al., 2010).

3.2.7 Blood Concentrations

In therapeutic settings, peak concentrations of methylphenidate may reach 7–8 ng/mL (Gualtieri et al., 1984; Pierce et al., 2010), although much higher concentrations (>20 ng/mL) can occur depending on the dose, frequency of administration, and formulation (Stevens et al., 2010). Treatment is not generally guided by plasma drug concentrations.

3.2.8 Breast Milk Concentrations

Methylphenidate is excreted in breast milk, but in such low concentrations that mothers with narcolepsy have no need to discontinue the drug. A case report published in 2007 described the values measured in a woman taking 15 mg of Ritalin per day. Maternal serum and breast milk were obtained at five time points: immediately before the morning dose at 8 a.m., just before the dose at noon, and 4, 8, and 21 h after the dose at noon. The first three samples were from foremilk, whereas the two last samples were from hindmilk. The maternal serum concentrations in the five samples were <0.3, 2.3, 3.8, 1.7, and <0.3 ng/mL, respectively. The corresponding milk concentrations were <0.3, 2.4, 5.9, 1.4, and <0.3 ng/mL. Presuming that the infant ingested the standard volume of 150 mL of milk per kilogram of body weight per day, the authors estimated the daily infant dose at 0.38 μg/kg of body weight, or less than 0.2 μg/day (Spigset et al., 2007). Furthermore, methylphenidate was not detected in breast milk 20–21 h after the previous dose, so the infant would not have been exposed to methylphenidate if breastfed immediately before the mother had taken her morning dose. This finding is consistent with the short plasma elimination half-life.

3.2.9 Tissue Disposition

Tissue disposition in humans has been poorly studied. In rats, after doses of 1 mg/kg (Kotaki et al., 1988), 10 mg/kg (Thai et al., 1999), or 20 mg/kg (Patrick et al., 1984), tissue disposition was similar: kidney > lung > brain > heart > liver. Within the brain, methylphenidate binds exclusively to DA reuptake sites.

3.2.10 Urine, Sweat, and Saliva Concentrations

At least 80% is excreted in the urine, primarily as RA, within 24 h of administration. The remainder is excreted as 6-α-phenylpiperidine-2-acetic acid. A very small proportion is excreted unchanged (Srinivas et al., 1992). The testing of stored samples may be problematic because RA is unstable and nearly half will be lost during the first month of storage (Thai et al., 1999).

Methylphenidate (but not RA) can be detected in the sweat, but less than 0.1% is excreted in that fashion. It is also detectable after administration of oral or timed-release

preparations, and after the oral administration of fast- and extended-release formulations. Fast-release formulations of the drug can be detected in sweat patches 2 h after administration in concentrations of up to nearly 16 ng/patch when the patch is left in place for 24 h. Extended-release formulations do not appear in the sweat until at least 5 h after administration, reaching a maximum of 34.3 ng/patch after 24 h (Marchei et al., 2010a).

The pharmacokinetic properties of methylphenidate in oral fluid are similar to those observed in plasma. The oral fluid peak concentration after administration of an extended-release formulation (20 mg) was 31 ng/mL at 3.0 h, in contrast to the 2.4 ng/mL peak concentration in plasma. Oral-fluid-to-plasma-drug ratios are both concentration and pH dependent. RA was also found in oral fluid but at concentrations one-tenth of those found in plasma. Concentration profiles of both parent drug and metabolite in oral fluid were similar during 4 weeks of drug administration (Marchei et al., 2010b; Mariotti et al., 2013).

3.2.11 Postmortem Measurements

Data on the tissue disposition of the amphetamine analogs are sparse. A woman who died after injecting 40 mg of methylphenidate intravenously had a blood concentration of 2.8 mg/L (Levine et al., 1986). In that same case, the concentration in the liver was 2.1 mg/kg, while the bile contained 5.7 mg/L and the kidneys 3.0 mg/kg. Little accumulation of methylphenidate occurs in the body. The blood level of a woman who died during a Cesarean section, who presumably had not had any drug for a number of hours, was only 9 ng/mL (Lundquest et al., 1987). A paper published in 1999 described the findings in two individuals who had died after intentional methylphenidate overdose. Ethanol had also been consumed and, as a consequence, ethylphenidate was also detected, although in extremely small quantities (8 and 1 ng/mL) (Markowitz et al., 1999).

3.2.12 Toxicity by Organ System

3.2.12.1 Overview

Information about methylphenidate toxicity is confined to anecdotal case reports. These can be divided into two separate groups: (1) complications related to catecholamine toxicity and (2) complications related to the intravenous injection of talc-containing pills meant for oral consumption. The lungs and eyes bear the brunt of the latter insult, and, except in the case of drug abusers, medical complications are rare, and deaths rarer still. It is unwise to attribute a particular death or medical complication to methylphenidate simply on the basis of one or two case reports, as the presence of the drug could be completely unrelated to the complication being reported.

3.2.12.2 Integument

Occasionally, drug users grind up methylphenidate tablets and inject them subcutaneously (Zumwalt and Franz, 1983). Superficial skin abscesses may form, but they appear to be no different than the type of infection produced by "skin popping" heroin or cocaine. Myositis has also been reported as a consequence of injecting crushed pills (Gautschi and Zellweger, 2006), as has arterial thrombosis due to injection of the wrong vessel (Still et al., 2001).

3.2.12.3 Cardiovascular

A case report described the cardiac findings in a 19-year-old who died after snorting powder methylphenidate tablets. The myocardium displayed foci of localized, microfocal necrosis with infiltration by histiocytes and polymorphonuclear leukocytes—typical changes associated with catecholamine toxicity (Massello and Carpenter, 1999). A second case describes spontaneous multivessel coronary vasospasm leading to anterior myocardial infarction and cardiogenic shock in a man who was taking methylphenidate and, at the same time, being withdrawn from beta-blockers and calcium channel antagonists, making causation nearly impossible to determine. Suggesting that the event was the result of methylphenidate abuse seems something of a stretch (Bromberg-Marin et al., 2007). Equally questionable are the claims made in another report describing a 24-year-old with sudden death who was found to have a left anterior descending coronary artery (LAD) occluded by intimal hyperplasia and evidence of an old infarction. Intimal hyperplasia is a regular feature of chronic cardiac rejection, but is otherwise extremely rare, with only three cases (Simpson and Edwards, 1986; Ramondo et al., 2009; Bhavsar et al., 2011) having been reported previously in stimulant abusers (Cohle, 2013).

In fact, controlled studies show that neither amphetamine salts nor methylphenidate pose a significant cardiovascular risk. Except in cases of very large overdose, methylphenidate appears to cause minor increases in blood pressure and heart rate. There are no strong data to suggest that methylphenidate increases the corrected QT interval (QTc), although amphetamines appear to cause minor increases in heart rate and blood pressure over the long term. Sudden death remains an extremely rare event and there is no clear evidence to attribute this to methylphenidate. Some data even suggest that the risk of sudden death in treated children may be less than in the background population (Besag, 2009; Stiefel and Besag, 2010).

3.2.12.4 Pulmonary

Granuloma formation and pulmonary fibrosis have been recognized as complications of methylphenidate abuse for many years (Hahn et al., 1969; Waller et al., 1980; Levine et al., 1986), but new reports of this disorder are very uncommon (Williams et al., 1988; Nikano et al., 1998; Shlomi et al., 2008), suggesting that the practice of injecting methylphenidate has essentially been abandoned. If anything is unique about the histopathology of methylphenidate abuse, it remains to be identified. Presumably, the deposition of birefringent material contained in the methylphenidate preparations is followed by a granulomatous inflammatory reaction and focal thrombosis (Byers et al., 1975). The situation has never been studied experimentally, and nothing has been found to distinguish granuloma formation in stimulant abusers from the same alterations seen in opiate addicts.

More than a decade ago, panacinar emphysema, more pronounced in the lower lung fields, was described in a group of young intravenous methylphenidate Ritalin abusers who died of severe obstructive lung disease (Sherman et al., 1987; Schmidt et al., 1991; Stern et al., 1994; Ward et al., 2000). There have been no similar reports since 2000. Autopsy findings included variable degrees of vascular involvement by talc granulomas, but no interstitial fibrosis. X-rays of these individuals show a distinctive picture with prominent, or even massive, fibrosis in the upper lobes and with translucence and bullae formation in the lower lobes (Pare et al., 1989). In most respects, the clinical and pathologic findings are the same as those associated with α_1-antitrypsin deficiency, although tests for that disorder are inevitably negative. Obstructive lung disease is an uncommon complication of intravenous

drug abuse, regardless of the type, and its mechanism remains to be determined (Groth et al., 1972; Vevaina et al., 1974; Ward et al., 2000).

3.2.12.5 Gastrointestinal Tract

Only two case reports of hepatic dysfunction associated with methylphenidate have ever been published, both occurring in intravenous drug abusers (Mehta et al., 1984). In the cases described by Mehta et al., portal inflammation with hepatocellular disarray was diagnosed in the liver biopsy of an intravenous abuser who survived an episode of liver failure. A second case report described the autopsy findings in a polydrug user who died of amniotic fluid embolus. Examination of the heart showed evidence of biventricular hypertrophy and there were multiple granulomas in the liver and lungs. Pulmonary hypertension is the expected outcome when tablets of methylphenidate, or any other tablet designed for oral ingestion, are ground and injected. The woman described by Lundquest was exceptional in that she had a patent foramen ovale and the elevated pulmonary pressure caused a right-to-left shunt. Talc granulomas were found throughout the body, including the brain and kidneys (Lundquest et al., 1987). Interestingly, and significantly, methylphenidate has been evaluated for the treatment of depression post liver transplant. The investigators concluded that it was a safe and effective drug (Pultchik et al., 1998).

3.2.12.6 Nervous System

Long-term experimental studies failed to disclose evidence of neurotoxicity but did suggest that methylphenidate might be neuroprotective! (Ludolph et al., 2006) Even so, psychosis occurs, even in nonabusers. In one study, 6 of 98 children diagnosed with ADHD and treated with stimulant drugs developed psychotic symptoms during treatment (Cherland and Fitzpatrick, 1999). Young people appear to be particularly vulnerable if they are addiction prone (Kraemer et al., 2010). A handful of reports exist describing drug abusers who injected ground tablets of methylphenidate and then developed retinal artery occlusion (Schatz and Drake, 1979; Lederer and Sabates, 1982); however, this complication has never been reported as a consequence of normal therapeutic use and is quite rare.

Stroke is equally rare in methylphenidate users, it has never been reproduced in an experimental model, and only three case reports have ever been published, one a known polydrug abuser (Sadeghian, 2004). One stroke involved an 8-year-old boy being treated for ADHD. Arteriography showed no congenital malformation, but did show evidence of cerebral vasculitis. An extensive hematologic evaluation failed to disclose any coagulation abnormalities (Schteinschnaider et al., 2000). Since the original case report, a second case of vasculitis in a methylphenidate user has been described (Thomalla et al., 2006). Because only two such cases have been reported and millions of children take methylphenidate, it would appear that the associated risk is exceedingly low and, in fact, there may not be any connection at all. There are even ongoing experiments in which methylphenidate is being used as an adjunct in the rehabilitation of stroke patients (Paolucci and De Angelis, 2006; Barrett et al., 2007; Sivan et al., 2010).

3.2.13 Postmortem Toxicology

3.2.13.1 Preanalytic Considerations

Methylphenidate has a labile methyl ester linkage that will impart some instability. Hydrolysis results in RA that is also a metabolite. Hydrolysis also occurs in aqueous

alkaline solutions and during incubation with blood or plasma. The *R*,*R*′ isomer of methylphenidate reportedly hydrolyzes more rapidly than the *S*,*S*′ isomer (Ramos et al., 1999). Whole blood stability experiments have shown that methylphenidate is only stable when stored for up to 6 h at room temperature (Varela-Rey et al., 1999).

3.2.13.2 Recommended Analytic Methods

Methylphenidate cannot be detected using immunoassay kits designed for amphetamine or MA; however, it has been reported to give a false positive on urine amphetamine screening (Manzi et al., 2002). ELISA kits specifically intended for methylphenidate are available commercially; however, for forensic purposes, it is recommended that a chromatographic test be used. This ensures that there is no cross-reactivity with the metabolite, or other substances also present, and also allows other substances of interest to be detected.

Methylphenidate can be extracted using conventional solvents (e.g., butyl chloride or hexane/isopropanol mixture) and then chromatographed on conventional GC columns (e.g., BP-5) with detection by nitrogen phosphorus detector and/or MS. The *d*- and *l*-enantiomers of *dl*-threo-methylphenidate can be separated using heptafluorobutyryl-*l*-prolyl chloride derivatives on GC–MS (Beitia et al., 1999). GC–MS can also be used in other specimens. For example, methylphenidate was detected in hair using *N*-methyl-bisheptafluorobutyric amide derivatives in selected ion monitoring mode (Sticht et al., 2007).

Direct injection of diluted urine has been used to detect both methylphenidate and RA by LC–MS/MS (Eichhorst et al., 2004). More conventional methodology has been used to detect both methylphenidate and metabolite in plasma, oral fluid, and urine using only 0.1 mL specimen (Marchei et al., 2010b). Specimens were treated with acetonitrile to precipitate proteins, followed by evaporation to dryness, before applying a reconstituted aliquot into an ultrapure silica column. When electrospray ionization was used, detection limits were below 1 ng/mL.

3.2.13.3 Interpretation

Compared to the other amphetamines, methylphenidate has a relatively low V_d (roughly 1 L/kg compared to over 3 L/kg for MA). The lower the volume of distribution, the greater the likelihood that values measured at autopsy may provide a closer approximation to blood concentrations at the time of death unless there has been extensive putrefaction. Hence, if postmortem concentrations of the metabolite are present and are vastly in excess of those considered to be consistent with therapeutic usage, either an overdose or a recent intravenous injection should be suspected. Though the possibility of overdose is mentioned in review papers, only one case report has been published, and it described a 62-year-old woman who committed suicide by taking 3 g of the drug. Postmortem concentrations of methylphenidate were confirmed in peripheral blood at 1.1 mg/L, central blood at 0.98 mg/L, liver at 3.6 mg/kg, and vitreous humor at 0.80 mg/L. Little evidence of postmortem redistribution was evident (Cantrell et al., 2014)

3.3 Captagon

Fenethylline, also spelled phenethylline, is a synthetic prodrug (Figure 3.27), introduced to treat ADHD. Its brand name was Captagon. Once ingested, it is converted into *d*-amphetamine and theophylline. Approximately one quarter of a given dose is converted to

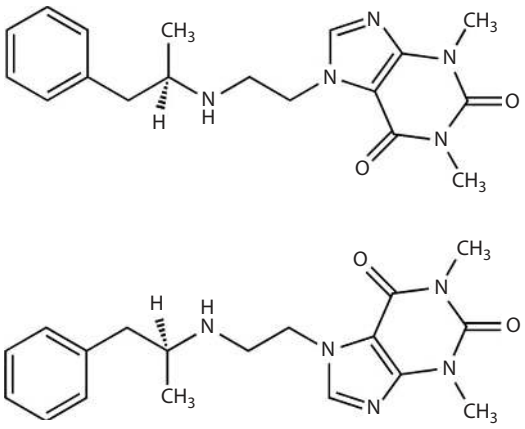


Figure 3.27 Fenethylline enantiomers.

amphetamine and 13% to theophylline; the remainder undergoes oxidative cleavage to produce metabolites such as carboxymethyl–theophylline and acetylaminoethyl–theophylline. The metabolism is essentially the same in rat and man (Yoshimura et al., 1988).

Captagon was withdrawn from the market many years ago, though an illicit product with the same name is still extremely popular, mainly in the Middle East, Arabian Peninsula, and Africa. Captagon sold on the black market is composed almost entirely of caffeine and amphetamine (UNDOC, 2010). It has a toxicity profile not very different from that of amphetamine.

Illicit Captagon is made from phenyl-2-propanone (P2P), a compound strictly controlled in most parts of the world except the Middle East (Figure 3.28). Jordan reported its annual legitimate requirements of P2P were 60,500 kg in 2009, accounting for more than half of the world's total P2P consumption. The Jordanian government claims that large quantities are legitimately needed for the purported formulation of P2P into products used for "cleaning and disinfection." However, P2P is not an essential ingredient in the formulation of cleaning and disinfection products—alternative, much less expensive, chemicals exist. It can safely be concluded that all of the P2P is going into MA production (UNDOC, 2010). Several reports of Captagon-induced psychosis have been published, but all of these reports dealt with the legitimate drug fenethylline. Since that drug is no longer contained in illicit Captagon, further discussion no longer seems relevant. General autopsy findings have never been reported in a Captagon-, let alone a fenethylline-related death, although the number of medical complications attributable to Captagon is increasing, with occasional reports of myocardial infarction and behavioral abnormalities (Simko, 1965; Ulucay et al., 2012).

3.4 Other Stimulants

A large number of other substances have emerged in recent years that have predominately stimulant-like properties; many of these also have entactogenic properties akin to ecstasy. These include the piperazines, beta-keto substituted amphetamines, 4-substituted amphetamines, and the phenethylamines. The substances with largely hallucinogenic properties are covered in Chapter 4.

Figure 3.28 Illicit Captagon (which was once a legal product) is widely abused across the Middle East. It is rarely sold as pure fenethylline but is compounded with other agents. This photograph shows various brands, sold under different names, across the Middle East. The popularity of this drug is said to be growing.

3.5 New Synthetic Stimulants

Over the last few years, the world has been flooded with new synthetic stimulant drugs that mimic many of the actions of the traditional amphetamines (MA, amphetamine, and MDMA). Some of these are designer analogs of naturally occurring stimulants, such as derivatives of khat (cathinone, a type of amphetamine, is the active principle), while others are totally new synthetic stimulants that, for as long as it takes new legislation to be enacted, remain legal. The latter include the piperazines (e.g., benzylpiperazine [BZP]) and pyrrolidinophenones. More recently, cathinone analogs including mephedrone, butylone, 4-methyl-*N*-ethylcathinone, flephedrone (4-fluoromethcathinone), and 3,4-methylenedioxypyrovalerone (MDPV) have become available as the new "legal highs." They include "Energy 1" brand, which is said to contain naphyrone (naphthylpyrovalerone, O-2482), but which is actually composed of cathinone analogs (Brandt et al., 2010a,b). All of these agents are likely to be scheduled by the time this book comes to press, though that hardly implies they will be unavailable.

3.6 Cathinone

3.6.1 History

Khat (*Catha edulis*) is an evergreen that grows at high altitudes in East Africa and the Arabian Peninsula. Its leaves contain a naturally occurring psychostimulant closely related in structure to ephedrine and amphetamine. Khat first came to the notice of Europeans in 1762, when the botanist Peter Forskal found it growing on the mountain slopes in Yemen (Pantelis et al., 1989; Bentur et al., 2008).

Historical references date the discovery of khat to the thirteenth century, when the Arab physician Naguib Ad-Din gave khat leaves to soldiers to relieve fatigue (Giannini and Castellani, 1983). Ad-Din might have been the first ever to give soldiers psychostimulants, making him one of the earliest to experiment with performance-enhancing drugs. Since Ad-Din's pioneering experiments, the practice has been repeated by many others, always with the goal of improving the performance of troops on the battlefield. Theodore Aschenbrand, a physician with the Prussian army, probably was the first to supply recruits with cocaine. This occurred during the Franco-Prussian war. Years later, both Japan and the Allies issued amphetamines to their troops during World War II (Karch, 1989) (and apparently still do—see Section 3.1.5 and Chapter 1). Today, Somali rebels use khat before going into combat.

In 1852, James Vaughn, an English surgeon, published an illustration and an account of khat chewing in the *Pharmaceutical Gazette* (Vaughn, 1852). Figure 3.29 is from Vaughn's paper. Vaughn speculated that the principal reason for khat's popularity was that its use was not forbidden by the Koran. Traditionally, khat chewing was a social event, with sessions often lasting for hours. In some areas of Africa where khat chewing is still popular (the World Health Organization estimates that there are still millions of khat users), houses often have a special room, called a *muffraj*, designed just for talking and smoking khat (Kennedy et al., 1983). However, khat is now widely abused by young soldiers in sub-Saharan Africa. When consumed in large doses, it can be expected to produce most of the same symptoms as excessive amphetamine ingestion (Odenwald et al., 2012).

The normal dose is 100–200 g of leaves and stems chewed over a 3–4 h period (Max, 1991). An occasional solitary individual will chew to increase his or her work capacity, and African soldiers have begun consuming prodigious quantities prior to combat. Users describe increased feelings of alertness and an improved ability to concentrate. Use is also said to make people friendlier and improve the flow of ideas. Nonetheless, use of this material conforms to most definitions of addiction. Chewers attempting to secure their daily supply of leaves will do so to the exclusion of all other activities. In Yemen, 4% of all arable land is used to grow khat, and 10% of the revenues in Djibouti are derived from taxes on khat (Max, 1991).

3.6.2 Cultivation and Manufacture

Although it grows in the United States (Wallace, 1998), most khat consumed in the United States is illegally imported. According to the UN, in 2005, 15 different nations reported khat seizures, amounting to 19 tons (by comparison, seized cannabis amounted to 115 tons). The rate of use of khat chewing (as opposed to production of synthetic variants) seems to be declining, at least in Africa where use has dropped by more than 7% in the last several years. On the other hand, there are reports that khat popularity is increasing in Scandinavia (Al-Samarraie et al., 2007).

Figure 3.29 Khat leaves. This drawing from 1852, published in the *Pharmaceutical Journal* of London, was the first illustration of khat to appear in the English literature. Khat abuse is still a problem in Africa, where some of the gratuitous violence in areas such as Somalia is attributed to it.

Analysis of khat leaves seized at the airport in Basel, Switzerland, showed the leaves contained, on average, 1 mg of cathinone, 0.86 mg of norpseudoephedrine, and 0.47 mg of norephedrine per gram of leaf (Widler et al., 1994). A common misconception is that khat leaves lose potency with aging and that cathinone is highly unstable once the plant is harvested. However, drying the plant material preserves the cathinone. Numerous seizures of dried khat (referred to as *graba* in the United States) have been made in recent years, and all have been found to contain cathinone, suggesting that drying the plant is a viable approach to preservation.

The stability of the drug is something of a mixed blessing as it makes khat exportation (and therefore, production of synthetic analogs) much easier. On the other hand, knowing that cathinone is stable makes forensic analysis of seized material a much more reliable process. A qualitative and quantitative study of seized dried khat samples showed that khat alkaloids are relatively stable for at least 3 years, and cathinone remains identifiable, stored at room temperature, for over 10 years. Drying the moist leaves either at room temperature or with gentle heat is all that is required to preserve cathinone in the dried material (Chappell and Lee, 2010).

3.6.3 Incidence and Epidemiology of Khat Usage

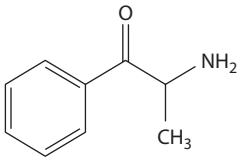
Khat is native to the sub-Sahara and is cultivated on the mountain slopes of Yemen, where use is endemic. Khat also grows well in California, the desert southwest of the United States, Oregon, and Florida where its popularity seems to be increasing. Khat chewing is prohibited in most European countries but is legal in the United Kingdom (Figure 3.30). With the exception of immigrants from the sub-Sahara, the U.S. population has not adopted the practice of khat-leaf chewing. Khat does not even rate a mention in any of the standard government surveys, including the Annual Household Survey and DAWN reports, yet, according to some estimates, 5–10 million people around the world chew khat on a daily basis (Balint et al., 1991). This situation is slowly changing: one of the natural components of khat is cathinone from which the psychoactive drug mephedrone is synthesized. In more recent times, cathinone itself has been available as an illicit drug. Hagigat (200 mg capsules) has been available in Israel as a natural stimulant and aphrodisiac (Bentur et al., 2008).

3.6.4 Absorption

The main psychoactive ingredients of khat are *S*-(−)-α-aminopropiophenone (cathinone), together with the less psychoactive phenylpropanolamine diastereomers *S*,*S*-(+)-norpseudoephedrine (cathine) and *R*,*S*-(−)-norephedrine, 3,6-dimethyl-2,5-diphenylpyrazine, and 1-phenyl-1,2-propanedione (Szendrei, 1980; Brenneisen et al., 1986).

Figure 3.30 Fresh khat leaves. Because they contain amphetamine-like substances, they are illegal in the United States, but use is still permitted in the United Kingdom and parts of Europe and Africa. (From the DEA website.)

Table 3.7 Physiochemical Properties and Pharmacokinetics of Cathinone

Chemical name	*S*-(−)-α-aminopropiophenone			
Physiochemical properties, structure, and form	Free base CAS 71031-15-7 MW 149.1 p*K*a 9.4 (cathine)			
Synonyms	Khat, qat, quat, gat, miraa, muhulo, musitat, tschat			
Pharmacokinetic parameters		Cathinone	Cathine	Norephedrine
	Bioavailability	45%	45%	59%
	T_{max} (h)	2.31 ± 0.65	2.62 ± 0.77	2.84 ± 0.42
	C_{max}	58.9 ± 18.8	71.2 ± 13.9	72.1 ± 12.2
	$T_{½}$	1.50 ± 0.81	5.22 ± 3.36	
	Taken from Toennes et al. (2003b) (volunteers chewing 44 g leaves)			
Common blood concentrations in drug users	Few data, but could be up to about 0.2 mg/L			
Metabolism	Oxidation of keto group and *N*-demethylation			
Urinary excretion	22%–52% of dose was recovered as cathinone, norephedrine, and norpseudoephedrine. Less than 7% excreted as cathinone			
Postmortem artifacts	Unknown but likely to be similar to amphetamines			
Interactions	None known, but likely to be similar to related amphetamines			

Cathinone is the keto analog of cathine and as such penetrates the blood–brain barrier far more quickly than cathine—hence its greater psychoactivity. The first two compounds occur in the leaves and are also produced by metabolic breakdown, so that any measurement of their blood concentration will include some drug that is ingested and some that is metabolized. Maximal plasma concentrations of cathinone, norephedrine, and norpseudoephedrine are achieved around 2–3 h. Norephedrine and norpseudoephedrine have longer elimination half-lives than cathinone (see Table 3.7).

3.6.5 Pharmacokinetics

When khat is chewed in a controlled setting, peak plasma levels occur some time between 1.5 and 3.5 h afterward, reaching a mean concentration of 83 ng/mL when it was given to five test subjects (Halket et al., 1995; Al-Motarreb et al., 2010). Volunteers who had been given leaves containing a total of 0.8 mg cathinone per kilogram body weight attained maximal plasma concentrations (127 ± 53 ng/mL) more than 2 h after they started to chew the leaves (127 min).

The elimination half-life of khat is on the order of 4.5 h (206 ± 102 min). Peak norephedrine levels in the most recent study were 110 ± 51 ng/mL for norephedrine and 89 ± 49 ng/mL for norpseudoephedrine (Widler et al., 1994). Urine levels have only been measured in six volunteers at 2, 4, 6, and 8 h after taking 0.5 mg/kg of optically pure (*S*)-(−)-cathinone. Resultant levels were from 0.2 to 3.8 mg/mL for the parent compound, 7.2 to 46 mg/mL for (*R*,*S*)-(−)-norephedrine, and 0.5 to 2.5 mg/L for (*R*,*R*)-(−)-norpseudoephedrine (Brenneisen et al., 1990).

Four volunteers chewed khat leaves equivalent to one quarter of that used in a typical khat session (average 44 g), and the results demonstrated that absorption occurred through the oral mucosa, which is where the major proportion of the alkaloids were absorbed. Little alkaloid was left in the plant material after a chewing session (about 9%). The terminal

elimination half-life of cathinone was about 1.5 ± 0.8 h, while the half-life of cathine was 5.2 ± 3.4 h (Toennes et al., 2003b).

When chewed in a controlled setting, an average dose of 45 g of leaf produces a peak plasma concentration of 0.06 mg/L (range 0.05–0.09) at about 2 h (Toennes et al., 2003). The terminal elimination half-life is about 1.5 h. At this dose, only small increases in blood pressure were observed as this dose is less than the usual dose taken. Cathine had a C_{max} of 0.07 mg/L at 2.6 h and a longer $T_{½}$ of 5 h. Norephedrine was detected at similar concentrations to cathine. Maximal plasma concentrations of cathinone were about 0.1 mg/L at 2 h following 0.8 mg/kg khat (Widler et al., 1994).

Unchanged drug is excreted in urine as are the metabolites cathine and norephedrine (22%–52% of dose was recovered) (Brenneisen et al., 1986). In another study of volunteers chewing khat leaves, less than 7% cathinone was excreted unchanged in urine; cathine and norephedrine were the prominent metabolites. However, only cathinone can be used to indicate exposure to khat, and it appears in urine for only about 1 day after ingestion (Fandino et al., 2002).

3.6.6 Clinical Studies

Khat chewing produces symptoms consistent with sympathetic activation, displaying both inotropic and chronotropic effects; use causes elevations in blood pressure, temperature, and respiratory rate, and inconsistent effects on heart rate. In isolated heart preparations, cathinone causes increased release of NE (Brenneisen et al., 1990; Hassan et al., 2000).

These effects are unequivocal. There are several case reports describing stroke and myocardial infarction in khat users, and some well-controlled case studies, the results of which leave little doubt about the connection between khat chewing and acute myocardial infarction (AMI). As patterns of khat consumption have evolved, so too has the timing of khat-related cardiovascular events. It is generally accepted that acute myocardial infarction and sudden death, for whatever reason, occurs in the early morning after waking and rising (Selwyn et al., 1991).

A prospective multicenter study of over 8000 subjects from six Middle Eastern countries showed khat chewers exhibited a significantly higher risk of cardiogenic shock, stroke, and mortality than did nonusers. Heavy khat chewers have a 39-fold increased risk of acute myocardial infarction over the general population (Al-Motarreb et al., 2005).

Khat chewers generally present with symptoms of AMI in the evening (Alkadi et al., 2002; Al-Motarreb et al., 2002, 2005; Croles et al., 2009). In a follow-up case-control study, khat chewing was found to be an independent risk factor for AMI. Moderate khat chewers were shown to be at high risk (OR = 7.62) and heavy khat chewers were at even higher risk (OR = 22).

Khat chewing causes chronic constipation, and the incidence of both constipation and hemorrhoids seems to be higher in countries where khat consumption is greatest (Al-Hadrani, 2000). There is also evidence of reduced milk production in nursing mothers (Kristiansson et al., 1987; Makonnen, 2000), consistent with the apparent ability of khat to induce numerous endocrine alterations. Khat significantly lowers the pulse frequency of luteinizing hormone (LH), the area under the LH curve, as well as mean plasma LH and mean plasma testosterone levels. In addition, plasma cortisol levels become significantly elevated and the elevation occurs in a dose-dependent manner. This constellation of findings raises the question of decreased sexual function in habitual users (Nyongesa et al., 2008).

Some retrospective and epidemiologic studies suggest there is a relationship between khat chewing and head and neck cancer (Abdul-Hamid et al., 2010), but evidence suggesting a link between khat chewing and head and neck cancer is equivocal. It has been reported that in some parts of Saudi Arabia, the only patients seen with oral cancers are those with long histories of khat chewing (Soufi et al., 1991), but this suggestion is not supported by biopsy studies of khat chewers that demonstrated that chewing khat rarely, if ever, was associated with signs of malignancy (acanthosis 97.5%, parakeratosis 50%, and orthokeratosis in 25%) (Ali et al., 2006).

The issue of khat-induced hepatic disease is also problematic. Animal studies and at least one human case report suggest hepatotoxicity, with elevated liver enzymes and histopathologic evidence of acute hepatocellular degeneration seen after 6 months of continuous exposure (Al-Mamary et al., 2002; Brostoff et al., 2006). A recent report in the *New England Journal of Medicine* described five khat chewers living in the United Kingdom, all with unexplained hepatitis. All chewed khat and had been counseled against its use. However, all had resumed use before their second visit to the clinic. All of the patients had had similar histopathologic findings characterized by multilobular hepatic necrosis; two of them had a background of chronic liver disease. Five of the six patients underwent orthotopic liver transplantation, accounting for 10% of the patients who received transplants because of fulminant or subfulminant hepatic failure during this 5-year period (Chapman et al., 2010). Reports of classic cirrhosis have become more common as the number of users increases (Peevers et al., 2010). Little more research has been performed in humans, but the results of tissue culture studies clearly demonstrate that khat triggers the generation of intracellular ROS and sequentially induces sustained activation of the c-Jun NH2-terminal kinase pathway. The result is increased cell apoptosis and decreased cell viability (Abid et al., 2013). Finally, periodontal disease is now a recognized complication of khat chewing. Many changes have been observed in the oral mucosa and in dentition. The mechanical and chemical irritation associated with khat chewing may result in the development of white mucosal lesions and dark pigmentation. Khat chewing may reduce gingival and periodontal inflammation, but this effect appears to be associated with the act of chewing itself (Yarom et al., 2010).

Patients hospitalized due to toxicity to cathinone present with headache, vomiting, hypertension, nausea, tachycardia, dyspnea, chest pain, and myalgia but also develop myocardial ischemia, pulmonary edema, and intracerebral hemorrhage (Bentur et al., 2008). Manic-like psychosis following heavy use of this substance has been reported (Giannini and Castellani, 1982).

3.6.7 Toxicologic Studies

Six drivers admitting to use of khat and suspected of DUI of psychoactive substances were found to have urine cathinone in concentrations ranging from 1.6 to 29 mg/L. Substantial amounts of cathine and norephedrine were also present (Fandino et al., 2002). The observed concentration range is too wide to permit any conclusions about impairment, and the same conclusion likely applies to the newer cathinone derivatives.

There are no published data on blood (plasma) concentrations in persons admitted to emergency units following excessive use of khat. However, calls to the Israeli Poison Center suggested that most of the presenting patients had consumed from ½ to 6 capsules containing about 200 mg cathinone (32 of the patients had ingested the capsules, and 2 had sniffed their contents) (Bentur et al., 2008).

3.6.8 Analysis

Commercial immunoassays to amphetamine- or MA-based kits are unlikely to detect khat users since cross-reactivity to the phenylpropanolamines is usually <1%. However, reports of cross-reactivity, probably with cathine and norephedrine in urine, have been observed when using the Mahsan-AMP300 on-site test (Toennes et al., 2003a).

Storage of urine at 4°C results in an 80% loss of cathinone in 3 months, but stability increases when samples are stored frozen at −20°C (Paul and Cole, 2001). Nonetheless, instability has been confirmed in urine and caution is advised even when storing at −20°C for more than 2 months (Marais et al., 2011).

Khat and related compounds can be measured by GC–MS in the same fashion as other amphetamine-like substances (Toennes et al., 2003b). Typically these can be run underivatized, but more commonly perfluoroacyl derivatives are used for GC-based methods. LC–MS methods have been described for a large number of illegal stimulants including cathinone (Wohlfarth et al., 2010).

3.7 Structural Analogs of Cathinone

3.7.1 Mephedrone

Mephedrone is a structural analog of cathinone. It was first synthesized in 1928 but did not become a recreational drug until in 2003. It gained notoriety in Europe, particularly in the United Kingdom, for its ready availability and for an unfortunately high incidence of hospital admission, even deaths. The EMCADDA indicates that over the first quarter of 2010, mephedrone was detected in some 20 EU member states (European Monitoring Centre for Drugs and Drug Addiction, 2010). Its use appears to be increasingly popular among established drug users (Schifano et al., 2011). Until recently, when it was scheduled by the DEA, mephedrone and even related compounds could be purchased on the Internet and from "head shops" labeled as "research chemicals" or "plant foods" (Figures 3.31 and 3.32). In the United Kingdom, 4-methylmethcathinone and other substituted cathinones are now categorized as class B drugs. Mixtures of these drugs are often sold under the name of "bath salts" and carry a warning "not for human consumption."

Figure 3.31 Mephedrone, one of the "bk"-type amphetamines, is readily available over the Internet. Typically, it is sold as a plant food, but as this advertisement makes clear, it is intended for insufflation.

Figure 3.32 Mephedrone is often sold as so-called bath salts, frequently combined with other cathinone derivatives. (From the files of the FDA, with permission.)

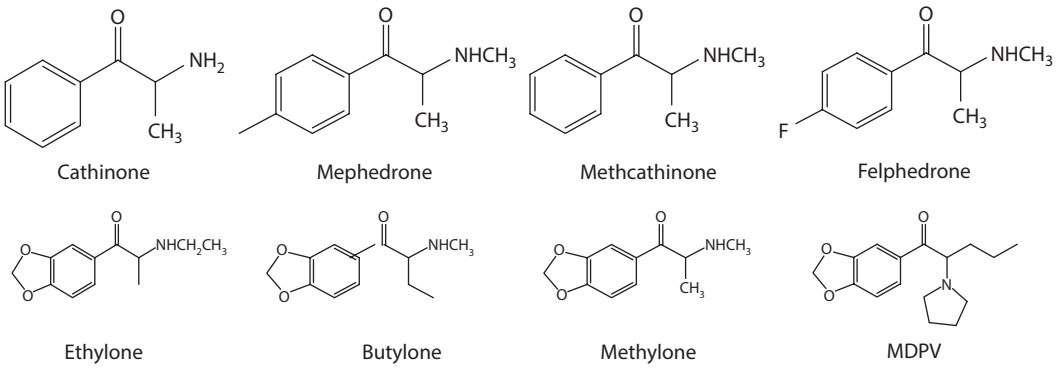


Figure 3.33 Molecular structure of compounds related to 4-methylmethcathinone.

Chemically, mephedrone is 4-methylmethcathinone (MMC), a synthetic derivative of the naturally occurring cathinone in *Catha edulis* that contains the beta-keto moiety (Figure 3.33). It is known as 4-MMC, meow meow, drone, bubbles, spice E, charge, M-cat, rush, ronzio, fiskrens, hammer, and plant food (Schifano et al., 2011). Mephedrone and its analogs exert extremely potent effects on serotonin and DATs. Some of the analogs are 10 times more potent serotonin blockers than methcathinone, 4-methylmethcathinone, and some may be equally potent both as serotonin and DA releasers (Cozzi et al., 2013).

Related compounds include ephedrone (4-ethyl-methcathinone) and methedrone (4-methoxymethcathinone), flephedrone (4-fluoromethcathinone), butylone (ketone analog of MBDB), methylone (ketone analog of MDMA), ethylone (ketone analog of MDEA), and MDPV. The beta-keto analogs of MA, MDMA, and MDEA are sometimes referred to as bk-MA, bk-MDMA, and bk-MDEA, respectively.

Mephedrone (and its analogs) can be synthesized by bromination of substituted propiophenone and reaction with the appropriate amine or from reduction of the hydroxyl of the substituted ephedrine to the ketone (Dargan et al., 2011).

A large number of other synthetic stimulants have also appeared on the market. These include 3-methylmethcathinone (3-MMC) (Backberg et al., 2015), methiopropamine (MPA; 1-(thiophen-2-yl)-2-methylaminopropane) sold as "Slush Eric" and "Blow" (Tuv et al., 2015), 5-APB (5-(2-aminopropyl)benzofuran) (Adamowicz et al., 2014), α-pyrrolidinovalerophenone (α-PVP; also known as Energy-3 (NRG-3)) (Eiden et al., 2013; Sykutera et al., 2015).

3.7.1.1 Usage

Mephedrone can be consumed by a variety of routes, particularly oral, nasal insufflation, and rectal. Oral doses are usually up to 100 mg but can exceed 200 mg and take up to 2 h for peak effect to be observed. Food retards absorption resulting in a delayed onset of action. The effects of the drug last for 2–3 h (Brandt et al., 2010). Nasal insufflation produces a rapid onset of action (within 30 min), and lower doses are required compared to oral ingestion—25–75 mg. The drug can also be taken rectally, requiring lower doses (about 100 mg; Brandt et al., 2010b; Schifano et al., 2011) and appears to show a more rapid onset of action than when taken orally.

3.7.1.2 Clinical Effects and Toxicity

The potency of mephedrone, and the other beta-keto analogs of the amphetamines, tends to be less than the parent amphetamine due to the presence of the beta-keto group. Mephedrone produces central and peripheral stimulant activity as well as "empathogenic activity" related to MDMA. Like the amphetamines, mephedrone appears to inhibit monoamine transporters, thereby enhancing neuronal effects.

Physiologic changes include anxiety, increases in heart rate and blood pressure, sweating, and flushing. Goosebumps, intensification of sensory experiences, and moderate sexual arousal are also reported. At higher doses perceptual changes have reported by users. Admissions to hospital are common, particularly in the United Kingdom, and treatment, as with any stimulant, is generally supportive, often with the administration of benzodiazepines (Wood and Dargan, 2012).

Confusion, psychoses, chest pain, nausea, vomiting, palpitations, sweating, bruxism, and hyperreflexia are also observed (James et al., 2010; Wood et al., 2010a; Regan et al., 2011; Wood and Dargan, 2012). The difficulty in attributing causation is that, in many cases, other substances have been used simultaneously. Withdrawal and addiction has been reported and is not surprising given the similarity of this drug to the amphetamines.

3.7.1.3 Fatalities

Deaths are presumably possible, although few have been reported and the details are sparse. In the United Kingdom, over 45 cases of suspected deaths from mephedrone have been reported, most of which have been confirmed by toxicology testing; however, details of these cases are at present sparse (Regan et al., 2011). In the majority of cases, other drugs have been used such as alcohol, cocaine, amphetamines, and opiates; hence, the true toxicity is unknown. The same is true in other localities (Aromatario et al., 2012; Adamowicz et al., 2013).

The death of a 22-year-old man was apparently caused by the ingestion of 0.2 g orally followed by 3.8 g intramuscularly. He developed malaise, blurred vision, chest discomfort, sweating, high blood pressure, and a mildly elevated heart rate. He became delusional. A serum mephedrone concentration was 0.15 mg/L. There were no signs of other physiologic changes and he was discharged with normal vital signs 6 h later (Wood et al., 2010b).

Table 3.8 Mephedrone Concentrations Detected in Postmortem Biological Samples

	Mephedrone Concentration		
Case	Urine (mg/L)	Blood (mg/L)	Hair (ng/mg)
1	Positive	22	N.A.
2	>0.50	3.3	4.2 and 4.7
3	Positive	5.7	N.A.
4	N.T.	1.2	N.A.

Source: Adapted from Torrance, H. and Cooper, G., *Forensic Sci. Int.*, 202(1–3), e62, 2010.

Note: N.T., not tested; N.A., not available for analysis.

In another recently reported case, a 36-year-old man was arrested after having injured himself severely by smashing windows in a rage of fury. He died despite resuscitation attempts. The forensic autopsy showed many superficial skin lacerations, bruises, and minor brain swelling, but there was no definitive cause of death. Toxicologic analysis showed a high concentration of mephedrone in femoral blood (5.1 mg/L) and traces of cocaine, MDMA, and oxazepam. The remaining dose of mephedrone in the stomach contents was estimated at 113 mg. Tablets that were found in the house of the deceased also contained mephedrone (Lusthof et al., 2011). Many of the symptoms described (particularly the glass-breaking behavior) sound very similar to those seen in cases of excited delirium that, until recently, was a disease that was more or less confined to cocaine abusers (and a few MA abusers—see Section 1.15).

Another recently reported mephedrone death was similar in that the autopsy findings were said to be "irrelevant." The blood and urine concentrations were 1.33 and 144 mg/L; cocaine and its metabolites were also present. Hair testing disclosed previous exposure to mephedrone, ketamine, and MDMA. It is difficult to know what to make of this paper since it is not even clear that microscopic examination of the heart was performed. The possible presence of underlying heart disease, even at the molecular level, makes it impossible to classify the cause of death with any certainty. However, the paper is not without value as it is the first to report mephedrone concentrations in the bile, lung, and brain (1.29, 0.79, and 0.89 mg/L [kg], respectively) (Gerace et al., 2014).

Concentration data in four fatalities are shown in Table 3.8 (Torrance and Cooper, 2010). Death was attributed to mephedrone in cases 1 and 2. Diazepam (0.1 mg/L) and amphetamine (0.34 mg/L) were also detected in case 1, but no other drugs were detected in case 2. Case reports now seem to be appearing with greater frequency (Aromatario et al., 2012; Adamowicz et al., 2013; Cosbey et al., 2013; Gil et al., 2013).

It is likely, in common with cocaine and amphetamine users, that death is more likely in persons with existing cardiovascular disease. It is unlikely that particular blood (or tissue) concentrations will have any particular value in predicting toxicity given the complexities of interpretation associated with the stimulant classes of drugs.

3.7.1.4 Analysis

Mephedrone has shown some cross-reactivity with MA in the ELISA assay of Immunalysis Corporation (Torrance and Cooper, 2010). The drug can be measured using conventional GC–MS. Pentafluoropropyl (PFP) derivatives have been used (Thompson et al., 2010), but the drug can be chromatographed underivatized following solid phase extraction (Torrance and Cooper, 2010).

3.7.2 Methylone

Methylone (3,4-methylenedioxymethcathinone [bk-MDMA], the beta-keto analog of MDMA) is the main ingredient of a liquid designer drug that appeared on the Dutch drug market, called "explosion" (Bossong et al., 2005). Another stimulant, mCPP (meta-chlorophenylpiperazine), was also detected in this seizure. Methylone has also been detected in Japan. Three percent of drug users in Ireland use methylone (McNamara et al., 2010). The drug has no doubt been encountered elsewhere as it is easily available over the Internet as shown in Figure 3.34.

In 2012, Cawrse described the tissue distribution in four fatalities. All four cases had detectable levels of methylone, with heart blood concentrations of 0.740, 0.118, 0.060, and 1.12 mg/L. Analysis of several tissue samples shows that methylone does not sequester in a particular tissue type after death. The average liver-to-blood ratio was 2.68. Two cases also had MDPV present, but insufficient data were collected to formulate a hypothesis on postmortem sequestration or redistribution (Cawrse et al., 2012).

Methylone acts on the plasma membrane catecholamine transporter and has a weak effect on the vesicular monoamine transporter (Baumann et al., 2013). It is metabolized by *N*-demethylation, reduction of the keto group, and oxidation of the tolyl moiety (Meyer et al., 2010). 4-Hydroxy-3-methoxymethcathinone (HMMC) is the major metabolite and can be used to confirm use of methylone. In rats, about 26% is excreted as HMMC over 48 h with less than 3% excreted as unchanged methylone (Kamata et al., 2006). Methylone, methcathinone, and presumably related substances can be detected in hair (Kikura-Hanajiri et al., 2007).

The clinical profile of this drug is not well characterized but presumably is similar to other drugs of the same class. In one case report, a highly agitated, incoherent, and confused young male presented to the emergency room with high heart rate, high blood pressure, dilated pupils, and sweating after a single ingestion of an Internet-derived substance

(a) (b)

Figure 3.34 (a and b) Advertisements for methylone taken from the Internet.

believed to be an hypnotic. Subsequent analyses indicated that the drug consisted of 60% methylone (120 mg) and 38% 5-MeO-MIPT (76 mg) (Shimizu et al., 2007).

3.7.3 Fluoromethcathinone

Recently, fluoromethcathinones have been reported (Figure 3.35; Brandt et al., 2011). These are known as flephedrone with common names as 3-FMC or 4-FMC. These fluorinated keto-amphetamines are likely to have a higher lipophilicity enhancing absorption into the brain and therefore, are likely to have enhanced central effects. They first became available at "head shops" and over the Internet. Customs authorities in England reported having confiscated a supply of capsules containing 3-fluoromethcathinone. These included an orange and white (lift), cream (sub coca dragon), yellow (high spirit), and cream and yellow capsules (NeoDove 2). Lift was found to contain 250 mg 3-fluoromethcathinone (Archer, 2009). In 2009, a powdery substance called Charge+ was also found to contain 3-fluoromethcathinone together with caffeine and lidocaine (Westphal et al., 2010). Isomeric fluoromethcathinones (regioisomers) are impossible to identify without more sophisticated MS. The main method recommended for detection is to use product ion MS investigation of HF-loss-ions [M+H_HF]+ generated by collision-induced dissociation of the protonated molecular ion during chemical ionization (Westphal et al., 2010).

The most recent edition of the UN World Drug Report for 2013 reports that fluorocathinones are especially popular in Asia. Hong Kong, China, has reported the emergence of a number of synthetic cannabinoids (such as JWH-018) and synthetic cathinones (4-methylethcathinone and butylone). Indonesia informed UNODC of the emergence of BZP. Singapore reported the emergence of a number of synthetic cannabinoids (including JWH-018) and synthetic cathinones (3-fluromethcathinone and 4-methylecathinone). According to UNODC, the phenomenon has spread to the Middle East as well. Oman authorities have reported the emergence of synthetic cannabinoids (JWH-018), while Japan reported the emergence of phenethylamines, synthetic cathinones, piperazines, ketamine, synthetic cannabinoids, and plant-based substances (UNODC, 2013b).

3.7.4 Piperazines (Table 3.9)

BZP (*N*-benzylpiperazine or 1-benzylpiperazine) is a schedule I controlled substance that has often been substituted for MDMA. It has been known as BZP, bliss, charge, herbal ecstasy, and legal X. The related pyrrolidinophenone 3-trifluoromethylphenylpiperazine monohydrochloride (TFMPP) is still not considered a controlled substance in the United States, but it too has been substituted for MDMA. It was first encountered in the United States in 2006, but it has been in fairly widespread use in Europe, Australia, and New Zealand for several years longer. TFMPP was briefly emergency scheduled in schedule I in the United States, but the scheduling expired in April 2004 and has not been renewed.

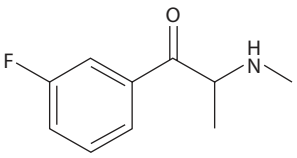
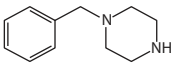
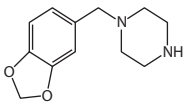
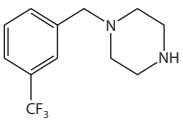
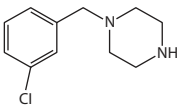
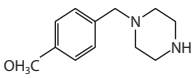
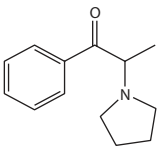


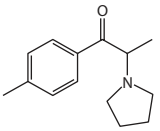
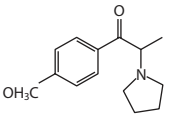
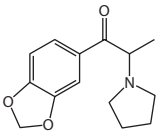
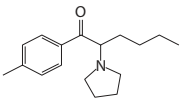
Figure 3.35 Molecular structure of fluoromethcathinone.

Table 3.9 Common Piperazines

Class	Common Name	Pharmacology	Comments	References
Piperazines				
	1-benzylpiperazine (BZP)	Cocaine-like inhibition of dopamine uptake, amphetamine-like dopamine release, agonist activity on postsynaptic dopamine receptors, and noradrenergic activity	3-OH and 4-OH metabolites and conjugates	Antia et al. (2009a), Gee et al. (2010), Thompson et al. (2010), Wood et al. (2010a,b)
	1-[3,4-methylendioxybenzyl]-piperazine (MDBP)	Likely to at least involve weak inhibition of serotonin uptake	Demethylenation and subsequent methylation and glucuronidation or sulfation	Maurer et al. (2004)
	1-[3-trifluoromethylphenyl]-piperazine (TFMPP)	Agonist on 5-HT_{1B}, 5-HT_{1C}, and 5-HT_2 serotonin receptors	4-OH metabolite, also dealkylation of the piperazine ring, with acetylation and conjugation. Often in combination with BZP	Antia et al. (2009b), Thompson et al. (2010)
	1-[3-chlorophenyl]-piperazine (mCPP)[a]	Serotonin mixed agonist–antagonist: anxiety, agitation, drowsiness, flushing, visual disturbances, and tachycardia		Hamik and Peroutka (1989)
	1-[4-methoxyphenyl]-piperazine (MeOPP)	Central serotoninergic activity	Metabolites: *O*-demethylation to 4-OH metabolite[b] and conjugation	Staack and Maurer (2005)
Pyrrolidinophenones				
	*R,S*-α-pyrrolidinopropiophenone (PPP)	Stimulant, possible release of dopamine and NE and inhibit monoamine transporters	Oxidation of pyrrolidine ring	Maurer et al. (2004), Sauer et al. (2009), Wohlfarth et al. (2010)

(*Continued*)

Table 3.9 (*Continued*) Common Piperazines

Class	Common Name	Pharmacology	Comments	References
	*R,S*-4′-methyl-α-pyrrolidinopropiophenone (MPPP)		Oxidation plus conjugation	
	*R,S*-4′-methoxy-α-pyrrolidinopropiophenone (MOPPP)		4-hydroxy-metabolite plus conjugates	
	*R,S*-3′,4′-methylenedioxy-α-pyrrolidinopropiophenone (MDPPP)		Demethylation plus conjugates	
	*R,S*-4′-methyl-α-pyrrolidinohexanophenone (MPHP)		Hydroxylation and oxidation with conjugation	

[a] Also a metabolite of antidepressants trazodone, nefazodone, etoperidone, and mepiprazole.
[b] 4-OH phenylpiperazine is also a metabolite of dropropizine and oxypertine.

Therefore, unlike its cousin BZP, TFMPP is not currently an illicit drug under U.S. federal law. However, some states have banned the drug in their criminal statutes making its possession a felony.

Some research on the properties has been performed. According to preliminary studies, TFMPP has affinity for the 5-HT_{1A} (K_i = 288 nM), 5-HT_{1B} (K_i = 132 nM), 5-HT_{1D} (K_i = 282 nM), 5-HT_{2A} (K_i = 269 nM), and 5-HT_{2C} (K_i = 62 nM) receptors and functions as a full agonist at all sites except the 5-HT_{2A} receptor, where it acts as a weak partial agonist or antagonist (Baumann et al., 2005). Unlike the related piperazine compound metachlorophenylpiperazine (mCPP), TFMPP has insignificant affinity for the 5-HT3 receptor (IC_{50} = 2373 nM) (Robertson et al., 1992). TFMPP also binds to the serotonin transporter (SERT) (EC_{50} = 121 nM) and evokes the release of serotonin. It has no effects on DA or NE reuptake or efflux.

Piperazines are used around the world as hardeners for epoxy resins, but also as antihistamines and anthelmintics. Interestingly, they are also used in the manufacture of Viagra and Marzine (cyclizine). Piperazines of various sorts are now available online or in "head shops" across the United Kingdom and, by extension, via the Internet in the United States. Head shops commonly sell this drug under the brand name of "pep pills." These chemicals present such a problem that New Zealand has established a new drug classification, "Class D," which requires certain drugs to be placed on a "restricted list" to be sold only under various strict licensing conditions. Analysis of some "legal high" products in the United Kingdom found that a product labeled as NRG-3 (mislabeled with the chemical structure of mephedrone) consisted of a mixture of 4-methyl-α-pyrrolidinopropiophenone (MPPP) and a cathinone derivative known as pentylone (Figure 3.36; Brandt et al., 2011). Other variations contained various cathinone derivatives.

A pyrrolidinophenone known as α-pyrrolidinovalerophenone appeared in Germany and in other European cities. It is intended for oral use. Little information is available on

Figure 3.36 Advertisement for typical packaging of BZP as seen in head shops and online.

its pharmacology and toxicity; however, it is similar in structure to pyrovalerone, which is a stimulant that acts by releasing DA and NE at nerve terminals; it may even be an inhibitor of monoamine transporters (Schauer et al., 2009). To date, only one death has been attributed to it (Eiden et al., 2013). A supplier of piperazines in the United Kingdom, called "spiritual highs," had sold four different products: two containing just BZP (in two strengths) and two containing BZP and TFMPP combined (again, in two strengths). The different mixtures allowed the products to be sold under separate, more lenient laws. In some areas, BZP gained popularity during the early 2000s, when it was sold as a legal alternative to amphetamine, MA, and MDMA.

Piperazines are synthetic and are *not* derived from plants of the *Piper* genus. The action of the most widely known piperazine, BZP, could be described as falling somewhere between those of amphetamines and those of MDMA, or even yohimbine. BZP has two main actions. It is an antagonist at the α_2-adrenoreceptor, disrupting negative feedback at the synapse, resulting in a larger stimulation-evoked release of an as yet to be specified neurotransmitter, most probably DA. However, BZP has only 1/1000 the potency of yohimbine. BZP also prevents the reuptake of NE (just like cocaine and some antidepressants). BZP acts as a stimulant in humans and produces euphoria and cardiovascular effects, namely, increases in heart rate and systolic blood pressure. BZP is about 10–20 times less potent than amphetamine in producing these effects (Staack and Maurer, 2005). A paper published in 2008 described BZP and TFMPP concentration where the drugs were present merely as an incidental finding. BZP was found at concentrations of 0.71, <0.50, and 1.39 mg/L, and 3-TFMPP was found at concentrations of 0.05 and 0.15 mg/L in postmortem blood (Elliot and Smith, 2008).

TFMPP is a more potent drug than BZP (Figures 3.37 and 3.38). The minimal dose is thought to be 25 mg with a maximum of 100 mg, which is much lower than that of BZP. Both BZP and TFMPP have an effect on the 5-HT receptors, and users report that the mix

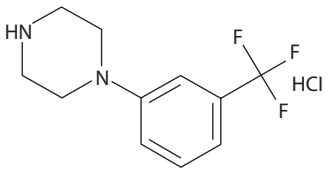


Figure 3.37 TFMPP molecular structure.

Figure 3.38 Packaged TFMPP as sold in a "head shop."

of the two drugs produces an effect that is similar to the euphoric high of MDMA, although neither BZP nor TFMPP appears to have this effect when used alone (Maurer et al., 2004). This has led some to conclude that the two drugs have a yet to be characterized synergistic relationship. No deaths from the use of either agent have been reported although they have been detected in some polypharmacy deaths.

Whether or not either product is addictive is an open question. An article in the *New Scientist* reported on studies showing that monkeys will self-administer BZP (the criteria for addiction), but no one has ever encountered an addict (Vince, 2006), or at least has ever written of such an encounter. Piperazine side effects are known to include severe agitation, nausea (lessened if taken on a full stomach), seizures, paranoia, hyperthermia, abdominal pain, and cardiac arrhythmias. Effects from longer-term use are not known. Both agents act as diuretics and extensive use may lead to dehydration, especially if consumed with alcohol.

3.7.4.1 Absorption and Pharmacokinetics

BZP is hydroxylated in the liver, but TFMMP undergoes demethylenation. *N*-dealkylation to piperazine, followed by further degradation to form the corresponding ethylenediamine or aniline derivatives, completes the process. As a rule, the phenylpiperazines are more extensively metabolized than the BZPs and virtually no unchanged drug is excreted. Glucuronidation occurs, but not to any great extent (Maurer et al., 2004).

In volunteers, plasma concentrations of BZP were found to peak at 0.26 mg/L at 75 min. Plasma concentrations of the major metabolites of BZP, 4-OH BZP and 3-OH BZP, were found to peak at 7 ng/mL (at 60 min) and 13 ng/mL (at 75 min), respectively. The elimination half-life for BZP was 5.5 h. BZP was detectable in plasma for up to 30 h following an oral dose. Six percent was excreted unchanged in urine (Antia et al., 2009a,b).

A controlled study of the administration of BZP/TFMPP (300 mg/74 mg) was curtailed early in an NZ study because of severe adverse events that occurred in subjects receiving these drugs, with or without alcohol (57.6 g). The adverse events included agitation, anxiety, hallucinations, vomiting, insomnia, and migraine. BZP/TFMPP significantly improved driving performance, decreasing the standard deviation of lateral position (SDLP) at −4.2 cm (95% CI −6.8 to −1.6, $p = .002$), while the effect of the alcohol was to increase SDLP: 2.3 cm (95% CI −0.3 to 4.9, $p = .08$) (Thompson et al., 2010). The blood concentrations of BZP and TFMPP peaked at 6.5 h with mean values of 0.6 and 0.04 mg/L, respectively.

3.7.4.2 Toxicology

BZP and 3-TFMPP have been confirmed in five misadventures where death was caused by trauma (road traffic deaths and a fatal fall). In all cases, other drugs and/or ethanol were also present, and in four cases, both substances were detected. They were found in postmortem blood at concentrations ranging from <0.50 and 1.4 mg/L in one case to 0.05 and 0.15 mg/L in the other. The substances were also detected in urine (Elliott and Smith, 2008).

An adult female with a plasma concentration of 0.2 mg/L BZP developed status epilepticus, hyperthermia, disseminated intravascular coagulation, rhabdomyolysis, and renal failure associated with BZP ingestion, and in another presentation to the emergency room, a man who had taken 2–3 "party pills" developed a similar pattern of toxicity from the combined use of BZP and MDMA. Blood concentrations 3 h after admission were 2.2 and 1.0 mg/L, respectively. Both cases required prolonged hospital care but survived. Symptoms, as might be expected, included hyperthermia, sweating, enlarged pupils, confusion, agitation, vomiting, anxiety, and palpitations and seizures (Gee et al., 2010). A postmortem blood concentration of BZP of 1.7 mg/L in a death attributed to this drug has been reported in combination with MDMA (Wikstrom et al., 2004).

3.7.4.3 Analysis

The piperazines will not be detected by any of the standard immunoassays although there was one report that BZP had some cross-reactivity with the enzyme multiplied immunoassay technique drugs of abuse (d.a.u.) kit for amphetamine compounds (de Boer et al., 2001). For positive identification, chromatographic assays are needed both to screen for these drugs and to ultimately confirm their presence. One method has been to use GC or GC–MS to screen and LC–MS/MS to confirm their presence in biological samples (Vorce et al., 2008). In one method for analyses in urine, acid hydrolysis is used to cleave conjugates followed by a liquid–liquid extraction and microwave-assisted acetylation (Staack and Maurer, 2005). It is preferable to use full-scan GC–MS such that all possible substances can be detected since selected ion monitoring may mean that a newer substance is not detected.

LC–MS/MS is highly selective and sensitive and requires little sample preparation. Methods have been published for piperazines and related compounds (Beyer et al., 2007; Thompson et al., 2010; Wohlfarth et al., 2010). As for selected ion monitoring (SIM) GC–MS, there is a risk of missing newer drugs if multiple reaction monitoring is used for drug screening instead of full scanning.

3.7.5 mCPP

One of the piperazines, 1-(3-chlorophenyl)piperazine or meta-chlorophenyl piperazine (mCPP), is of particular interest (Figure 3.39). It is sometimes sold as an MDMA

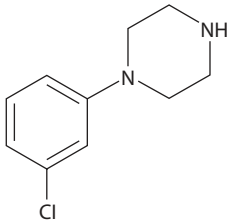


Figure 3.39 Structure of the mCPP molecule.

substitute. It is a metabolite of trazodone (an antidepressant), and in many U.S. jurisdictions, it is sold legally; however, it is being increasingly encountered as an MDMA look-alike. Recent reports indicate that, in some areas, it is also being used as a cocaine adulterant (Staack and Maurer, 2005). More is known about the pharmacology of mCPP than any of the other drugs in this group. It causes neuronal release of 5-HT and prevents reuptake of 5-HT by seemingly interfering with the 5-HT transporter (Baumann et al., 1995). There is also evidence that it causes the release of DA (Hamik and Peroutka, 1989).

There is only one published pharmacokinetic study for mCPP. Maximum mCPP concentrations varied 2.3-fold after intravenous infusion and 8-fold after oral administration, and absolute bioavailability ranged from 12% to 84%. The drug's elimination half-life ranges anywhere from 2.4 to 6.8 h after intravenous infusion and from 2.6 to 6.1 h after oral administration. This enormous degree of variation is attributed to CYP2D6 polymorphism (Feuchtl et al., 2004). The experimenters also showed that, compared to placebo, the drug causes increased plasma concentrations of ACTH, cortisol, and prolactin.

In one case, an overdose to mCPP produced a plasma concentration of 0.3 and 2.3 mg/L in urine. The concentration of mCPP in plasma was approximately six times higher than the usual concentration measured in patients under trazodone treatment (26–108 ng/mL, average 56 ng/mL) (Kolaleva et al., 2008). A more recent report describes a 20-year-old asthmatic with a fatal respiratory arrest following the ingestion of approximately 20 mg of mCPP. Blood concentrations could not be determined as the body had already been embalmed before the death came to the attention of the authorities (Gaillard et al., 2013).

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Hallucinogens

4

4.1 Introduction

It is difficult to say which drugs really should belong in this category as there is no universally accepted method of classification. Hallucinogens are said to share five common features: (1) changes in mood and perception dominate in proportion to any other effects the drugs might exert; (2) minimal memory or intellectual impairment occurs; (3) use is not associated with either stupor or excessive agitation; (4) side effects from autonomic nervous system stimulation are minimal; and (5) craving and addiction do not occur (Hollister, 1967). In addition, members of this group bind to 5-HT_2 receptors. Traditionally, hallucinogens have been divided into two groups: phenylalkylamines (drugs such as mescaline, 4-methyl-2,5-dimethoxyamphetamine [DOM], and 4-bromo-2,5-dimethoxyamphetamine [DOB]) and the indolealkylamines (psilocybin, bufotenine, lysergic acid diethylamide [LSD], and harmaline) (Table 4.1).

To some extent, certain anesthetics, including dissociative anesthetics, and some amphetamine derivatives share common effects with the hallucinogens, making any sort of classification problematic. What distinguishes putative new members is the fact that while they may be hallucinatory, they usually also produce agitation and sympathetic activation. Hollister did not include medical complications when he wrote his set of criteria. If he were writing today, his criteria would have been different; the ingestion of some designer amphetamines certainly can lead to agitation, sometimes with fatal results. Our understanding of the toxicology of these drugs and, to some extent, the neurochemistry of the hallucinogens in general has advanced considerably in the last decade, as has the number of different hallucinogens available. Hollister's initial scheme is followed in this chapter. Except for the designer amphetamines, none of the hallucinogens discussed is associated with unique or specific pathologic lesions.

It seems probable that Hollister's classification of hallucinogens will require revision in the near future. Many more synthetic agents are available today than when Hollister's definition was first proposed, and the neurochemistry of their action is better understood. It may ultimately be that these drugs are classified by their chemical, rather than psychological effects. Chemical structures such as piperazines (e.g., benzylpiperazine [BZP], trifluoromethylphenylpiperazine), phenethylamines (e.g., the drug 2C or the D-series of ring-substituted amphetamines, benzodifurans, cathinones, aminoindans), tryptamines (e.g., dimethyltryptamine, alpha-methyltryptamine, ethyltryptamine, 5-methoxy-alpha-methyltryptamine), piperidines, and related substances such as desoxypipradrol and diphenylprolinol all have a slightly different mode of action and effect. Some, such as *Salvia divinorum*, interact only with κ receptors, putting them in a class by themselves. As of this writing, both scientific and public health interests are focused on the new amphetamine and cathinone derivatives, which not only are difficult but appear to be genuinely dangerous and almost impossible to regulate. Accordingly, a cathinone-derivative subsection has been added to this edition.

Table 4.1 Hallucinogens

A.	*Indolealkylamines*
	Tryptamine derivatives
	Mescaline
	Substituted amphetamines
	TMA
	DOM
	PMA
	DOB
	4-Bromo-2,5-dimethoxyphenethylamine
	Nutmeg
	DOC
	2,5-Dimethoxy-4-iodophenethylamine
	Piperazines
	BZP
	TFMPP
	mCPP
B.	*Hallucinogenic amines*
	MDMA
	MDA
	MDEA
	4-MAX (U4Euh, EU4EA, U4EA), aminorex
	Methcathinone
C.	*Phenylalkylamines*
	Simple tryptamines
	DMT
	Ayahuasca (harmine and harmaline)
	Bufotenine
	5-MeO-DMT (5-methoxy-*N*,*N*-dimethyltryptamine)
D.	*Psilocybin*
E.	*Ergolines*
	Lysergic acid diethylamide (LSD)
F.	*Bk-amphetamines*

4.2 Production and Consumption

Drug Enforcement Administration (DEA) figures relating to current hallucinogen use are difficult to obtain and usage difficult to estimate. The UN Office on Drug Control and Crime (UNODC) reports that only one mescaline laboratory was seized in 2009 (in Chile). When the last government report was issued, beta-keto amphetamines, the so-called *bk*-amphetamines (mephedrone, butylone, methylone), had not yet come to public attention, and many were not even in existence. The usefulness of the UN figures is limited by the fact that they combine hallucinogenic and nonhallucinogenic drugs in one category. There really is no way to separate methamphetamine production from 3,4-methylenedioxymethamphetamine (MDMA) production.

The National Survey on Drug Use and Health is the primary source of statistical information on the use of illegal drugs, alcohol, and tobacco by the U.S. civilian population

and follows 23 million individuals aged 12 and above. It reports that in 2011, there were 1.1 million persons aged 12 or older who had used hallucinogens for the first time within the prior 12 months. This number is of considerable concern, given that none of the new cathinone derivatives nor bk-amphetamine had yet gained any market share. This estimate was similar to the estimates from 2006 to 2010 (ranging from 1.1 to 1.3 million), but was higher than the estimates from 2003 to 2005 (ranging from 886,000 to 953,000).

The number of individuals 12 years old and older admitting to at least one episode of use was 358,000 in 2011, which was similar to the number in 2010 (381,000), but higher than the estimates from 2003 to 2007 (ranging from 200,000 to 271,000). Past year initiates of PCP decreased by nearly a third from 123,000 in 2002 to 48,000 in 2011. In 2011, the number of individuals admitting to Ecstasy use in the prior year was 922,000, which was similar to the number in 2010 (949,000), but lower than the number in 2009 (1.1 million). The estimate was 1.2 million in 2002; it declined to 642,000 in 2003 and then increased by about 50% between 2005 (615,000) and 2011 (922,000). Most (61.3%) of the recent Ecstasy initiates in 2011 were aged 18 or older at the time they first used Ecstasy.

In addition, 0.7 million individuals had tried *S. divinorum*, and another 0.7 million had tried *N*,*N*-dimethyltryptamine (DMT), α-methyltryptamine (AMT), or 5-MeO-DIPT at least once in their lifetime. Among young people aged 12–17, females were more likely than males to have used Ecstasy in the past year (1.4% vs. 1.0%). Among those aged 12–17 years, males were more likely than females to have used *S. divinorum* in the past year (0.9% vs. 0.3%). In 2006, young adults aged 18–25 were more likely than youths aged 12–17 or adults aged 26 or older to have ever used LSD, Ecstasy, or *S. divinorum* in the last year (Office of Applied Studies, 2009). The 2014 edition of the UN World Drug Report mentions mephedrone use in England and Wales but makes no mention of any other cathinone derivatives (UNODC, 2014).

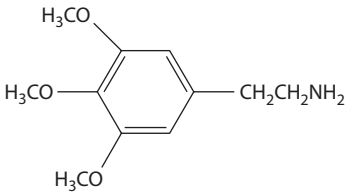
Hallucinogens are neither produced in the same way nor sold by the same organizations that produce heroin and cocaine, making it difficult or impossible to offer reliable broad production estimates. With the possible exception of MDMA, production of these drugs is decentralized. Production and consumption are subject to wide geographic variation. The UN estimates that manufacture of drugs marketed as *Ecstasy* ranged from 55 to 133 metric tons in 2008. MDMA is becoming important in many parts of the developing world, including Asia, but MDMA use is declining in Europe and Australia in favor of synthetic psychotropic substances sold as faux Ecstasy tablets and other drugs such as *bath salts*. MDMA manufacture increased in North America in 2008, especially in Canada (mainly for U.S. export) (UNODC, 2010). No reliable figures exist for cathinones or bk-amphetamines.

4.3 Tryptamine Derivatives

Mescaline derivatives remain the most popular hallucinogens (Table 4.2), and MDMA is by far the most widely used mescaline analog. Evidence suggests that these compounds may be more toxic than had previously been appreciated, and increasing numbers of deaths attributable to MDMA are being reported (Mascola et al., 2010). But in the absence of an effective worldwide database (in the United States, at least, there is no reporting system for specific types of abused drugs), it is impossible to tell how much more popular these drugs have become. Many European countries have reported declines in MDMA use.

Table 4.2 Physiochemical Properties and Pharmacokinetics of Mescaline

Chemical name	3,4,5-Trimethoxybenzeneethanamine or 3,4,5-trimethoxyphenethylamine.
Physiochemical properties, structure, and form	Ingested as *buttons* or powder, also mescaline sulfate or mescaline hydrochloride; CAS 54-04-6; MW 211.26; pK_a 9.6.
Source	*L. williamsii* (peyote cactus), also seen in other cactus species such as *Echinopsis pachanoi* and *E. peruviana* and in *Acacia berlandieri*.
Dose and pharmacokinetics	Common doses 200–500 mg; bioavailability unknown; 3.88 mg/L after 500 mg oral dose (Charalampous, 1971); 14.8 mg/L after 5 mg/kg given intravenously (Mokrasch et al., 1959).
Common blood concentrations in drug users	Little data available but likely to range into mg/L concentrations.
Blood terminal elimination half-life	About 6 h (Charalampous, 1971).
Metabolism	Oxidation to carboxylic acid, *N*-acetylation, and *O*-demethylation; no interaction with CYP2D6 (Wu et al., 1997).
Urinary excretion	About 55%–60% excreted in urine unchanged (Charalampous et al., 1966). Major metabolite is 3,4,5-trimethoxyphenylacetic acid (Demisch et al., 1978).
Postmortem artifacts	Unknown.
Interactions	Unknown.
Published major papers or reviews	Carstairs and Cantrell (2010).



This is in line with reports of manufacturing difficulties in a number of European countries in recent years, leading to the sale of various other substances in place of MDMA in *Ecstasy* tablets. Nonetheless, levels of MDMA use in the Czech Republic, Latvia, Slovakia, and the United Kingdom remain high (UNODC, 2014).

4.3.1 Mescaline (Figure 4.1)

4.3.1.1 History

Louis Lewin was one of the first to systematically study mescaline. Mescaline is derived from the cactus referred to as either *Lophophora williamsii or Anhalonium lewinii*. This small cactus can be found growing in dry areas and upon rocky slopes throughout the southwestern United States (Figure 4.2). Plants grow singly or in clusters, but either way it is an inconspicuous plant and can be difficult to find. Unless it is flowering, it tends to look

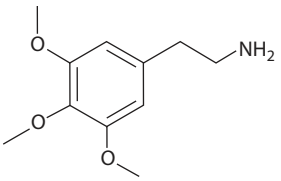


Figure 4.1 Mescaline and the *designer* amphetamines. Whether small recreational doses of these drugs are hallucinogenic is difficult to say, but all of these agents can impair judgment, and their use occasionally leads to fatal accidents.

Figure 4.2 Peyote buttons. Even though it grows wild throughout the American southwest, the cactus can be very difficult to find. Except when it is in bloom, it tends to resemble a small rock. (From Anon., *Microgram*, XXXVI, 226, 2003.)

like a small rock. Indian shamans have used the dried tops of the plants, known as peyote buttons, for centuries. In the early 1800s, the Apaches, Kiowas, and Comanche of the Great Plains began to chew the buttons and incorporated them into their religious rites. The practice spread quickly among the Plains Indians, who combined mescaline use with elements of Christianity. Today, their ceremonies still begin with the chewing of peyote buttons, followed by nights of prayers and singing. This sect is now known as the Native American Church and has more than 200,000 members (Barron et al., 1964). The emblem of this church is shown in Figure 4.3. Mescaline, or 3,4,5-trimethoxy-β-phenethylamine, is the active principle found in peyote cactus. The average mescaline content of the plant is about 6%. It is also found in some other species (Figure 4.4).

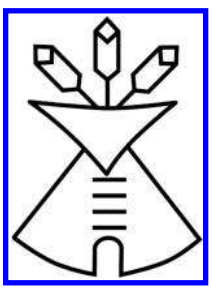


Figure 4.3 The emblem of the Native American Church.

Figure 4.4 The button-shaped knob at the tip of the stem contains most of the psychoactive components. (From Anon., *Microgram*, XXXVI, 226, 2003.)

Lewin and Henning reported the first systematic chemical and pharmacologic studies in 1888 (Lewin and Henning, 1888). Lewin's work attracted the attention of the famous American neurologist S. Weir Mitchell. Mitchell, who was a prolific writer and a pioneer in the study of peripheral nerve injuries, was also interested in toxicology and psychiatry (Metzer, 1989). He obtained some peyote buttons and tried them on himself. He went on to publish an account of his experiences in the *British Medical Journal* (Metzer, 1989). He believed that the plant might be of great value in the study of psychological disorders, but he also warned of the abuse potential. The famous sexologist Havelock Ellis also dabbled with mescaline and described the many benefits to be derived from its use (Mitchell, 1896). Neither the benefits nor the epidemic of abuse ever really materialized. Similar claims now being made for MDMA have considerably more substance.

4.3.1.2 Production

Mescaline is extracted from the cactus, first by drying and then grinding the plant tops. The ground material is then soaked in methanol for a day, filtered, and acidified. After the alcohol has evaporated, the solution is neutralized and the mescaline extracted with chloroform. Less sophisticated chemists *cook* the cactus in a pressure cooker, producing a tarry material that can be formed into small pills. Some clandestine producers may even apply an enteric coating or place the tarry material in gelatin capsules with the hope of reducing the nausea induced by mescaline use.

4.3.1.3 Mechanism of Action

Hallucinogens such as mescaline cause concentrations of extracellular glutamate within the prefrontal cortex to increase in two different ways. First, they are agonists on receptors located on cortical and pyramidal neurons causing glutamine to be released. Second,

they act as agonists on postsynaptic serotonin (5-hydroxytryptamine) 2A (5-HT_{2A}) receptors. The receptors specifically involved are located on large glutamatergic pyramidal cells located in deep cortical layers (layers V and VI). These cells, in turn, project to layer V pyramidal neurons. Increased glutamate release activates α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and *N*-methyl-D-aspartate receptors located on cortical pyramidal neurons. When enough glutamate has accumulated, there is increased expression of brain-derived neurotrophic factor (BDNF), leading to enhanced firing of glutamatergic projection neurons and increased extracellular glutamate levels in the prefrontal cortex (Vollenweider and Kometer, 2010). Interestingly, BDNF can be measured in serum and has been studied in Alzheimer's patients, where levels appear to correlate with the severity of the disease. It would be interesting to make similar measurements in hallucinating patients, but this research has not been undertaken (Konukoglu et al., 2012).

4.3.1.4 Clinical Syndromes

Healthy volunteers given a 0.5 mg dose of mescaline exhibit symptoms of psychosis indistinguishable from those normally associated with acute schizophrenia. Single-photon emission computed tomography (SPECT) imaging of human volunteers during mescaline-induced psychosis showed increased regional flow in the frontal lobes bilaterally (Hermle et al., 1998). Otherwise, the symptoms associated with mescaline abuse are mostly those of sympathetic nervous system stimulation. Transient rises in heart rate, blood pressure, and temperature may occur (Kapadia and Fayez, 1970). In spite of these observations, no episode of clinically significant hyperthermia and/or excited delirium has ever been reported following the use of this drug. The most commonly reported effects include hallucinations, tachycardia, agitation, and mydriasis (Carstairs and Cantrell, 2010).

4.3.1.5 Tissue Concentrations

When dogs are injected subcutaneously with mescaline, the highest drug concentrations are found in the liver and kidneys. Concentrations in the liver, spleen, and kidneys are three to six times higher than the concentration found in the bloodstream. Brain levels tend to parallel the blood levels (Kapadia and Fayez, 1970). Animals metabolize mescaline differently than humans, who excrete it mostly unchanged (Cochin et al., 1951); other aspects of mescaline's human pharmacokinetics may also differ from experimental models. Human tissue levels have been measured in a handful of cases: one mescaline user, who died of a head injury, was found to have a blood concentration of 9.7 mg/L, a concentration eight times higher than that found in the liver (Reynolds and Jindrich, 1985). In 2003, a case report described blood and tissue levels in another mescaline user who had been shot. Concentrations of the drug were 2.95 mg/L, 2.36 mg/L, 8.2 mg/kg, and 2.2 mg/kg in blood, vitreous, liver, and brain, respectively (Henry et al., 2003). There have been no additional reports.

4.3.1.6 Toxicology and Pathology

No mescaline-related deaths or emergency room visits have ever been reported in any DAWN survey, and only two case reports describing mescaline-related deaths have appeared in the world literature. No specific pathologic findings have been identified. A search of the California Poison Control System database (1997–2008) uncovered 31 single-substance exposures to peyote or mescaline. Almost all of these (97%) were intentional and the drug was taken orally, with only individual patients who insufflated mescaline powder. None of

these subjects died or even required hospital care (Carstairs and Cantrell, 2010). According to the Drug Enforcement Agency, the hallucinogenic dose of mescaline is about 0.3–0.5 g and the effects last about 12 h.

4.3.1.7 Toxicology

4.3.1.7.1 Preanalytic Considerations There are little data regarding mescaline analysis; however, it does appear to be stable in plasma for at least 3 days at 4°C, or up to 1 month at –20°C (Habrdova et al., 2005; Beyer et al., 2007). It has also been found to be stable in urine for at least 1 week (Bjornstad et al., 2008).

4.3.1.7.2 Preferred Analytic Methods Mescaline is not difficult to detect in biological specimens since concentrations are high and the molecule is relatively small. Extraction can occur with use of butyl chloride or by use of mixed-mode solid-phase cartridges, as with other phenethylamines (Habrdova et al., 2005; Beyer et al., 2007); however, derivatization of the primary amine (e.g., with heptafluorobutyric anhydride) is recommended (Habrdova et al., 2005). Liquid chromatography–mass spectrometry (LC–MS) techniques can also be used without derivatization of the drug (Beyer et al., 2007; Bjornstad et al., 2008). This allows the detection of many other drugs, including other hallucinogens.

4.3.1.7.3 Postmortem Interpretation Since mescaline toxicity has never been found to be the actual cause of death in any reported case (although in the case reported by Nolte, it was an indirect cause), postmortem blood concentration measurement would be of little help in determining the cause of death.

4.3.2 Substituted Amphetamines

Phenethylamines are compounds in which the core chemical structure is a benzene ring substituted with a 2-aminoethyl chain. Although phenethylamine itself is not a controlled substance, it has a chemical structure that constitutes the skeleton of many phenethylamines listed in schedule I of the Controlled Substances Act (CSA), all of which are classified as hallucinogens. Phenethylamines also appear on the CSA's list of schedule II and IV drugs. Phenethylamine is sometimes substituted on the benzene ring or the 2-aminoethyl chain, or both, with various moieties resulting in the production of different physiological and psychological effects.

Many of the mescaline-type analogs that have been substituted at position 4 (e.g., escaline, proscaline, buscaline) are, on a weight-for-weight basis, much more potent than mescaline. Alkoxylated mescaline homologs (e.g., metaescaline, metaproscaline) are less potent. A series of thiomescaline analogs have also been synthesized, with substitutions at both positions 3 and 4. The clinical effects of these agents have never been systematically studied, and virtually nothing is known about their pharmacology, in either humans or animals. Since 1947, when researchers produced the first psychoactive mescaline analog (2,4,5-trimethoxyamphetamine [TMA]), molecular manipulations have been used to produce a long list of psychoactive derivatives. With the exception of MDMA, considerably more is known about the conformational chemistry of these molecules (Kovar, 1998) than about their clinical effects. Late in the last decade, a new class of designer drug emerged—the bk designer drugs such as mephedrone (bk-4-methylmethamphetamine) (Nicholson et al., 2010; Wood et al., 2010), butylone (bk-MBDB) (Meyer et al., 2010), and

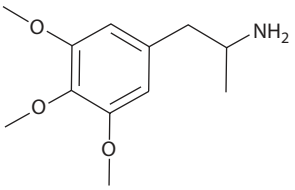


Figure 4.5 2,4,5-Trimethoxyamphetamine molecule.

methylone (bk-MDMA) (Bossong et al., 2005; Prosser and Nelson, 2012). The toxicity of these compounds remains poorly characterized, but anecdotal reports suggest that it may be significant.

4.3.2.1 TMA

TMA was the first of the synthetic phenethylamines found to be active in man. It is said to have twice the psychoactive potency of mescaline (Shulgin, 1973). It was first synthesized in 1933 but was not used as a psychedelic until 1962. Shulgin says TMA produces the same effects as mescaline, but with a lower therapeutic index. The amount required to cause hallucinatory or psychedelic experiences is not very different from the amount needed to produce toxicity (Chesher, 1990). The structure of TMA is essentially the same as that of the mescaline, except that it has a one-carbon side chain (Figure 4.5). After it was first synthesized, there followed a series of synthetic mescaline molecules with ever-longer side chains. All of these compounds are potent serotonin (5-HT_2) receptor agonists (Ewald and Maurer, 2008).

Very little is known about TMA metabolism or toxicokinetics. Gas chromatography–mass spectrometry (GC/MS) of urine from rats given TMA has shown that this drug may be metabolized by several different routes. TMA, like all the designer drugs in this group (DOM, 4-iodo-2,5-dimethoxyamphetamine [DOI], 4-chloro-2,5-dimethoxyamphetamine [DOC], DOB, 4-bromo-2,5-dimethoxymethamphetamine, and TMA-2), is a potent serotonin 5-HT_2 receptor agonist (Ewald and Maurer, 2008).

TMA-like drugs are mainly metabolized by *O*-demethylation or, in case of DOM, by hydroxylation of the methyl moiety. CYP2D6 is the only cytochrome P450 (CYP) isoenzyme involved in the main metabolic steps, but only very small amounts of the metabolites are formed. Similarly, all the drugs in this group are non-mechanism-based competitive inhibitors of CYP2D6, though the degree of inhibition varies from drug to drug. Interactions with other CYP2D6 substrates is thought to be possible, but unlikely (Ewald and Maurer 2008). According to Shulgin, the dosage is 100–250 mg and the effects last for 6–8 h (Shulgin and Shulgin, 1991). There are no reports of death, nor are specific pathologic lesions identifiable.

4.3.2.2 DOM

Methyl-2, 5-dimethoxyamphetamine (DOM; Figure 4.6) was first synthesized in 1963, shortly after TMA (Shulgin, 1979). Abuse was first reported in 1967. Two different clandestine labs started producing DOM at the same time. One illicit lab called the drug DOM and the other named it STP (*serenity, tranquility, and peace*). In doses of less than 3 mg, the effects of DOM are said to be similar to those of mescaline. Higher doses cause hallucinations and unpleasant side effects that may last for as long as 8 h (Snyder et al., 1968).

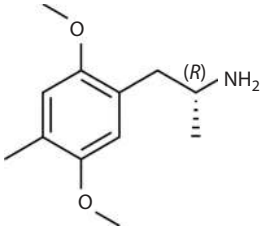


Figure 4.6 4-Methyl-2,5-dimethoxyamphetamine molecule.

DOM disappeared rather quickly from the streets, probably because large doses cause a very unpleasant experience. DOM, STP, and TMA all are metabolized in the same fashion (see Section 4.3.2.1). Regardless of the dose, approximately 20% appears unchanged in the urine with peak excretion occurring at between 3 and 6 h, the period when intoxication is most intense. Hallucinations produced by higher doses are associated with nausea, diaphoresis, and tremor, all symptoms of serotonin excess and almost certainly a result of DOM's potent stimulation of serotonin receptors.

Moderate elevations occur in heart rate and systolic blood pressure, but not in diastolic pressure. Blood and tissue levels have never been determined, either in the living or dead. The pathologic changes associated with its use, if any, are unknown. The closely related drug (2,5-dimethoxy-4-propylthio-beta-phenethylamine), which is usually referred to as (2C-T-7), in which Shulgin ranked nearly as high as mescaline for its pleasurable effects (Shulgin and Shulgin, 1991), has been linked with at least one fatality (Curtis et al., 2003). It shares structural and pharmacodynamic features with MDMA. In one case report, DOM was initially identified on routine screening of postmortem urine collected from a 20-year-old male who died in a local emergency room. He reportedly had insufflated 35 mg of 2C-T-7. Postmortem testing revealed the following concentrations: heart blood, 57 ng/mL; femoral blood, 100 ng/mL; urine, 1120 ng/mL; and liver, 854 ng/g (Curtis et al., 2003). No case of serotonin syndrome secondary to use of DOM has ever been reported. There are no human pharmacokinetic studies and no additional autopsy studies since 2003, but it is known that 2,5-dimethoxyamphetamine-derived designer drugs interact with CYP isoenzymes to form metabolites that inhibit CYP2D6 (Ewald and Maurer, 2008).

4.3.2.3 PMA

Paramethoxyamphetamine (PMA; Figure 4.7) is a potent hallucinogen and the only member of this group that truly qualifies as a dangerous drug. The first PMA-related deaths were reported in Canada in the mid-1970s (Cimbura, 1974). They were followed not long thereafter by a series of fatalities in Australia (Byard et al., 1998; Felgate et al., 1998; James and Dinan, 1998; Byard et al., 1999; Ling et al., 2001). PMA-related deaths are still occasionally reported from Australia (Dams et al., 2003; Johansen et al., 2003; Lamberth et al., 2008). The first PMA-related death in the United States was not reported until the summer of 2000 and deaths remain extremely uncommon.

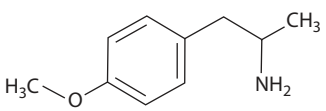


Figure 4.7 Paramethoxyamphetamine molecule.

A report published in 2001 described a series of 22 patients with PMA poisoning. All had been admitted to a major metropolitan teaching hospital over a 2-year period (1996–1998). These patients presented with tachycardia (64%), hyperthermia (temperature >37.5°C; 36%), coma (41%), seizures (32%), arrhythmias (23%), and QRS intervals ≥100 ms (50%). Two patients had severe hypoglycemia (blood glucose level, <1.5 mmol/L) accompanied by hyperkalemia (K^+ concentration, >7.5 mmol/L). It may be that hypoglycemia and hyperkalemia are specific to PMA poisoning (Ling et al., 2001).

In another Australian case series, PMA poisonings accounted for most of the severe reactions among people who believed they had taken Ecstasy (Jaehne et al., 2005). PMA and MDMA both facilitate the release, and prevent the reuptake, of 5-hydroxytryptamine (5-HT) but, in addition, PMA is also a potent inhibitor of monoamine oxidase type A (MAO-A), an enzyme responsible for the catabolism of 5-HT, and this characteristic may contribute to its increased toxicity. In humans, coadministration of MDMA with the reversible MAO-A inhibitor moclobemide has led to increased evidence of toxicity with ensuing fatalities (Freezer et al., 2005).

Animal studies from the early 1960s suggested that the hallucinogenic potency of PMA is nearly as great as that of LSD (Byard et al., 1998), but, unlike LSD, PMA may cause instances of marked hypertension and hyperthermia, and in fact, most of the reported fatalities have been as a consequence of extreme temperature elevation. Decedents are always young, usually in their mid-1920s, but with a different sex distribution than other abused drugs, as PMA fatalities are just as likely to be female as male. Several mechanisms have been proposed to explain the severe hyperthermia experienced by some PMA users.

Cocaine and MDMA alter body temperature by impairing cutaneous vasodilation. Both drugs act centrally to increase the output of neural pathways that control vasodilation (Vongpatanasin et al., 1999; Pedersen and Blessing, 2001; Crandall et al., 2002). In addition, the neurotransmitters serotonin and dopamine are thought to be involved in controlling body temperature, particularly when the relationship between D_1 and D_2 receptors becomes distorted (see Section 1.15). Antagonists to serotonin and dopamine receptors prevent MDMA-induced hyperthermia, but not always predictably and not always completely (Malberg et al., 1996; Mechan et al., 2002). PMA and MDMA are chemically related, so it may well be that they both control temperature in the same way, though this analogy has never been specifically proven, nor would similarities between the two drugs explain why the incidence of hyperthermia is so much higher in PMA than MDMA users (Daws et al., 2000; Seeman et al., 2005).

In six fatalities reported from Australia, femoral blood PMA concentrations ranged from 0.24 to 4.9 mg/L (mean, 2.3 mg/L), while liver PMA levels ranged from 1.4 to 21 mg/kg (mean, 8.9 mg/kg). Other amphetamines were found in five of the six cases, confirming the impression that when PMA is detected, it is probably present as an adulterant. Blood PMA concentrations in nonfatal cases are usually less than 0.5 mg/L (Felgate et al., 1998). Dams et al. studied whole body distribution in a fatal case involving the combined ingestion of amphetamine, 3, MDMA, 3,4-methylenedioxyamphetamine (MDA), and PMA (Table 4.3). Tissue distribution of all four drugs was very similar to that of amphetamine (though of course the amount ingested was not known). This suggests that the V_{ss} of the drugs is similar as well (Dams et al., 2003). The most recent case report described a 20-year-old man who died 10 days after being admitted to hospital with fever and extreme rhabdomyolysis, including cardiac muscle. The patient's antemortem blood concentration of PMA was 2.3 mg/L, and just as in the decedent described earlier, traces of both methamphetamine

Table 4.3 Compounding Difficulties Caused by the Postmortem Redistribution Process

Sample	PMA	MDMA	MDA	AMP
Subclavian blood (μg/L)	2,012	1,917	614	239
Femoral blood (μg/L)	1,634	1,129	436	198
Vena iliaca blood (μg/L)	1,618	1,421	493	203
Inferior vena cava blood (μg/L)	2,058	1,801	507	218
Right atrial blood (μg/L)	2,058	1,624	574	248
Pulmonary artery blood (μg/L)	2,952	2,212	475	279
Left pulmonary vein blood (μg/L)	2,120	1,693	526	261
Right pulmonary vein blood (μg/L)	2,427	1,941	610	329
Aorta ascendens blood (μg/L)	2,031	1,726	558	252
Right pleural blood (μg/L)	1,794	1,375	446	207
Left pleural blood (μg/L)	3,814	3,243	912	450
Pericardial fluid (μg/L)	2,373	2,335	615	318
Vitreous humor (μg/L)	2,101	1,633	577	292
Urine (μg/L)	932	791	369	522
Bile (μg/L)	50,012	25,420	11,655	9425
Muscle of the right cardiac ventricle (μg/g)	2,176	1,650	469	235
Muscle of the left cardiac ventricle (μg/g)	2,422	1,815	293	290
Right lung, upper lobe (μg/kg)	4,614	2,580	925	592
Right lung, median lobe (μg/kg)	4,460	2,535	1,023	693
Right lung, lower lobe (μg/kg)	3,164	1,281	676	395
Left lung, upper lobe (μg/kg)	3,742	2,138	661	475
Left lung, lower lobe (μg/kg)	4,390	2,358	875	543
Liver (μg/kg)	8,904	6,657	744	857
Stomach contents (μg/L)	73,103	33,168	14,308	5478
Right kidney (μg/kg)	5,669	4,058	3,888	746
Left kidney (μg/kg)	4,716	3,411	2,891	534
Spleen (μg/kg)	4,390	3,050	1,454	666
Iliopsoas muscle (μg/kg)	1,654	1,528	592	221
Abdominal adipose tissue (μg/kg)	131	317	44	67
Brain, frontal lobe (μg/kg)	4,081	2,258	919	330
Brain, temporal lobe (μg/kg)	4,188	2,289	1,035	358
Brain, parietal lobe (μg/kg)	4,040	2,514	1,026	773
Brain, occipital lobe (μg/kg)	3,357	1,932	918	910
Brainstem (μg/kg)	3,200	1,951	761	346
Cerebellum (μg/kg)	2,371	978	491	664

Source: Data adapted from Dams, R. et al., *J. Anal. Toxicol.*, 27(5), 318, 2003 July–August.

Note: It shows concentrations of PMA, MDMA, MDA, and amphetamine in one case of polydrug overdose.

and amphetamine were present, though actual postmortem measurements were not given (Lamberth et al., 2008).

PMA toxicity should be suspected when severe or atypical reactions occur in individuals who believe they have taken Ecstasy. The problem is that so many of the new, not yet scheduled, bk-amphetamines can produce exactly the same constellation of symptoms.

The evidence strongly suggests that many who think they are buying MDMA are actually purchasing material contaminated with PMA (Byard et al., 1998) or with one of the bk-amphetamines. PMA poisoning should also be considered as the possible etiology when hallucinating patients present with both hypoglycemia and hyperkalemia (Ling et al., 2001). The same can probably be said of a related compound, *para*-methoxymethamphetamine (see following paragraph).

p-Methoxymethamphetamine (PMMA) is an amphetamine-related designer drug. It may be sold under its own name, found as the only ingredient in faux Ecstasy tablets or found in combination with 3,4 methylenedioxymethamphetamine (Ecstasy, MDMA) or even with other substances such as PMA, amphetamine, and ephedrine. Any one of these drugs is capable of causing many of the same problems as PMA. In fact, PMA is a metabolite of PMMA.

The human pharmacokinetics and toxicology are unknown. However, when rats were injected with a 40 mg/kg subcutaneous dose of PMMA, a maximum plasma concentration of 4014 ± 1122 ng/mL was reached 30 min after dosing, although the appearance of metabolites was considerably delayed. PMMA had an approximate half-life of 1.0 h, a volume of distribution of 6.4 L/kg (higher than what might be expected in humans). The rate of plasma clearance was 4.4 L/h. Concentration of PMMA in tissue exceeded that in plasma, and the highest concentrations were found in the lungs (C_{max} 43 ± 10 μg/g). Penetration through the blood–brain barrier by PMMA and its *N*-demethylated metabolite PMA is likely. The maximum brain/plasma concentration ratio of PMMA (16) and PMA (12) was reached after 8 h (Rohanova and Balikova, 2009).

4.3.2.4 DOB

DOB (also called bromo-DMA, brolamfetamine, and Bromo-DMA; Figure 4.8) is another potent hallucinogen synthesized by Shulgin in 1967 (Shulgin and Shulgin, 1991). It has pronounced sympathomimetic effects but appears to be far less toxic than PMA and has a longer duration of action. Use still seems largely confined to Australia (Buhrich et al., 1983). It is occasionally sold as MDMA or found as an adulterant in MDMA tablets, but sometimes is sold under its own name. Whether this state will persist, given the introduction of drugs such as methylone and mephedrone, remains to be seen. There are already reports of mephedrone being used as an MDMA adulterant and even sold as faux MDMA. Because it is so potent, DOB can be absorbed into blotter paper and misrepresented as LSD (Shulgin, 1981). The problem with distributing DOB in the form of postage stamps is that DOB is considerably more toxic than LSD. During manufacture, the drug may migrate to the corners or bottom of the sheet of stamps. Users buying squares from the center of the sheet often receive less DOB than they paid for, while those buying squares from the margins of the sheet often get more than they bargained

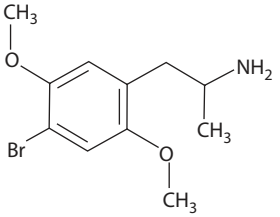


Figure 4.8 4-Bromo-2,5-dimethoxyamphetamine molecule.

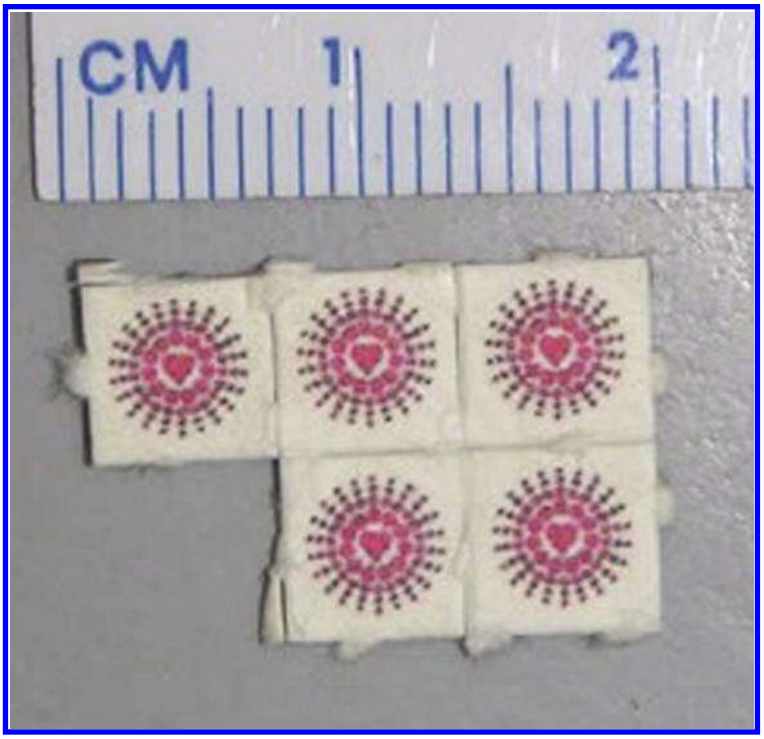


Figure 4.9 4-Bromo-2,5-dimethoxyamphetamine impregnated in a blotter. This dosage form is only occasionally reported. (From *Microgram,* November 2006.)

for (Figure 4.9). This may explain why so many bad experiences have been associated with use of the drug (Delliou, 1980, 1983).

Little is known about the human pharmacokinetics of this drug. Rats administered a 20 mg/kg dose of DOB, either orally or by subcutaneous injection, had a peak plasma DOB concentration of 320 ng/mL and a 203 ng/L peak of the metabolite 1 h post dosing. DOB itself is rapidly absorbed, with concentrations slowly decreasing over 32 h. After subcutaneous injection, high plasma levels of the unchanged parent drug and relatively reduced formation of its metabolite 2M5H4BA were observed. The maximum plasma concentration of DOB was 1.1 mg/L reached 1 h after subcutaneous injection; a peak metabolite concentration of 0.2 mg/L was not reached until 8 h later. DOB tissue concentrations exceeded those in plasma and the highest values were found in the lungs, where drug accumulation occurred with prolonged retention for as long as 32 h after the subcutaneous dose (Berankova et al., 2007). After oral administration, there is extensive first-pass metabolism. Once the drug has reached the liver, it is mainly metabolized by *O*-demethylation or, in the case of DOM, by hydroxylation of the methyl moiety by CYP2D6 (Ewald and Maurer, 2008).

Like other members of this subfamily, DOB is a potent serotonin 5-HT_2 receptor agonist. That action may explain why symptoms of intoxication, which do not occur for 3–4 h after ingestion, include pupillary dilation, increased heart rate and blood pressure, and increased temperature, although no cases of serotonin syndrome have been reported in DOB users. The effective dose is said to be between 2 and 3 mg.

DOB is associated with more morbidity than other mescaline analogs (Winek et al., 1981; Buhrich et al., 1983). Diffuse vascular spasm of the sort associated with ergotism

Table 4.4 Blood Levels in a Case of Fatal 4-Bromo-2,5-Dimethoxyamphetamine Intoxication

Tissue	Concentration (mg/L)
Blood	0.90
Bile	0.64
Vitreous	0.51
Brain	0.25
Liver	9.00
Kidney	1.10

Source: Winek, C.L. et al., *Clin. Toxicol.*, 18(3), 267, 1981. With permission.

has been reported (Bowen et al., 1983), and grand mal seizures have also been described (Delliou, 1983). This syndrome has not been reported in conjunction with use of the other *designer* amphetamines, but it is a well-known complication of LSD use. Scant autopsy information about DOB is available. One reported case (Winek et al., 1981) described the findings in a 21-year-old woman who was found dead at the wheel of her parked car. Gross autopsy findings included cerebral edema with uncial herniation. The lungs were minimally congested. Microscopic findings were not reported. Blood and tissue concentrations for this particular case are shown in Table 4.4. Another case reported from Germany described two men who took what they thought was LSD; it was not, and in the one who survived, the serum DOB concentration was 13 ng/mL, while the other, who died, had a concentration of 19 ng/mL (Berankova and Balikova, 2005).

4.3.2.5 4-Bromo-2,5-Dimethoxyphenethylamine (2C-B)

The first reports of this drug came from Florida in 1979. It has been sold in different markets, under different names, ever since. In the United States, at one time or another, it has been sold as Nexus, 2C-B, bromo, toonies, Venus, Bromo, Erox, and XTC, but the dealer is essentially free to call the drug whatever they wish.

Nothing is known of 2C-B's pharmacokinetics in humans. In rats given subcutaneous 2C-B, the estimated half-life was 1.1 h and estimated volume of distribution 16 L/kg. There is near immediate uptake by the lung with gradual release. As lung drug concentrations decrease, brain concentrations increase. The 2C-B brain/serum ratio reached a maximum of 14 and remained over the value of 6.5 for 6 h when the experiment was terminated (Rohanova et al., 2008).

Threshold effects are noted at an oral dose of approximately 4 mg. The drug is said to produce euphoria with increased body awareness and enhanced receptiveness to visual, auditory, olfactory, and tactile sensation. Oral doses of 8–10 mg produce stimulant effects. Doses in the range of 20–40 mg are said to produce LSD-like hallucinations. It is also reported that doses greater than 50 mg lead to the occurrence of extremely frightening hallucinations and morbid delusions. Onset of subjective effects following 2C-B ingestion occurs between 20 and 30 min after ingestion, with peak effects occurring at 1.5–2 h. Effects of 2C-B can last up to 6–8 h. Detection of this drug can be problematic because it does not react with the usual ELISA screening tests for amphetamines (Shulgin and Shulgin, 1991; Ambrose et al., 2010).

The drug is very closely related to DOB and is sometimes referred to as α-desmethyl DOB (Figure 4.10). 2C-B shares common properties with 2,5-dimethoxy-4-methylamphetamine (DOM) and DOB, including a high affinity for central 5-HT receptors. In addition

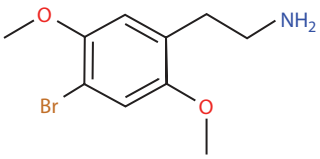


Figure 4.10 4-Bromo-2,5-dimethoxyphenethylamine structure.

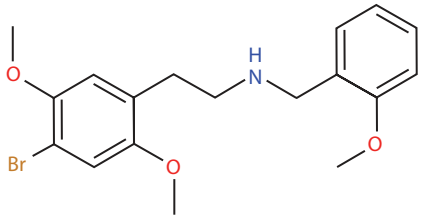


Figure 4.11 *FLY* is just one of many phenethylamine analogs. The presence of two *winglike* furan groups is said to give the molecule the appearance of a fly.

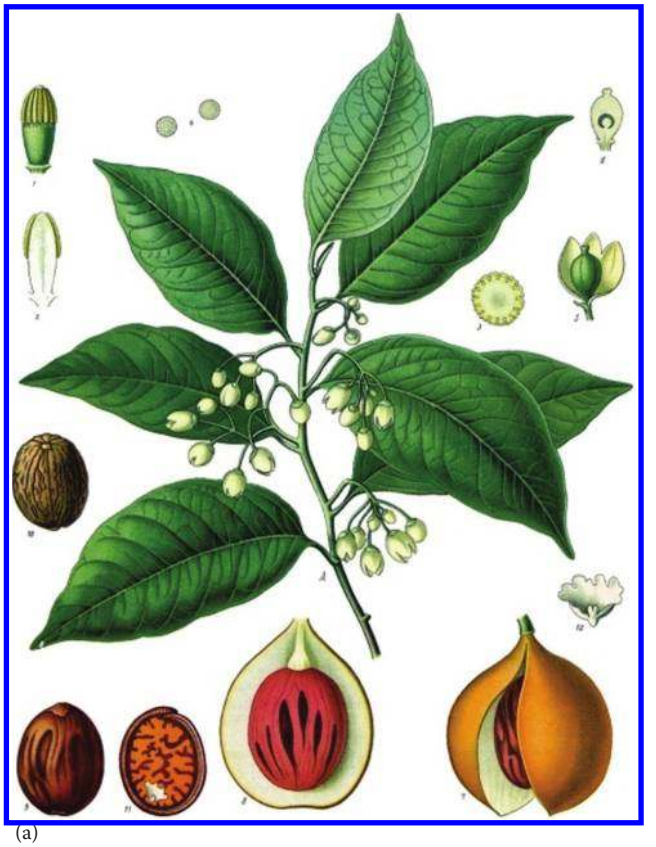
to its serotonergic actions, 2C-B has been shown to act as an α_1-receptor agonist. In animal experiments, its administration is associated with smooth muscle contraction in rat aorta suggesting that 2C-B could cause vasospasm and that regular use could lead to vascular remodeling (Lobos et al., 1992). This could explain why there are no reported deaths from 2C-B abuse, but there are reports of cerebral vasculitis and stroke (Lee et al., 2003; Drees et al., 2009; Ambrose et al., 2010).

A derivative of 2C-B is called 2-C-BFLY (Figure 4.11). It is another phenethylamine analog, one of a series of drugs known as the FLY compounds. The *FLY* designation allegedly derives from the two *winglike* furan or dihydrofuran rings that are fused on the opposite sides of the central benzene ring, producing an insect-like appearance with the bromine at the head and the ethylamine or isopropylamine group as the tail. FLY is occasionally encountered in crime labs but the incidence of use is not known. All the members of the FLY family are alleged to be potent hallucinogens, but virtually nothing is known about the extent of use, metabolism, or toxicity (Reed and Kiddon, 2007). As yet this drug is not scheduled in the United States, but it may yet be banned because it appears to fall under the scope of the Federal Analogue Act.

4.3.2.6 Nutmeg

Myristica fragrans Houtt. is a tall tropical evergreen tree, the fruit of which contains a large central seed, called the nutmeg, which is used as a spice (Figure 4.12). It has been used as a hallucinogen since the Middle Ages (Beck and Morgan, 1986). Even though it does not contain any amphetamines, and in spite of the fact that none of its components (elemicin, myristicin, and safrole) are converted to amphetamines, they seem to produce much the same effect and are included here with the other substituted amphetamines (Beyer et al., 2006). Safrole is the preferred, but strictly controlled, precursor used to manufacture MDMA, even though it is not itself psychoactive.

Reports of nutmeg intoxication are rare (Lavy, 1987; Demetriades et al., 2005; Forrester, 2005) and reports of death rarer still. Intoxication produces a typical anticholinergic syndrome. Common presenting complaints are hallucinations, palpitations, and feelings of impending doom (Abernethy and Becker, 1992). After the ingestion of a single 400 mg



(a)

(b)

Figure 4.12 Nutmeg: (a) appearance of plant, (b) nutmeg fruit. (From Wikipedia.)

dose of myristicin (still believed by many to be the hallucinatory component of nutmeg), onset of symptoms occurred in 1–2 h and resolved within 24 h. This dose of myristicin is equivalent to the amount of myristicin present in 40 g of nutmeg (Truitt, 1967).

Recent studies show that nutmeg contains a previously unrecognized furan with the formula $C_{13}H_{18}O_3$ and it may be that this compound shares a common method of action with some of the newly discovered FLY compounds (see Section 4.3.2.5). Other nutmeg constituents have been shown to be inhibitors of CYP3A4 and CYP2C9 activity (Kimura et al., 2010), but whether this effect has anything to do with nutmeg's hallucinogenic properties is not known. No clinical test would indicate the presence of any nutmeg constituent, and it is unlikely to be detected in postmortem samples, presupposing, of course, that nutmeg is ever the cause of death (Forrester, 2005).

4.3.2.7 4-Chloro-2-5-Dimethoxyamphetamine

According to Shulgin and Shulgin (1991), the three halo-amphetamine derivatives of this compound all share roughly the same potency; however, there are no controlled studies. All of the derivatives have long half-lives, but little else is known about them. They can be smoked or insufflated. A normal average dose of DOC is said to range from 1.5 to 3.0 mg. Doses as low as 10 mg have been known to cause restlessness leading to stupor. Onset of drug effect requires 1–3 h, with a peak effect and plateau at 4–8 h, followed by a gradual resolution.

Some users may still remain symptomatic 24 h after taking the drug. As with many of the drugs in this group, the mechanism of action is not known, but as with DOB and DOI, its effects seem to be mediated by a partial agonist activity at the 5-HT_{2A} serotonin receptor and by a high binding affinity for the 5-HT_{2B} and 5-HT_{2C} serotonin receptors (Shulgin and Shulgin, 1991). DOC is a potent CYP2D6 inhibitor (Ewald and Maurer, 2008), and since this enzyme is involved in the metabolism of most xenobiotics, the potential for multiple drug interactions exists, though none has been demonstrated. DOC is excreted in the urine but only one case has ever been reported (Ovaska et al., 2008) and, in any event, it would not be detected by routine clinical testing.

This molecule (Figure 4.13) is unscheduled in the United States, but it is likely that it would be considered an analog (of DOB), in which case sale for human consumption or possession with the intent to ingest could be prosecuted under the Federal Analog Act. DOC is scheduled in many other countries including Canada, Germany, New Zealand, Sweden, and the United Kingdom. In the United States, the other analogs, including 2,5-dimethoxyamphetamine, DOB, and DOM, are schedule I controlled substances. This analog is sometimes misrepresented as LSD.

4.3.2.8 2,5-Dimethoxy-4-Iodophenethylamine (Figure 4.14)

As with all amphetamine analogs that possess a chiral center, the *d* isomer of this drug is more potent than the *l* isomer. In common with the other members of this group, this drug is a potent 5-HT_{2A} serotonin receptor agonist. 5-HT_{2A} receptors are expressed at

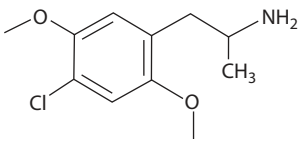


Figure 4.13 4-Chloro-2,5-dimethoxyamphetamine molecule.

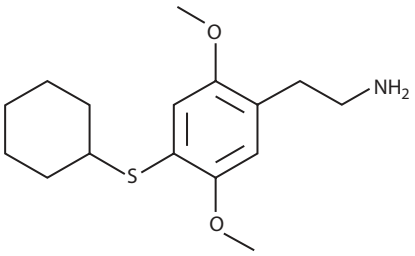


Figure 4.14 2,5-Dimethoxy-4-iodophenethylamine.

an especially high density in the frontal cortex. Stimulation of these receptors affects working memory, cognitive processes, and affective disorders such as schizophrenia. Stimulation of 5-HT_{2A} receptors appears to be the shared mechanism of action for all hallucinogenic drugs (Vollenweider et al., 1998; Marek et al., 2000; Williams et al., 2002), and studies with radioactively labeled 2,5-dimethoxy-4-iodophenethylamine (INBMeO) have shown that INBMeO selectively acts at 5-HT_{2A} receptors located in the frontal lobe (Nichols et al., 2008).

The potential for serious toxicity exerted by these drugs was not recognized until recently, and use appears to be more widespread than many had thought. In 2014, the Texas Poison Control Center described its experience with 25 NBOMe exposures in 2012 and 2013; 76% involved 25I-NBOMe, 12% 25C-NBOMe, and 12% an unknown NBOMe. Eighty-eight percent of the patients were male; the mean age was 17 years (range, 14–25 years). The exposure route was 72% ingestion alone, 12% inhalation alone, 4% ingestion and inhalation, and 12% unknown route. The most common clinical effects were tachycardia (52%), agitation (48%), hallucinations (32%), hypertension (32%), confusion (24%), and mydriasis (20%). Two patients died (Forrester, 2014).

Two deaths and two episodes of severe toxicity almost certainly related to INBOMe have recently been reported, and the descriptions strongly suggest that INBOMe produces a recognizable pattern of symptoms (Tang et al., 2014; Waltersheid et al., 2014). At least one, and possibly more, of the decedents described by Waltersheid et al. thought they were taking LSD. One, aged 21 years, experienced the onset of a sudden violent rage and subsequently became unconscious. Except for multiple contusions and abrasions sustained while he was agitated, the autopsy was unremarkable. The other man was at a rave party when he abruptly became violent and agitated, following much the same pattern as the first man. Routine toxicological testing disclosed only marijuana in the younger of the two (ages 21 and 15 years), but time-of-flight mass spectrometry revealed the presence of 25I-NBOMe in both men. The findings were further confirmed by tandem mass spectrometry (Waltersheid et al., 2014).

At nearly the same time the first two deaths were reported, a report describing two cases of serious toxicity was published (Tang et al., 2014). The two cases involved males aged 17 and 31 years. They had ingested drugs labeled as *NBOMe* or *Holland film*. Within minutes, they developed confusion, agitation, hypertension, tachycardia, hyperthermia, sweating, and dilated pupils. More importantly, they also experienced grand mal convulsion, rhabdomyolysis, and grossly abnormal liver function. Nonetheless, both men survived with only symptomatic support, primarily benzodiazepines. Urine samples from both patients were analyzed using liquid chromatography–tandem mass spectrometry (LC–MS/MS) following glucuronidase digestion and solid-phase extraction. Identification was based upon

comparison of the retention time and enhanced product ion scan with reference standards. In both urine samples, 25B-NBOMe was detected. Additionally, 25C-NBOMe was identified in one of the urine samples.

4.3.2.9 Analytic Methods

These designer amphetamine-like substances require MS methods for detection since, with the possible exception of PMA, cross-reactivity with standard amphetamine or methamphetamine immunoassays would not be expected.

Chromatographic-based analyses that are adequate to detect the known hallucinogenic stimulants include GC–MS methods involving derivatization with acetic anhydride (Kudo et al., 2007) or heptafluorobutyryl anhydride (Peters et al., 2003). Tandem LC–MS methods tend to require less sample preparation (mixed-mode solid-phase extraction or liquid extraction) and can be adapted to detect these and other stimulants and indeed other psychoactive drugs (Wohlfarth et al., 2010).

4.4 Hallucinogenic Amphetamines

4.4.1 MDMA (Table 4.5)

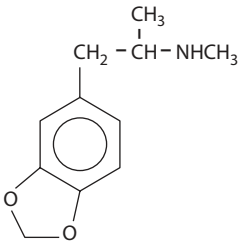
4.4.1.1 History

Merck's historical archive, located in Darmstadt, Germany, contains documents indicating that MDMA was first produced in 1912 and named *Methylsafrylamin*. Merck applied for a patent, but did so only to protect MDMA as a precursor chemical for another product, styptic hydrastinine. MDMA was never intended for use as an appetite suppressant. There is also evidence that Merck scientists did not perform any serious or fundamental pharmacologic testing on MDMA (which they had renamed *Safrylmethylamin*) until 1927. These tests were halted for economic reasons but some additional testing was done in the 1950s and then abandoned (Bernschneider-Reif et al., 2006). Later in the 1950s, the U.S. Army contracted with a group of researchers at the University of Michigan to perform MDMA toxicity studies. The results of the Michigan study remained classified until 1973, when they were finally released. The studies showed that MDMA was somewhat less toxic than MDA but more toxic than mescaline (Hardman et al., 1973). MDMA was classified as a schedule I drug in 1985.

MDMA causes the release of dopamine, 5-HT, and norepinephrine within the central nervous system. There is also evidence that it interferes with 5-HT reuptake transporters (Eisner, 1989; Green et al., 2003). Indeed, the results of studies with *knockout* mice rather convincingly demonstrate that transporter-mediated norepinephrine release has a critical role in the cardiovascular and stimulant-like effects of MDMA in humans (Hysek et al., 2011). All of these compounds have an effect on behavior, and in the case of MDMA, the result is said to be increased empathy; hence, it was described an *empathogen* or *entactogen* (from the Greek *en*, meaning "inside," which can also mean "to produce," and the Latin *tactus*—"touch") (Nicholas, 1986).

MDMA is immensely popular in Europe, where the number of MDMA tablets seized increased from less than 500,000 in 1997 to more than 30 million in the year 2000 (Interpol, 2006). However, since that peak was reached, use and availability have been decreasing steadily. This is thought to be a consequence of restrictions on the importation of MDMA

Table 4.5 Physiochemical Properties and Pharmacokinetics of 3,4-Methylenedioxymethamphetamine

Chemical name	*N*-α-dimethyl-1,3-benzodioxole-5-ethanamine or 3,4-methylenedioxymethamphetamine or 1-(1,3-benzodioxol-5-yl)-*N*-methylpropan-2-amine
Physiochemical properties, structure, and form	Soluble in water, also available as hydrochloride salt CAS 69610-10-2 (base) MW 193.25 pK_a 8.7 V_d 3–7 L/kg 
Synonyms	Adam, Ecstasy, E, M&M, MDM, XTC
Pharmacokinetic parameters	C_{max} 0.2 mg/L after 100 mg p.o. T_{max} 1–2 h after p.o. $T_{½}$ 6–10 h
Common blood concentrations in drug users	Up to about 0.5 mg/L
Metabolism and metabolites	*N*-demethylated form (MDA, also active), ring opening monohydroxy (HMMA, HMA), dihydroxy forms, and their conjugates
Urinary excretion	MDMA ~ 25%, MDA ~ 1%, HMMA 23%, 3,4-diOH-methamphetamine (20%)
Postmortem artifacts	Increases in concentration occur postmortem
Interactions	With other serotonin-active drugs (tramadol, SSRI, SNRI, MAOI) and drugs that require CYP2D6 for metabolism
Key papers	de la Torre et al. (2004), Kolbrich et al. (2008a), Abraham et al. (2009), Mueller et al. (2009), Meyer and Maurer (2010), Turillazzi et al. (2010), and Pilgrim et al. (2011)

precursors. Importation of the most important precursors is regulated by international treaties, but compliance has always been an issue. According to the UN, Chinese control efforts have significantly diminished production (UNODC, 2014). Consumption, at least as gauged by dosage units, peaked in 2008 at 18.3 million doses, dropping to just over 15 million in 2010 (U.S. Department of Justice, 2011). This downward trend seems likely to continue as more and more alternative *designer* products come to market (see Figure 4.15 for MDMA seizures) (DEA, 2006; U.S. Department of Justice, 2011).

4.4.1.2 Incidence and Epidemiology

No MDMA-related deaths are listed in the DAWN report for 1999 (Kissin et al., 2000). The emergency room component of the most current DAWN report lists 8621 MDMA-related visits (CI 5,985–11,257). Whether any were fatal is not known (SAMHSA, 2006). The last reliable medical examiner component to be issued by DAWN was for 2002, and it does not mention MDMA. The situation is far different in Europe and Asia. In a 2006 U.K.

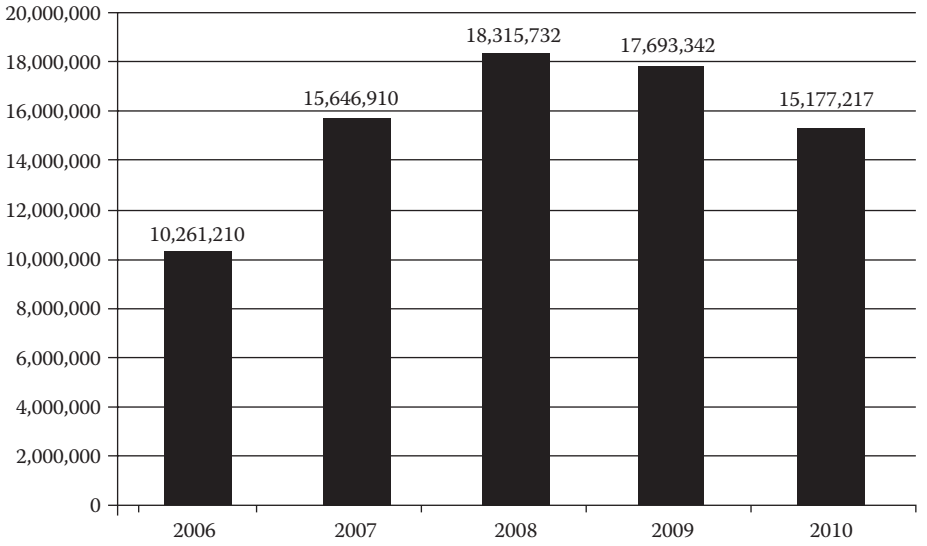


Figure 4.15 3,4-Methylenedioxymethamphetamine seizure amounts in the United States, in dosage units, 2006–2010. (Compiled from National Seizure System data, run date November 5, 2010.)

survey, based upon medical death certificates, coroners' reports in the United Kingdom tended to underestimate the problem of MDMA, MDA, and MBDB deaths occurring from 1994 to 2003 found a total of 394 deaths, although suicides are rare and deaths are almost always accidental. In 42% of the deaths identified, MDMA was the only drug detected. All of the other deaths occurred in polydrug abusers, making causality assessment all but impossible. The study also found that the number of MDMA-related deaths appeared to be increasing yearly, but that state of affairs does not appear to obtain in the United States, where MDMA deaths are uncommon.

4.4.1.3 Illicit Production

Safrole, the active ingredient in nutmeg, can be used to prepare the starting ketone (3,4-(methylenedioxy)phenylpropanone) by oxidization with hydrogen peroxide in an acid medium. Safrole can no longer be bought without authorization, though it is still available via the Internet. If safrole can be obtained and oxidized, it is then combined with methylamine in alcohol. Aluminum powder, freshly treated with mercuric chloride in ethanol, is added to the mixture, which is then boiled for several hours. MDMA is then distilled off under pressure (Verweij, 1991a, b). The final product is compressed into a tablet that is usually embossed with a logo (Figure 4.16). Other routes of synthesis are possible.

In Southeast Asia, MDMA is made by reductive amination of 3,4-methylenedioxyphenylacetone (MDP2P or PMK) with methylamine and sodium borohydride. Gassing an acetone solution of the free base with commercial hydrochloric acid gas produces the hydrochloride salt. The amphetamine is then synthesized via the Leuckart reaction and crystallized as the sulfate salt. Some of these laboratories may produce a remarkable amount of drug. There is evidence that the same synthetic route is favored in Europe as well as the United States and Asia (Palhol et al., 2002).

In 2005, a laboratory seized outside of Jakarta was found to be producing 60–90 kg of MDMA per batch, corresponding to 428,000–642,000 tablets/day, based on a standard

Figure 4.16 Tablets confiscated from a megalab located outside of Jakarta. Tablets are colorful and tend to carry logos, but the logo is no guarantee of quality. Forensic labs have noticed that the percentage of 3,4-methylenedioxymethamphetamine (MDMA) contained in each tablet tends to decrease with time and that the MDMA is replaced with other drugs, especially methamphetamine. (From the website of the DEA.)

dosage unit of 140 mg of MDMA per tablet. In 2005, the DEA discovered large quantities of *Ocotea cymbarum* (also referred to as Brazilian sassafras, *O. cymbarum* oil, *O. cynbarnum*, *O. cymbarium*, and oil of *Ocotea*) at several clandestine MDMA laboratories located in the northeastern United States. *O. cymbarum* oil is made by distilling the trunk bark of a tropical tree native to Brazil, Colombia, and Paraguay. The distillate typically contains between 80% and 94% safrole (see Section 4.5.5) plus small amounts of MDA. Sales of *O. cymbarum* are not controlled.

O. cymbarum oil is available via the Internet, even at online auction sites, and also through chemical, aromatherapy, and perfume companies, but most of the product is diverted to illicit MDMA production. *Ocotea* is also occasionally diverted from legitimate domestic businesses such as the manufacture of fragrances, flavoring agents, and insecticides. The DEA estimates that a 5 gal drum of *O. cymbarum* oil, if used correctly as a precursor, would yield an estimated 49,000–108,000 tablets containing 120 mg of MDMA each. Much of the MDMA available in the United States is still produced in clandestine laboratories located in the Netherlands and Belgium. MDMA is then transported from Europe to the United States by couriers on commercial flights, via mail and package delivery services, and even by air cargo and maritime vessels.

Once MDMA is produced, it is converted into tablets, each with a recognizable logo (see Figure 4.16). The tablets quickly develop names based upon the imprinted logo. Brand loyalties evolve, and users ask for particular tablets by name (tablets with the Mitsubishi label were once particularly popular, with a very loyal following). Some of the logos often have whimsical themes, ranging from an imprint of McDonald's Golden Arches to the Rolex trademark symbol, the Mercedes symbol, and even the skull and crossbones. A recognized logo is, however, no guarantee of quality, safety, or purity. Once the makers have developed a following for a pill with a particular logo, they then begin substituting cheaper ingredients in the pill such as methamphetamine or even PMA (a much more dangerous drug than MDMA) (Lora-Tamayo et al., 2004).

4.4.1.4 Metabolism

At least 16 different MDMA-related compounds have been discovered. Part of the reason for proliferation of metabolites is this drug's complex metabolism, which involves two main metabolic pathways: (1) *O*-demethylation is followed by catechol-*O*-methyltransferase (COMT)–catalyzed methylation and/or glucuronide/sulfate conjugation; and then (2) there is *N*-dealkylation, deamination, and oxidation to the corresponding benzoic acid derivatives, which are conjugated with glycine (de la Torre et al., 2004). Given that the polymorphic enzyme CYP2D6 partially regulates *O*-demethylation, one might suppose that individuals with the poor metabolizer phenotypes might be at higher risk of acute toxicity, and there is some evidence to support this notion. When volunteer extensive CYP2D metabolizers were given two doses of MDMA over a 2-week period, CCYP2D inhibition did occur, but only in women. It is speculated that this difference may account for the fact that after using MDMA, women require psychiatric care at a much higher rate than men (Yubero-Lahoz et al., 2011). However, it is important to note that after chronic use, all subjects, independent of genotype, phenotypically become poor metabolizers (de la Torre et al., 2004).

Except perhaps for mood disorder in women, there is no evidence that *poor CYP2D6 metabolizers* are more prone to manifest acute toxicity, unless MDMA is used with other serotoninergic-active drugs such as tramadol, moclobemide, and the serotonin reuptake inhibitor antidepressants (Pilgrim et al., 2011). The possibility also exists that MDMA-induced CYP2D6 inhibition could enhance the toxicity of other drugs (Moeller and Kraemer, 2002; Schifano, 2004). CYP2D6 is not the only polymorphic enzyme involved in MDMA metabolism.

COMT activity is also key to MDMA metabolism. It plays a critical role in determining the fraction of MDMA that is converted to potentially neurotoxic metabolites. Thus, CYP-mediated demethylation of MDMA or its *N*-demethylated metabolite, 3,4-(±)-methylenedioxyamphetamine, gives rise to the catechols *N*-methyl-α-methyldopamine and α-methyldopamine, respectively. Methylation of these catechols by COMT limits their oxidation and conjugation to glutathione, a process that ultimately gives rise to neurotoxic metabolites (Herndon et al., 2014).

The tendency for mood disorders in women is almost certainly a consequence of COMT polymorphisms. In a controlled study of human volunteers, it was clearly demonstrated that female subjects displayed more intense physiological (heart rate and oral temperature) and more negative effects (dizziness, sedation, depression, and psychotic symptoms) than the males. The difference appears to be largely accounted for by a functional COMT polymorphism at codon 158 producing a valine (val) to methionine (met) substitution (Val158Met, rs4680), resulting in three genotypes (val/val, val/met, and met/met). Individuals with the *met* allele have a lower enzyme activity, which leads to higher levels of extracellular dopamine (Pardo-Lozano et al., 2012). There is an expanding body of evidence that suggests women are more likely to develop MDMA-associated hyponatremia (see Section 4.4.1.5) because of low-activity polymorphisms of both CYP2D6 and COMT (Aitchison et al., 2012).

The half-life of MDMA is somewhere between 4 and 7 h, but the greater the dose administered, the longer the half-life. In other words, the more drug that is taken, the more slowly it is broken down. The half-life for MDA is substantially longer than that of the parent compound, with peak plasma concentrations that are only one-tenth as great.

MDA itself is psychoactive. Illicitly produced MDMA is a racemic mixture, and clinical evidence indicates that the different enantiomers are metabolized at different rates (Moore et al., 1996). Enantiomeric effects on tissue distribution are known, and it is clear that the racemic form of the molecule has an enormous impact on drug distribution.

4.4.1.5 Clinical Syndromes

The most feared complications of MDMA use are serotonin syndrome (now increasingly referred to as *serotonin toxicity*), hyperthermia with rhabdomyolysis, and hyponatremic encephalopathy. The first disorder is characterized by the rapid onset of confusion, diaphoresis, diarrhea, increased muscle tone, and cardiac arrhythmias. There may also be shivering, myoclonus, and increased deep tendon reflexes.

There is no laboratory test for serotonin syndrome; therefore, diagnosis is by symptom observation and investigation of the patient's history. There are two competing definitions of the syndrome. The first set of criteria was introduced in 1991 by Harvey Sternbach, a professor of psychiatry at UCLA. However, researchers in Australia later developed a system known as the Hunter serotonin toxicity criteria, which seem to have better sensitivity and specificity, 84% and 97%, respectively, than the original criteria proposed by Sternbach (Ables and Nagubilli, 2010).

Serotonin syndrome in its most severe form leads to hyperthermia, rhabdomyolysis, multisystem failure, and death. Risks for developing the syndrome are higher in individuals taking SSRI and monoamine oxidase inhibitor (MAOI) drugs. Most adult drug users take multiple drugs, and it is worth remembering that many of these other drugs may have weak, but quite real, SSRI and MAOI activity (methadone, tramadol, dextromethorphan, and meperidine).

Hyperthermia can also occur in the absence of other obvious symptoms classically associated with serotonin syndrome, occasionally with lethal outcome. In humans, MDMA increases core body temperature regardless of ambient temperature, and it also causes substantial increases in metabolic rate (Freedman et al., 2005). The results of animal studies suggest that the higher the ambient temperature when MDMA is ingested, the greater the increase in metabolism that results. Even in the absence of overt evidence of significant hyperthermia, MDMA (and amphetamines in general) can induce brain hyperthermia. Just how great an increase occurs depends upon an individual's state of activity and on prevailing environmental conditions. MDMA seems to exacerbate drug-induced hyperthermia via mechanisms that are still not fully understood (Kiyatkin and Bae, 2008).

Laboratory results suggest that MDMA has the ability to uncouple skeletal muscle mitochondria (leading to heat generation); in combination with hypothalamic–pituitary–adrenal activation, body temperature increases via a series of complex neurologic mechanisms (Hargreaves et al., 2007). Just as recent animal studies suggest that MDMA produces more hyperthermia when it is used in a warm environment than in a cold one (Brown and Kiyatkin, 2004), so too does some of the human experimentation. In one of the few controlled human studies ever performed, the physiological and subjective effects of MDMA were measured in 18 volunteers under both cold (18°C) and warm conditions (30°C). MDMA produced significant elevations in core body temperature under both conditions, even without any exercise. The metabolic rate in both warm and cold conditions also increased, and significant elevations in blood pressure and heart rate were noted (Freedman et al., 2005). MDMA increased core body temperature regardless of

ambient temperature. In short, MDMA causes heat to be produced while, at the same time, preventing its dissipation.

In addition to hyperthermia and rhabdomyolysis, all of the usual complications also occur. The other dangerous consequence of MDMA ingestion is the so-called Ayus–Arieff syndrome. Hyponatremia produces cytotoxic cerebral edema, which in turn leads to a neurogenic pulmonary edema, and the pulmonary edema leads to hypoxia, which impairs brain cell volume regulation and results in a vicious circle ending in encephalopathy (Ayus et al., 1992). The true incidence of this syndrome is not really known, but over 25 reports of Ecstasy-associated hyponatremic encephalopathy have appeared in the literature in the literature, and over half of them are fatalities. Almost all cases are reported in young females between the ages of 15 and 30, with a serum sodium of ≤130 following the ingestion of just one dose of Ecstasy (Moritz et al., 2013).

Nearly all of the usual complications associated with amphetamine abuse have, at one time or another, been seen with MDMA abuse. There have been reports of cerebral sinus thrombosis, subarachnoid and intracerebral hemorrhage (De Silva and Harries, 1992; Gledhill et al., 1993), and even aplastic anemia (Marsh et al., 1994). The occasional chronic MDMA user may develop compartment syndrome when attempting to inject crushed tablets into the femoral or other major vessels (Swan et al., 2006). Pneumothorax and pneumomediastinum have also been reported in conjunction with MDMA use, but would not normally be expected (Rejali et al., 2002).

When intracranial bleeding does occur, it is usually as a consequence of a preexisting malformation such as undiagnosed aneurysm or arteriovenous malformation (Hughes et al., 1993; Auer et al., 2002; Drees et al., 2009). Several reports of cerebral infarction were published in the early 1990s (Manchanda and Connolly, 1993; Hanyu et al., 1996); few new cases have been published recently (Goldstein and Mordish, 2006). Given the very great number of users today and the paucity of new cases, intracerebral infarction or hemorrhage should not be high in the differential diagnosis. Similar considerations apply to the one report of MDMA-related spongiform encephalopathy (Bertram et al., 1999), where the victim may have used other drugs in the past.

Finally, myocardial hypertrophy has been documented as a complication of regular MDMA use (Patel et al., 2005). Hypertrophy has been recognized as a complication of cocaine use for years (Karch et al., 1995) although it was only recently that the mechanism became apparent—cocaine combines with myocyte DNA leading to increased production of calmodulin kinase II, leading, in turn, to elevated concentrations of intracytosolic calcium with subsequent myocyte hypertrophy (Henning and Cuevas, 2006). Both abnormalities favor the occurrence of sudden cardiac death (Ruan et al., 2007).

4.4.1.6 Interpretation of Blood and Tissue Concentrations

Postmortem measurements are unreliable indicators of toxicity. One reason may be that MDMA is usually sold as a racemic mixture. The *S*-enantiomer is metabolized faster than the *R*-enantiomer (roughly 5 vs. 15 h), and both of the isomers have different pharmacologic properties (Brunnenberg and Kovar, 2001). The fact that there is complete overlap in blood concentrations between users who are experiencing symptoms and those who are not (Henry et al., 1992) further complicates the picture as is true for all abused stimulants. Drug concentrations in MDMA fatalities may be extremely high, often as much as four or five times higher than concentrations measured in controlled studies with doses of MDMA comparable to those used by *recreational* users. This is because MDMA has a high

volume of distribution (>5 L/kg) and partly because it undergoes postmortem redistribution that is (as for most drugs) site dependent. When the origin of the blood sample is not specified, interpretation of concentrations should not be attempted.

MDMA postmortem redistribution is described in a paper by Elliott, published in 2005 (Elliott, 2005). He analyzed multiple tissues taken from five MDMA users who were treated at the hospital just before they died, so that both admission and postmortem blood samples were available. Admission MDMA concentrations ranged between 0.6 and 4.3 mg/L, while those of MDA ranged from 0 to 0.1 mg/L in antemortem serum/plasma. Postmortem blood samples of MDMA and MDA from the same individuals ranged from 0.5 to 28 mg/L and 0.02 to 1.3 mg/L, respectively. In every single case, the postmortem values were higher than the antemortem values. Selected values from this study are shown in Table 4.6.

In one case, the MDMA concentration in femoral blood was 2.8 mg/L, while the concentration measured at the same time in *heart* blood was 9.1 mg/L. Although not shown in Table 4.6, the concentrations in the brain were even higher: 10 mg/kg in the medulla and 14 mg/kg in the cerebellum. Still another factor that complicates postmortem interpretation is the presence of MDA. It is produced as a minor metabolite of MDMA, but it is also manufactured and sold in its own right (see Section 4.4.2). Determining the origin of the MDA can usually be accomplished by looking at the other drugs found in the gastric contents. If MDA is found in isolation at significant concentrations, that is good evidence that its presence has nothing to do with MDMA degradation.

MDMA blood concentrations in recently published case series seem to be higher than in cases described in the early 1990s. Table 4.7 is composed of data from a 2006 paper on MDMA use in Taiwan. In another study, MDMA concentrations in five patients who survived serious bouts of toxicity were said to have ranged from 200 to 970 ng/mL, while

Table 4.6 Selected Values from 3,4-Methylenedioxymethamphetamine Postmortem Redistribution Study by Elliott

	MDMA/MDA	MDMA/MDA
Specimen	Case 4	Case 5
Antemortem serum	4.33	1.08
PM, L femoral	7.25/0.21	NA
PM, R femoral	6.19/19	2.6/.02 (side?)
PM, heart	28.39/1.33	NA

Source: Adapted from Elliott, S.P., *J. Anal. Toxicol.*, 29(5), 296, 2005.

Table 4.7 Postmortem 3,4-Methylenedioxymethamphetamine Concentrations

Matrix	Highest	Range	Mean SD	SD	Mean ± 2 SD
Urine (n = 10)	67.115 μg/mL	<0.011–0.174	0.061	0.049	0.038–0.160
Bile (n = 8)	130.952 μg/mL	<0.011–0.146	0.057	0.045	0.000–0.147
Gastric (n = 12)	40.515 μg/mL	<0.004–0.463	<0.004–0.046	0.130–0.086	0.000–0.346
Heart (n = 15)	40.412 μg/mL	<0.014–0.045	0.069	0.053	0.000–0.174
Antemortem urine (n = 22)	33.31 μg/mL	0.128–0.211	0.101	0.052	0.000–0.205
Hair (n = 6)	55.91 ng/mg	0.128–0.211	0.160	0.032	0.096–0.224

Source: Data adapted from Liu, R.H. et al., *J. Anal. Toxicol.*, 30(8), 545, 2006.

concentrations in five car-accident victims varied from 50 to 340 ng/mL (Henry et al., 1992). Several authors have reported plasma concentration measurements in suspects arrested for driving while intoxicated. The concentration ranges are so wide as to be nondiagnostic, ranging from less than 50 ng/mL to nearly 0.6 mg/L, and as high as 2.1 mg/L in one traffic fatality attributed to MDMA; it appears that in all of these cases, MDMA was almost always coingested with other drugs. Nonetheless, elevation in postmortem concentrations of MDMA and MDA should always be expected. Furthermore, the degree of elevation cannot be reliably predicted, except to say that blood collected from the heart is always likely to have higher concentrations than blood from peripheral sites.

Postmortem MDMA measurements bear no predictable relationship to antemortem values (Gamma, 2004; Elliott, 2005). It also bears repeating that MDMA has two stereoisomers and that both chiral forms have different volumes of distribution and different activity. It is entirely possible that a reported MDMA plasma concentration may not reflect the presence of any psychoactive MDMA in the plasma. The possibility always exists that the presence of MDMA may just be an incidental finding. Dowling et al. (1987) described one asthmatic with a blood MDMA concentration of 1.1 mg/L, but his severe chronic lung disease was the cause of death, not his drug use. MDMA users are very likely to be using more than one drug (see Section 4.4.1.5); if death is to be attributed to MDMA, then at least some plausible mechanism, such as water intoxication or hyperthermia with rhabdomyolysis, should be demonstrable.

4.4.1.7 Neurotoxicity

Like other amphetamines, MDMA acts on the heart and the central nervous system to cause release of catecholamines (including 5-HT) and prevent their reuptake. Neurotoxicity in animals is manifested by damage to serotonergic neurons. In the rat model, even one dose of MDMA causes degeneration of 5-HT-containing neurons, but animals treated with massive and repeated doses of MDMA eventually recover and at 1 year after treatment have no apparent lesions (Battaglia et al., 1987).

Interspecies variation in the response to MDMA is considerable. The monkey is much more sensitive to the 5-HT-depleting effects of MDMA than is the rat (Ricaurte et al., 1985), while the mouse experiences dopamine-related changes without any alteration of the serotonergic system. Baboons given substantial doses of MDMA had little or none in their plasma but had high plasma concentrations of 3,4-dihydroxymethamphetamine, pointing to much more extensive first-pass metabolism of MDMA in baboons than in humans, an effect that cannot be ignored (Mueller et al., 2011). There are other differences as well. The brains of animal models never demonstrate any evidence of gliosis, a change that is considered an important anatomic marker for brain tissue damage in man. It is an open question whether the findings in animal models can reliably be extrapolated to human beings (Burgess et al., 2000; Curran, 2000; Turner and Parrott, 2000).

In 2002, a paper was published claiming to have demonstrated that multiple studies, all done at the same laboratory and conducted over a span of approximately 2 years, had shown that MDMA produced not only serotonergic neurotoxicity, but that in addition, it caused severe dopamine neurotoxicity in two different nonhuman primate species (Ricaurte et al., 2002). This conclusion was met with disbelief by most of the research community, and the authors subsequently retracted the paper (Ricaurte et al., 2003). The authors were unable to reproduce their original research, leading them to investigate their laboratory records. They discovered that in the controversial studies they had published,

methamphetamine (which does damage dopaminergic neurons) had been mistakenly used instead of MDMA (Ricaurte et al., 2003). No one now suggests that MDMA has the potential to damage dopaminergic cells, and this now seems to be a dead issue best forgotten.

It has not been possible to recreate the brain lesions observed in humans. The results of functional MRI imaging show no evidence of injury (de Win et al., 2008), but they do show that long-lasting increases in cortical excitability occur, possibly through loss of serotonin input to cortical and subcortical regions. Some have even suggested that functional magnetic resonance imaging (fMRI) demonstration of cortical hyperexcitability may be a biomarker for MDMA-induced serotonin neurotoxicity (Bauernfeind et al., 2011). Other recent studies have shown decreased cerebral cortical serotonin transporter binding in chronic MDMA users (Kish et al., 2010).

Chronic paranoid psychosis has been reported on a number of occasions, and some recent fMRI findings suggest that MDMA-induced psychosis may be associated with hippocampal remodeling (Creighton et al., 1991; Schifano, 1991; Williams et al., 1993; Landabaso et al., 2002; Vecellio et al., 2003; Van Dam et al., 2008; Nifosi et al., 2009; Potash et al., 2009; Angelucci et al., 2010). The experience in Europe has been that MDMA abusers may present with a diverse group of psychiatric syndromes including, but not limited to, toxic psychosis (Morland, 2000). Nothing distinguishes the psychotic symptoms of MDMA users from those seen in individuals with any other type of toxic psychosis.

Even though there is a wealth of experimental evidence, much of it derived from PET and fMRI scanning of human volunteers, suggesting that MDMA is toxic to serotonergic neurons, there is no clinical evidence that humans ever develop the typical symptoms of 5-HT depletion (disorders of sleep, mood, appetite), and the most recent evidence suggests that depressive symptomology simply does not occur in occasional users (Falck et al., 2008). The fact that MDMA users are at risk for serotonin syndrome (Padkin, 1994; Parrott, 2001) suggests that 5-HT excess, not deficiency, is more of a threat.

Functional MRI has shown the brain areas that are significantly activated by low oral doses of MDMA; these include the midbrain raphe nuclei, hippocampus, hypothalamus, amygdala, and the corticostriatal circuit composed of the dorsal thalamus, sensory motor cortex, and basal ganglia. The onset of brain activation correlates well with the rise in plasma MDMA concentrations (measured in monkeys) (Meyer et al., 2006). Users of MDMA are also at increased risk for seizure activity. It is thought that seizure onset after MDMA is related mainly to its acute systemic effects (e.g., hyponatremia and hyperthermia as discussed earlier). However, additional mechanisms may be involved. Amphetamines exert profound effects on different monoaminergic systems, any one of which might participate in lowering the seizure threshold. Unfortunately, the chronic effects of MDMA abuse on seizure threshold really have not been explored (Giorgi et al., 2006).

4.4.1.8 Cardiovascular Toxicity

Modest oral doses of MDMA cause increases in heart rate (increases of up to 30 beats/min), blood pressure (average increase of 7 mm in diastolic pressure), and myocardial oxygen consumption. These increases are comparable to those induced by a dobutamine infusion of 20–40 mg/kg/min. However, unlike dobutamine, MDMA has no measurable inotropic effects (Lester et al., 2000). In the only large, controlled, postmortem study of MDMA cardiotoxicity, it was determined that heart weights were significantly higher in MDMA-related deaths than in controls (Patel et al., 2005). The results are comparable to the changes seen in the hearts of cocaine and methamphetamine abusers, which generally

are 10%–12% greater than predicted (Karch et al., 1998). In a 1996 report, Milroy et al. described the findings in seven MDMA-related fatalities. Hepatic damage was the most frequent abnormality (see Section 4.4.1.10), but one of the decedents, who was assumed to have suffered a sudden cardiac death, was also found to have myocardial fibrosis, a very common finding in methamphetamine abusers (Figure 4.17).

Some MDMA users have shown classic signs of amphetamine/catecholamine toxicity with hyperadrenergic symptoms including fever, tachycardia, and hypertension, as well as rhabdomyolysis, renal failure, and disseminated intravascular coagulation (Chadwick et al., 1991; Campkin and Davies, 1992). A report published in 1988 described an MDMA user, previously diagnosed with Wolff–Parkinson–White syndrome, with sudden cardiac death. At autopsy, in addition to the presence of an aberrant conduction pathway, myocardial fibrosis was also evident. It is impossible to say whether it was preexcitation or reentry, or both, that led to the individual's demise (Suarez and Riemersma, 1988).

One factor that favors reentry is that amphetamine analogs increase plasma 5-HT levels. This, at least in theory, could cause contraction of pulmonary arteries and/or stimulate mitogenesis in pulmonary artery smooth muscle cells (which would favor the occurrence of atrial fibrillation) (Zolkowska et al., 2006).

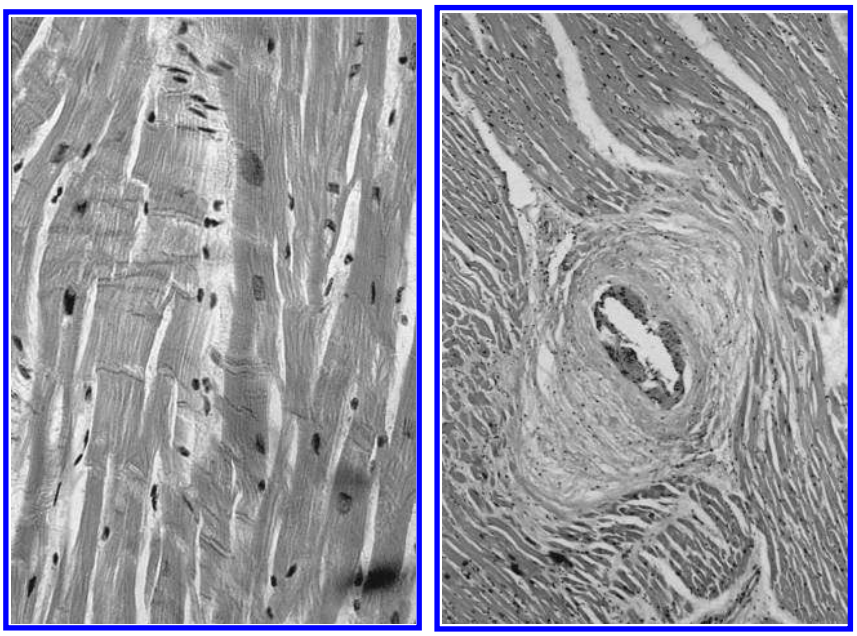


Figure 4.17 (a) Myocardium of a 28-year-old man with sudden cardiac death. He was thought to be a chronic 3,4-methylenedioxymethamphetamine user. Note areas of extremely intense contraction band necrosis. CBN is a common lesion in stimulant abusers because of their increased plasma catecholamine concentrations. (b) Zone of perivascular fibrosis from the same heart showing contraction band necrosis in (a). The combination of perivascular fibrosis, microvascular disease, and contraction band necrosis is nearly diagnostic for chronic exposure to high concentrations of catecholamines, which can only be explained by the presence of a pheochromocytoma or by chronic stimulant abuse. (From (a and b) H & E stain; courtesy of Professor Chris Milroy, Sheffield Forensic Centre, U.K.)

Two cases of aortic dissection in MDMA users have been reported (Duflou and Mark, 2000). Dissection is also a known complication of methamphetamine abuse, but the mechanism in these cases is simply not known. Special stains are generally unrewarding, and evidence of medial degeneration is conspicuously absent. Presumably, the MDMA-related cases and the methamphetamine-related cases share common mechanisms, but since so few episodes have been reported, it is impossible to say.

MDMA strongly binds to the 5-HT_{2B} receptor. It is known that chronic exposure to high levels of 5-HT, either from metastatic carcinoid tumors or the anorectic drug fenfluramine, are associated with proliferative disease and thickening of cardiac valves, mediated through 5-HT_{2B} receptors. Whether this applies to MDMA remains debatable, but in a recent controlled study of chronic MDMA users (29 subjects each with two age-matched controls), there was a significantly higher incidence of very mild tricuspid regurgitation (Droogmans et al., 2007).

4.4.1.9 Hepatotoxicity

Reports, mainly from Europe, continue to describe patients with severe hepatitis, sometimes with fulminant liver failure (Fineschi and Masti, 1996; Andreu et al., 1998; Schwab et al., 1999; Brncic et al., 2006; Shenouda et al., 2010). The etiology is not clear but the results of in vitro animal studies of liver mitochondria suggest cytotoxicity caused by MDMA is linked to mitochondrial failure (Nakagawa et al., 2009). While a great deal of laboratory work has been undertaken, almost all has been in rats, and it is not certain that these findings can be generalized to humans. Small animal studies have been shown to induce oxidative stress, lipid peroxidation, and TNF-α apoptosis in the rat liver. Put another way, regardless of the species, MDMA use leads to lipid peroxidation and reduced glutathione levels, that is, MDMA induces a state of oxidative stress in the liver (Ninkovic et al., 2004). The original finding has been confirmed in more recent studies. Other data suggest a possible association of specific human leukocyte antigen phenotypes and MDMA-induced hepatotoxicity (Brncic et al., 2006; Cerretani et al., 2011).

Liver failure might also be the result of hyperthermia and multiorgan failure, but the evidence is lacking. The only feature that MDMA users with liver failure have in common is that they tend to present with symptoms of liver damage indistinguishable from those of infectious hepatitis, with centrilobular necrosis and microvascular steatosis (Milroy et al., 1996).

4.4.1.10 Preanalytic Considerations and Analysis

MDMA and its main metabolite MDA are relatively stable molecules. Concentrations are unaltered after 12 h at ambient temperature and 2 days at 4°C (Scheidweiler et al., 2008). In urine, both substances are stable for up to 2 years when urine is stored frozen, but only 7 days when it is stored at 37°C (Jimenez et al., 2006). Similar stability results have been observed in water and urine (up to 21 weeks), serum (up to 17 weeks), and blood (up to 5 weeks) when stored at –20°C, 4°C, and 20°C (Clauwaert et al., 2001). In contrast, a small temperature-dependent loss in water and oral fluid occurred when stored for 10 weeks (Concheiro et al., 2005). Similar stability considerations are likely for most of the other hallucinogenic amphetamines (MDA, 3,4-methylenedioxyethylamphetamine [MDEA], PMA, etc.).

There are numerous methods published for the analysis of MDMA, MDA, and related amphetamines (MDEA, PMA, etc.). Immunoassay methods are commercially

available for urine, blood, and oral fluid analyses where antibodies are directed to *methylamphetamines*. These show generally good cross-reactivity to MDMA, while *amphetamine* kits allow detection of MDA (Stout et al., 2004; Laloup et al., 2005). Trazodone and its metabolite meta-chlorophenylpiperazine (mCPP) have been shown to cross-react with at least two Ecstasy/amphetamine kits (Anon., 2010; Logan et al., 2010; Baron et al., 2011).

Confirmatory analyses using mass spectral methods abound. These include GC–MS (Peters et al., 2005; Kolbric et al., 2008b; da Silva et al., 2010). Extraction techniques include conventional solvent extraction from basified specimen and mixed-mode solid-phase extraction methods. Increasingly popular are methods that allow a large number of similar compounds to be detected in one assay in a variety of matrices (Weinmann et al., 2000; Peters et al., 2003).

LC–MS/MS methods are also available and can be used to screen a range of related substances including other illicit drugs in blood, oral fluid, and even urine. A variety of extraction methods are used including solid-phase column extraction and protein precipitation (Cheze et al., 2007; Sergi et al., 2009; Bjork et al., 2010).

Chiral methods to separate parent and metabolites into the enantiomers are also available (Pizarro et al., 2003; Rasmussen et al., 2006).

4.4.2 3,4-Methylenedioxyamphetamine (Table 4.8)

MDA is produced as a minor metabolite of MDMA, but it is also manufactured and sold in its own right. It is frequently misrepresented as MDMA. Even moderate doses of MDA can produce marked sympathetic stimulation, with resultant tachycardia and hypertension (Gunn et al., 1939).

Table 4.8 Physiochemical Properties and Pharmacokinetics of 3,4-Methylenedioxyamphetamine

Chemical name	MDA, 1-(benzo[1,3]dioxol-5-yl)propan-2-amine, or 1-(1,3-benzodioxol-5-yl)propan-2-amine.
Physiochemical properties, structure, and form	Soluble in water, also available as hydrochloride salt. CAS 4764-17-4 (base). MW 179.22. pK_a 9.7.
Synonyms	Tenamfetamine, love drug, love pill, speed.
Pharmacokinetic parameters	C_{max}, after 100 mg MDMA, 13 ± 4 ng/mL; bioavailability not known.
Metabolism and metabolites	Likely to be ring opening monohydroxy, dihydroxy forms, and their conjugates.
Urinary excretion	About 1% when consumed as MDMA orally.
Postmortem artifacts	Increases in concentration are likely postmortem.
Interactions	No clinically significant interactions reported.
Key papers	Thiessen and Cook (1973), Kunsman et al. (1996), Maurer (1996), and de la Torre et al. (2004).

4.4.2.1 History

MDA has not always been used for recreational, or even medical, purposes. After World War II, intelligence agencies around the world tried to discover and develop chemical agents that might serve as *truth serums* or incapacitating agents. These agencies were sure that psychedelics might help them find truth. Drugs were given in various settings to knowing and unknowing subjects without their consent. One famous case occurred in 1953, when MDA was given to a psychiatric patient named Howard Blauer. The experiment proved fatal. The army had contracted with several physicians at the New York State Psychiatric Institute to explore new chemicals from the Edgewood Arsenal, and one of these, with a chemical warfare code number of EA-1298, was MDA. The last and lethal injection into Blauer was an intravenous dose of 500 mg (Shulgin and Shulgin, 1991).

MDA was also considered for possible commercial development by Smith Kline & French, under the code name SKF-5, trade name Amphedoxamine, which is said to be an effective anorectic. The drug enjoyed some popularity in the early 1960s before MDMA became widely available. MDA was even patented as an anorectic agent and as an antitussive (Lukaszewski, 1979), though it never saw commercial distribution. Today, it is important only because it is a major metabolite of MDMA. No evidence indicates that MDA is currently synthesized or sold in the United States or Europe.

4.4.2.2 Clinical Syndromes

MDA is a chiral compound, and according to Shulgin's unpublished studies, the *d* form is substantially more potent than the *l* form. Since chiral separation is rarely carried out in medical examiners' offices, MDA chirality can be a source of forensic confusion. The pharmacokinetics of MDA have never been seriously studied, and what is known about its pharmacokinetics is largely a function of the study of MDMA metabolism. Nonetheless, based upon the behavior of other chiral drugs (methadone comes to mind immediately), it seems likely that chirality has an effect on both MDA metabolism and the effects that it exerts on the subject. There simply is no way a pathologist (or toxicologist) can look at an isolated postmortem measurement and be sure he or she is looking at mostly active or mostly inactive drug.

The effects of a 150 mg dose of MDA peak at 1.5 h and may last for as long as 8 h. The half-life of MDA is on the order of 24 h. One report compared MDMA and MDA concentrations in a case of polydrug overdose. These results are shown in Table 4.9. MDA undergoes oxidative cleavage of the methylenedioxy ring, producing methoxy and/or hydroxy metabolites, which then undergo conjugation (Marquardt and DiStefano, 1974). The results of pharmacokinetic studies in human volunteers have been reported from at least three separate studies.

In rats, the *d* isomer of MDA is extremely arrhythmogenic, and even moderate doses can provoke ventricular tachycardia. This may explain some reported cases of MDA-associated sudden death. Illicitly manufactured MDA is always a racemic mixture (Lukaszewski, 1979), but the proportions of each isomer present may vary. Blood and tissue concentrations reported in several fatalities have ranged from 6 to 26 mg/L (Cimbura, 1972). In one driving fatality, the deceased was a 29-year-old man with no known history of drug abuse. The concentrations of MDMA in clotted blood, sodium fluoride–potassium oxalate anticoagulated blood, vitreous humor, and urine were 2.3, 2.1, 1.1, and 119 mg/L, respectively. The concentrations of the metabolite MDA were less than 0.25 mg/L in blood and vitreous and 3.9 mg/L in the urine (Crifasi and Long, 1996), but it is hard to say whether such

Table 4.9 Postmortem Distribution of 3,4-Methylenedioxymethamphetamine and 3,4-Methylenedioxyamphetamine Found in One Polypharmacy Death (Cocaine and Heroin Were Also Present)

Bile (ng/mL)	
MDMA (*d*)	58
MDMA (*l*)	15
MDA (*d*)	0.5
MDA (*l*)	1.2
Blood (ng/mL)	
MDMA (*d*)	1.6
MDMA (*l*)	1.3
MDA (*d*)	0.8
MDA (*l*)	0.8
Liver (ng/mL)	
MDMA (*d*)	5.0
MDMA (*l*)	1.4
MDA (*d*)	0.3
MDA (*l*)	0.4
Urine (ng/mL)	
MDMA (*d*)	302
MDMA (*l*)	227
MDA (*d*)	8
MDA (*l*)	18
Vitreous (ng/mL)	
MDMA (d)	1.2
MDMA (*l*)	0.7
MDA (*d*)	0.2
MDA (*l*)	0.04

Source: Adapted from Moore, K.A. et al., *Forensic. Sci. Int.*, 83(2), 111, 1996.

precision is meaningful, given all the possible preanalytic sources of error. In another case report, it appeared that MDA was actually the cause of death. The decedent was a 26-year-old individual whose clinical history suggested arrhythmia. At autopsy, fresh thrombosis was found in a severely obstructed (75%) left main coronary artery. Microscopic features were not described (Nichols et al., 1990).

4.4.3 MDEA (Eve) (Figure 4.18)

4.4.3.1 Introduction

Chemically, drugs like MDEA fall between amphetamines on the one hand and phenylethylamine hallucinogens on the other, with considerable overlap between. Until 1997, the rates of MDEA use in Europe and the United Kingdom were roughly similar. Now, survey

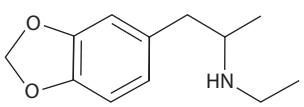


Figure 4.18 3,4-Methylenedioxyethylamphetamine molecule.

results suggest that MDEA is confined to some isolated pockets in Germany (Schifano et al., 2006). As clinical and postmortem screening techniques improve, and there are now a number of very comprehensive methods for measuring all of the drugs in this group in most matrices, it may transpire that MDEA is more popular than is commonly thought (Casteele et al., 2005; Concheiro et al., 2005). Further confirmation that MDEA has fallen out of favor derives from the discipline sometimes known as *sewer epidemiology*, where the presence of drugs such as cocaine can be ascertained directly from the water. These studies are now performed fairly regularly, and MDEA is rarely found on the lists of drugs that have been recovered.

Taiwanese chemists analyzed 181 MDMA tablets from confiscated drugs received over a 3-year period ending in 2005. The MDMA content of the tablets varied from 16 to 193 mg/tablet; 66%–71% of the tablets seized each year contained only MDMA, and when the tablets contained only MDMA, the drug content of each tablet varied from 89 to 133 mg. As has been observed in the United States and in Europe, MDMA tablet content decreases over time and other, less expensive drugs are substituted. In the Taiwanese study, adulterants commonly found mixed with MDMA included caffeine (18%), methamphetamine (7%), MDEA (7%), amphetamine (4%), MDA, ketamine, ephedrine, diazepam, chlorzoxazone, and nicotinamide (Teng et al., 2006). It should only be a matter of time until the bk-amphetamines begin to appear as adulterants.

4.4.3.2 Physiological Effects in Humans

fMRI of brain glucose utilization in human volunteers shows that both MDMA and MDEA cause similar neurochemical alterations, most marked in the frontostriatocerebellar regions, areas implicated in the actions of most psychotropic drugs (Schreckenberger, 2006). One fatality in an individual with an enlarged heart and nonspecific histologic changes has been described. The subject's blood contained 2.0 mg/L of MDEA (Hopster et al., 1996). A second report described the findings in a 19-year-old who died after taking 10 tablets of pure MDEA. His symptoms progressed from apparent intoxication to profuse sweating, followed by aggressive behavior and hallucinations. Respiratory failure quickly supervened. The only significant autopsy finding was passive congestion. The serum MDEA was 12 mg/L in femoral vein blood, 22 mg/L in *heart* blood, and 201 mg/L in the urine (Weinmann and Bohnert, 1998). As with MDA and MDMA, the *S*(+) isomers of MDEA have a much higher affinity for the dopamine transporter and, therefore, exert more activity. The dispositions of the different enantiomers in humans are not known. The effects and lethality are difficult to access with any certainty, as the only data available seem to be approximately a decade old.

Some, but not all, of MDEA's effects have been replicated in animals. For example, MDMA, MDA, and MDEA all cause an initial drop in the temperature of experimental animals, then predictable elevations in core temperature. That is not always the case for human MDEA users. MDEA also produces a transient fall in the diastolic pressure of experimental animals that is not seen in humans. MDEA is an α_1-adrenoceptor antagonist with a pK(B) of 4.79 ± 0.12, and this action is thought to explain the increase in temperature seen after the initial hypothermia (Bexis and Docherty, 2006).

4.4.3.3 Illicit Synthesis

MDEA is relatively easy to make; production requires no expensive or bulky equipment. More than 20 different synthetic pathways are known, and nearly all source materials are available over the Internet. The simplest methods begin with the alkylation

of MDMA. Other popular precursors include safrole from sassafras, nutmeg, and dill. The final product is usually mixed with excipients such as sorbitol or glucose. Pills are stamped from the same dyes that clandestine drug makers use to make MDMA tablets (Freudenmann and Spitzer, 2004). Depending on the intent of the manufacturer, any number of active agents, such as opiates or cocaine (or even methadrone or methylone), may also be added to the mix, making this a particularly dangerous recreational drug. The average dose of MDEA in an illicit pill is said to be between 64 and 176 mg (Freudenmann and Spitzer, 2004).

4.4.4 4-MAX (U4Euh, EU4EA, and U4EA) and Aminorex

4-Methylaminorex (4-MAX) and aminorex belong to a group of compounds known as oxazolines (Figure 4.19). Aminorex was sold in Europe by McNeil Laboratories in the 1960s under the brand names Menocil and Apiquel. It was promoted for appetite suppression and weight reduction, but had to be withdrawn from the market when its use was linked with the development of fatal pulmonary hypertension (Ioannides-Demos et al., 2006). The average aminorex-treated patient who developed pulmonary hypertension (now called idiopathic arterial pulmonary hypertension [IAPH]) had been taking the drug for at least 9 months, in doses ranging from 10 to 40 mg/day (Follath et al., 1971). Unfortunately, levamisole, for reasons that are not understood, has now become the principal adulterant found in cocaine (and is beginning to appear in heroin as well), and levamisole converts directly to aminorex, although the rate of conversion from levamisole to aminorex is not known.

The first reports of 4-MAX as a drug of abuse came from Florida during the mid-1980s. Since then, rare, sporadic seizures have continued (Gaine et al., 2000). Instead of being sold under its own name, methylaminorex has more often been misrepresented as methamphetamine. Based largely on concerns that methylaminorex had the potential to become a low-cost substitute for cocaine or methamphetamine, it was classified as a schedule I substance in April 1989. No death or emergency room visit has been attributed to this drug since then, at least not within the United States. There is evidence that 4-MAX continues to be a problem in the former Soviet Republics. Unfortunately, nothing new about the abuse of this drug, either in the United States or Europe, has been written in the last 5 years, though a great deal more is now known about IAPH, including the fact that symptoms are insidious and nonspecific and the diagnosis difficult to make. Thus, the number of people at risk for developing IAPH is not known and cannot even be approximated without more information about use prevalence.

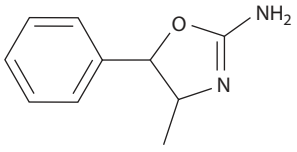


Figure 4.19 4-Methylaminorex is essentially the same molecule as aminorex, except with a methyl group adjacent to the nitrogen. Aminorex, a metabolite of levamisole, the main adulterant found in cocaine today, causes pulmonary hypertension. Whether or not 4-methylaminorex exerts the same function is not known.

Methylaminorex is synthesized in a one-step reaction by condensing phenylpropanolamine with cyanogen bromide. It could also be produced starting with norpseudoephedrine, although with the tight controls on this agent in the United States, the use of that route seems increasingly unlikely, especially since phenylpropanolamine has already been withdrawn from the market. When production laboratories have been raided, the *cis*-(+)-isomer is the form of the drug most often found. Methylaminorex produces the same effects as the other amphetamines, causing substantial increases in brain dopamine release and decreases in tryptophan hydroxylase activity (Hanson and Magill, 1962). Since serotonin excess is thought to be responsible, at least partially, for the development of IAPH, aminorex must also act as a serotonin agonist, though the mechanism is far from clear (Bertol et al., 2011). The discovery that aminorex could cause fatal pulmonary hypertension effectively stopped all further research on this substance (Seiler, 1975; Frank et al., 1993).

Pharmacokinetic studies of aminorex and methylaminorex were done before sophisticated measurement techniques became available. Aminorex absorption is rapid: a single 15 mg oral dose produces a peak plasma concentration of 40 ng/mL at 2 h. Concentrations decline slowly after that, dropping to 5 ng/mL at 24 h. The reported half-life for aminorex in humans is 7.7 h. Studies have not been done on 4-MAX, but the similarities to aminorex are so great that it should behave in much the same way. Most of a given dose is eliminated unchanged in the urine (WHO, 1991).

The chemistry of aminorex is somewhat better understood than a decade ago. Three metabolites have been identified by high-performance LC–MS/MS with thermospray ionization: norephedrine, 5-phenyl-4-methyl-2-oxazolidinone, and 2-amino-5-(*p*-hydroxyphenyl)-4-methyl-2-oxazoline. Stability studies have shown that in aqueous solution aminorex degrades very slightly to norephedrine upon standing. There is no evidence for glucuronidation, which means that P450 plays only a negligible role in its metabolism; rather, it is excreted primarily unchanged but undergoes some slight oxidative deamination and aromatic hydroxylation. Hydrolytic degradation back to the synthetic precursor can also occur (Henderson et al., 1995).

4.4.5 Other MDMA Homologs

The 2-butanamine-2-homolog of MDMA (*N*-methyl-1-1(3,4-methylenedioxyphenyl)-2-butanamine) has been produced by German clandestine chemists (Figure 4.20). Use of the drug is said to result in a pleasant, introspective state, devoid of hallucinogenic effects. Nothing is known about the pharmacology of this drug (Rosner and Ouednow, 2005).

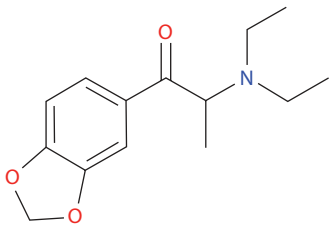


Figure 4.20 Butanamine molecule.

4.5 Phenylalkylamines

4.5.1 Simple Tryptamines (Figure 4.21)

Tryptamines are monoamine alkaloids that occur naturally in plants and animals. It is believed that small amounts are present in the human brain as well, where they act as neurotransmitters. Tryptamines contain an indole ring structure and are related to the amino acid tryptophan. 5-HT and melatonin are both classified as tryptamines. Interestingly, the acacia plant contains large amounts of tryptamine, and it has been suggested that Moses, when he received the Ten Commandments, was actually intoxicated on an acacia-based beverage.

AMT and 5-methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) are now scheduled drugs. Both of these compounds are tryptamine derivatives and share chemical and pharmacologic similarities with other tryptamine hallucinogens, specifically α-ethyltryptamine (AET) and (DMT). AMT and 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT) are stimulants/hallucinogens. AMT can produce nervous tension, irritability, restlessness, insomnia, blurred vision, pupillary dilatation, hallucinations, and dextroamphetamine-like mood-elevating effects. 5-MeO-DMT can produce talkativeness, disinhibition, pupillary dilatation, nausea, jaw clenching, muscle tension, and overt hallucinations with both auditory and visual components. Clinical studies of these drugs have never been performed and their safety for human consumption is not known.

4.5.2 Bufotenine (Figure 4.22)

Bufotoxins are found in the parotid gland, venom, and skin of a variety of toads. They can also be found in some other amphibians and in plants, especially mushrooms (Siperstein et al., 1957). More than 90% of the plants and animals containing bufotoxins are of New World origin (Weil and Davis, 1994). The exact composition of the venom varies greatly depending on the specific source. Toad glands can contain a mixture of toxins, including 5-MeO-DMT; other closely related compounds called bufagins—bufotalin, bufotenine, bufotionine—and cardiac glycosides; and potentially significant amounts of epinephrine, norepinephrine, and 5-HT.

Bufotoxin consists of three major types of endogenous glycosides, all of which are digitalis-like substances that circulate mainly in the plasma of the toad, *Bufo marinus*. One fraction is present in fresh plasma and is composed of chromatographically homogeneous

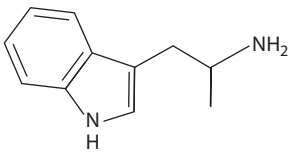


Figure 4.21 Simple tryptamine molecule.

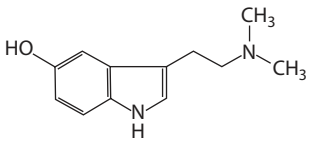


Figure 4.22 Bufotenine molecule.

polar conjugates, principally bufadienolide 3-sulfates, and ATPase inhibitors that range in activity from weak to very strong. Another group, found within the parotids, is of variable and unpredictable composition. 5-Hydroxy-DMT (bufotenine) shares structural and mass spectral similarities with psilocybin and is a potent hallucinogen (Aboul-Enein, 1974).

The results of receptor-binding studies suggest that bufotenine has approximately the same affinity for 5-HT_{2A} and 5-HT_{2C} receptors as LSD. It was the active ingredient in the South American hallucinogenic snuffs described by early Amazon explorers more than 400 years ago (Monardes, 1559). Under U.S. law, bufotenine is a controlled substance, as are the related bufadienolides: resibufogenin, bufalin, and cinobufagin. The Chinese medication *Chan Su* and the West Indian *love stone* both contain bufadienolides, which, because they are potent cardiac glycosides, may cause potentially lethal arrhythmias. Taken in excess, these compounds can produce all the symptoms of digitalis poisoning, which can be successfully treated with digoxin-specific Fab fragments (Brubacher et al., 1996; Ashok et al., 2011).

Archeological evidence indicates that the use of bufotenine-containing snuff dates back several thousand years. In spite of its ancient origins, the drug received little attention until a California wildlife instructor was arrested in 1994 for the possession of bufotenine, which he had collected from four pet toads. At about the same time, police from Australia began to encounter people smoking the dried skin of the Australian cane toad. Occasional samples of bufotenine began appearing at crime laboratories in the early 1990s. At the same time, *toad smoking* began to receive extensive publicity in the lay press (Ramsay et al., 1976; Gallagher, 1994).

Bufotenine is present in at least four different species of toads, and not just in their skin. The digitalis-like component of the poison can be detected in toad plasma and internal organs (Lichtstein et al., 1993). It can also be found in Asian herbal remedies prepared in China from either dried toad skins or milked parotid secretions. One, *Chan Su*, is also used topically to treat skin ailments. Toad products are also added, in minute amounts, to other Asian proprietary mixtures in hopes of strengthening the heart. Given the recent changes in DEA scheduling, it is not clear whether *Chan Su* remains legal when prescribed by herbalists practicing in the United States.

Confiscated samples of bufotenine have been described as resinous, reddish brown cubes, reminiscent of root beer barrel candies that have been sucked on. It is believed that abusers shave off some of the resin and smoke it at the end of a cigarette. The dose used is not known, and resultant blood levels have never been measured. A fairly extensive literature on the botany and chemistry of bufotenine now exists, but essentially nothing is known of its pharmacokinetics or pharmacodynamics.

In addition to the toxin, toad tissue also contains bufogenins, a group of steroid derivatives. At least one is a potent vasoconstrictor, and its effects are not entirely blocked by antidigoxin antibodies (Bagrov et al., 1993). Dogs poisoned with toad secretions develop drooling, seizure activity, cyanosis, and cardiac arrhythmias (Palumbo et al., 1975). The homodynamic effects, at least, seem to be due to the combined effects of the glycosides and catecholamines (Ojiri et al., 1991).

Human toad poisoning does occur, although death is rare. A 1986 case report described a child who developed status epilepticus after mouthing a toad (Hitt and Ettinger, 1986). Profound drooling, seizure activity, arrhythmias, and cyanosis have all been described in *Chan Su* users (Chern et al., 1991; Kwan et al., 1992; Yei and Deng, 1993; Jan et al., 1997; Chi et al., 1998; Kuo et al., 2009). Toxicity is also reported to be common in dogs (Roberts et al., 2000).

4.5.3 DMT (Figure 4.23)

DMT is a component of South American hallucinogenic snuffs. It can be isolated from Old and New World plants, and even European mushrooms. DMT is almost always found together with 5-OH-DMT. The Indian term *ayahuasca* is sometimes used to describe a mixture of plants containing DMT and harmaline. Harmine/harmaline (Figure 4.24) is usually obtained from the *Banisteriopsis caapi* vine and DMT from the leaves of the *Psychotria viridis* bush. None of these plant substances is, by itself, psychoactive when taken orally. Harmine/harmaline is said to cause hallucinations, but only at highly toxic levels. Smaller doses are simply tranquilizing. DMT is not orally active (hence its early use as a snuff), but it can be made active by combining it with a MAOI. In fact, ayahuasca is effective orally because the harmala alkaloids in the *B. caapi* vine are potent short-acting MAOIs. Most tryptamines are inactivated by MAOIs. For that reason, MAOIs can be used to potentiate the effects of tryptamines and to make DMT and 5-MeO-DMT (toads produce it by methylation of 5-OH-DMT to form 5-MeO-DMT) active orally (Weil and Davis, 1994).

MAOIs fall into two classes: irreversible and reversible. Irreversible MAOIs (e.g., the hydrazides iproniazid and phenelzine) bind permanently to the enzyme and cause MAO inhibition lasting 1–2 weeks. They are used clinically to treat depression. Reversible MAOIs, such as moclobemide, which is also used as an antidepressant, and the β-carbolines harmine and harmaline, are effective for a much shorter time, perhaps up to 24 h. Recreational drug users around the world have empirically learned to use mainly harmine and harmaline to prolong their drug-induced experience. In experimental animals, harmaline causes neuronal cell loss and caspase-3-mediated apoptosis in cerebellar granular cells and Purkinje cells, as well as on the inferior olivary neurons (Iseri, et al., 2011). The effect in humans is not known.

4.5.4 5-Methoxy-*N,N*-Dimethyltryptamine (Figure 4.25)

This is a relatively common hallucinogen and police confiscations are not infrequent. Sometimes, it is sold as blotter acid. In 2010, 5-MeO-DMT, its salts, isomers, and salts of isomers were all placed on schedule I of the CSA in the United States.

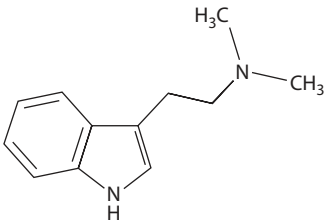


Figure 4.23 *N,N*-dimethyltryptamine molecule.

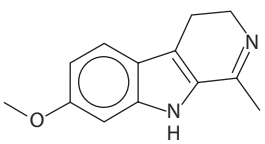


Figure 4.24 Harmaline molecule.

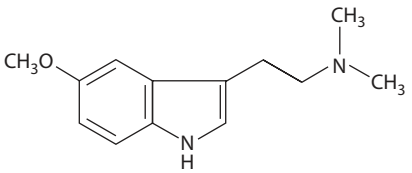


Figure 4.25 5-Methoxy-*N,N*-dimethyltryptamine structure.

The toad *Bufo alvarius* does not contain 5-MeO-DMT but it does contain the precursor 5-OH-DMT, which can be transmethylated to form 5-MeO-DMT (Weil and Davis, 1994). 5-OH-DMT can also be synthesized from standard laboratory chemicals (Shulgin and Shulgin, 1991). Of course 5-MeO-DMT also occurs naturally. It is mainly used in the ayahuasca brews of South America, but occasionally also in traditional South American shamanic snuffs. Like other naturally occurring alkaloids, it is not active when it is taken orally, so it must be smoked. According to Shulgin the dose is 10–25 g. When smoked, it is about four times as potent as DMT. Shulgin found in some of his early experiments that effects are felt in less than 60 s, peak in 2–3 min, and disappear after 20 min. When it is taken orally, 5-MeO-DMT is taken in combination with MAOIs (Callaway and Geyer, 1992).

Controversy exists about the relative toxicity of the combination. A report published in the summer of 2006 described a 25-year-old white male found dead in the morning after consuming a cocktail of β-carbolines and hallucinogenic tryptamines. According to the authors, no cause of death was evident at autopsy. Blood taken from the heart was found to contain DMT (0.02 mg/L), 5-methoxy-DMT (1.88 mg/L), tetrahydroharmine (0.38 mg/L), harmaline (0.07 mg/L), and harmine (0.17 mg/L) (Table 4.10). The medical examiner ruled that the cause of death was hallucinogenic amine intoxication and the manner of death was undetermined (Sklerov et al., 2005). Two years earlier, the death of another young man was attributed to the same combination of drugs (Brush et al., 2004). These are the only reported cases suggesting significant toxicity.

In theory, this combination of drugs could cause something very much like acute catecholamine toxicity. Unfortunately, no details of the autopsy were given in either of the reported cases, and whether or not there was identifiable myocardial necrosis is not known. One might expect that it would have been had it been diligently sought. The other factor making assessment impossible is that no genetic screening was done of the heart, and there is no way to rule out death from heritable channelopathy or cardiomyopathy or for that matter simple myocarditis; a substantial proportion of all cases of myocarditis can only be identified by DNA resequencing.

Table 4.10 Blood Levels in an Alleged Ayahuasca Death (Values Are in Nanograms)

Substance	Heart Blood	Peripheral Blood	Urine	Liver	Brain
DMT	20	10	890	ND	570
5-MeO-DMT	1888	1200	9590	1,638	150
Tetrahydroharmine	380	240	6002	13,240	430
Harmaline	70	40	6002	360	40
Harmine	170	80	1150	2,310	160

Source: Adapted from Sklerov, J. et al., *J. Anal. Toxicol.*, 29(8), 838, 2005.

4.5.5 Ergolines (Figure 4.26)

The structural skeleton of ergoline is contained in many different alkaloids including the psychedelic LSD. Ergoline derivatives, such as ergotamine, are used clinically to cause vasoconstriction by interacting with the type 1 5-HT receptor. Drugs in this group are used to treat migraine and sometimes Parkinson's disease. However, the most important ergoline is LSD. The UN Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances strictly controls the precursors needed to produce LSD. There are three main classes of ergoline derivatives. LSD belongs to the first group of water-soluble ergolines, that is, the amides of lysergic acid.

Harmine and harmaline are indole alkaloids and it has recently been demonstrated that harmine, harmaline, harmalol, harmol, and harman are all CYP3A4 and CYP2D6 inhibitors in humans (Zhao et al., 2011). Whether this observation is of clinical significance is not known, but there is potential for any number of unanticipated drug interactions. *Peganum harmala* is just one of at least eight plant families, some Old World, some New, containing harmine and harmaline (Figure 4.27). They are profoundly hallucinogenic. In the New World, *Banisteriopsis*, a malpighiaceous tropical genus, is the main source of the psychoactive snuff. Autopsies of cattle that have consumed too much of the drugs show little more than passive visceral congestion (Bailey 1979a,b).

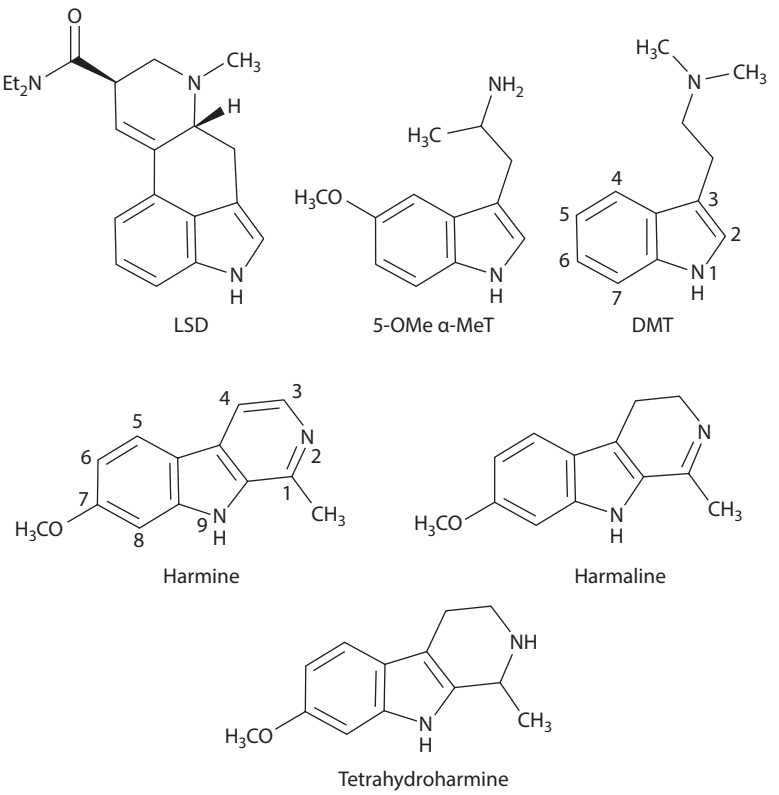


Figure 4.26 The relationships between the ergolines harmine and harmaline and LSD, the α-alkyltryptamine hallucinogen 5-methoxy-α-methyltryptamine, the *N*,*N*-diallyltryptamine hallucinogen *N*,*N*-dimethyltryptamine, and the β-carbolines harmine, harmaline, and tetrahydroharmine.

Figure 4.27 Extracts of the harmaline alkaloids are readily available over the Internet. A concentrate is shown in this figure.

Seeds of *P. harmala* (Syrian rue) contain roughly 3% harmine/harmaline. *B. caapi* has been found to contain from 0.18% to 1.36% β-carbolines, with the concentration of harmine being from 0.057% to 0.635% (McKenna et al., 1984). According to anecdotal reports, the ingestion of 1 g of *P. harmala* seeds will inhibit MAO sufficiently to make DMT orally active. Harmine and harmaline are hallucinogenic even in the absence of DMT. The effective dose is thought to be approximately 300 mg (Ott, 1999). These drugs have few emotional or *psychedelic* effects but produce strong visual hallucinations. For that reason, Amazon natives often add larger amounts (75–100 cm of stem per dose) of *B. caapi* to ayahuasca brew in order to produce MAO inhibition (Luna, 1984). One of the characteristic features of these mixtures is that they produce nausea before inducing any psychiatric effects. DMT is usually sold on the black market as a brownish solid material that smells like mothballs. Users cut off small pieces and smoke them by placing them at the end of a cigarette, often a marijuana cigarette. DMT is sometimes referred to as the *businessman's high* because a single inhalation will produce a 5–10 min *trip* that is entirely gone in 30 min (Chamakura, 1993).

Only one alleged case of ayahuasca poisoning associated with recreational abuse has ever been reported or studied. Autopsy was performed within 24 h of death and no gross lesions were apparent. Blood levels from various sites were measured (see Table 4.10); however, these levels cannot be reliably used to determine the cause of death for a number of reasons: (1) the decedent, a 25-year-old, could well have suffered from a heritable channelopathy, but no testing was done; and (2) nothing is known about blood levels in the living or the behavior of any of these compounds after death, nor is anything known about the toxicokinetics of smoked DMT; however, controlled double-blind studies with intravenously administered drug have been performed in experienced hallucinogen users. With doses of 0.2 and 0.4 mg/kg (which are fully hallucinogenic), effects were experienced almost instantly, peaking within 2 min and disappearing in 20–30 min. Measured blood

levels corresponded to the subjective effects of the drug. Peak levels varied widely from subject to subject and ranged from 32 to 204 ng after a 0.4 mg/kg dose.

Hallucinogenic drugs such as DMT are serotonergic agonists, or at least partial agonists, and, in addition, have adrenergic and dopaminergic properties. DMT causes hormonal, autonomic, and cardiovascular effects. Pupils dilate and, in some studies, levels of cortisol, prolactin, corticotropin, growth hormone, and β-endorphin all increase in a dose-dependent manner. In other human studies, these changes have not been confirmed, which may be a function of the dosage given. Values return to near baseline within 30 min. Increases are also observed in heart rate and blood pressure. Body temperature also rises, although that change lags slightly behind the others (Strassman and Qualls, 1994; Strassman et al., 1994). The risk of addiction is thought to be negligible (Gable, 2007). Overall, the risk compared to other drugs seems to be very low. In spite of earlier work suggesting hormonal and blood pressure changes, in the most recent human study, where freeze-dried ayahuasca was given (two doses, 12 h apart, 0.75 mg/kg), the only statistically significant findings were mild decrease in heart rate and blood pressure and a marked rise in growth hormone secretion (Dos Santos et al., 2012).

4.6 Psilocybin (Figure 4.28)

4.6.1 History

Psilocybin-containing mushrooms were probably first used by the Aztecs, but until the 1960s they were not known outside of Mexico. The name psilocybin is derived from the Greek roots *psilo*, meaning "bald," and *cybe*, meaning "head," presumably because of the shape of the mushrooms from which the active compounds are derived. The structure of the molecule was established by Albert Hoffman at Sandoz Pharmaceuticals in 1958. Hoffman had succeeded in synthesizing LSD just a few years earlier. For some time, Sandoz marketed pure psilocybin under the brand name Indocybin.

Psilocybin can be found in three different genera of mushrooms: *Psilocybe*, *Panaeolus*, and *Conocybe*. All three varieties grow naturally in the northwestern and southeastern portions of the United States. Related or identical forms grow wild in Central and South Americas, as well as in Southeast Asia and India. Large quantities are cultivated for illegal distribution. The most common species is *Psilocybe cubensis*. It grows wild in the manure of cattle, water buffalo, and other ruminants, including deer and possibly kangaroos. In Southeast Asia, farmers collect droppings from these animals and systematically grow the fungi in disused rice paddies (Allen and Merlin, 1992).

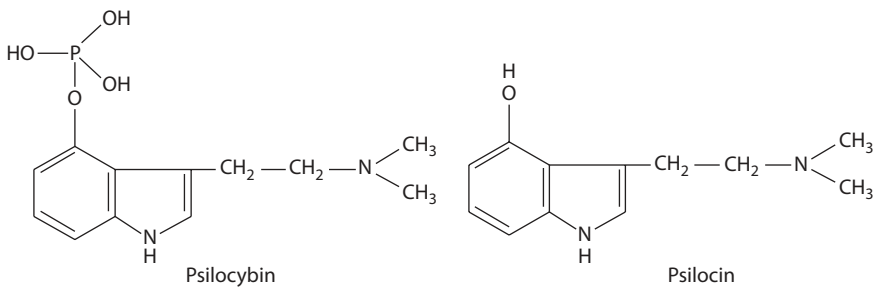


Figure 4.28 Psilocybin and psilocin molecules.

All three genera contain the tryptophan derivatives of psilocybin. *P. cubensis* is generally the preferred cultivar and on average yields 10 mg of psilocybin per gram of fresh mushroom, which is equal to an average dose. Psilocin is 1.5 times more potent than psilocybin, but because the latter oxidizes more slowly, both contribute almost equally to the effect of the mushroom (Leikin et al., 1989). During the early 1980s, growing kits complete with spores were advertised in magazines. They are now illegal (Schwartz and Smith, 1988).

Identifying wild *Psilocybe* is difficult and dangerous. Psilocybin-containing mushrooms grow side by side with the poisonous *Galerina autumnalis*. *Galerina* species have rust-brown-colored spores, while the spores of *Psilocybe* species are gray to lilac. Some, but not all, species can be distinguished from poisonous mushrooms by their reaction to room air; when *Psilocybe* mushrooms are cut, they oxidize and turn blue within 30–60 min. Unfortunately, some poisonous mushrooms can do the same thing. Pathologists are much more likely to encounter cases of mushroom poisoning than they are to encounter psilocybin-associated medical problems!

4.6.2 Physiological and Psychological Effects

After oral doses of up to 15 mg, psilocybin produces no significant alteration in heart rate, blood pressure, or neuroendocrine function, although profound psychological alterations do occur (Gouzoulis-Mayfrank et al., 1999). The mechanism by which these drugs exert their psychotropic effects may have recently been identified. The effects of hallucinogenic drugs, such as psilocybin and LSD, require the presence of the 2AR 5-HT receptor, and the symptoms they produce resemble many of those seen in schizophrenia. Metabotropic glutamate receptor #2 (mGluR2) interacts through specific transmembrane helix domains with the 2AR 5-HT receptor, along with another member of an unrelated G-protein-coupled receptor family. Together, these groups form functional complexes within the brain cortex. When a hallucinogenic drug interacts with the 2AR–mGluR2 complex, a set of unique cellular responses occurs and normal behavioral responses are disrupted (Gonzalez-Maeso et al., 2008).

4.6.3 Pharmaco- and Toxicokinetics

Psilocybin is detectable in plasma 20–40 min after oral administration (Hasler, 1997 reviewed in Passie et al., 2002). Psilocybin is metabolized in the liver, where it is chiefly transformed into the active metabolite psilocin, detectable in plasma 30 min after administration (Hasler et al., 1997; Lindenblatt et al., 1998; Passie et al., 2002), with psilocin first appearing in plasma 15–50 min after oral administration of 0.2 mg/kg psilocybin. Psilocin half-life ranges between 2 and 3 h; it is detectable 6 h after oral administration (Hasler et al., 1997; Lindenblatt et al., 1998). The two studies reported similar but not identical findings, with peak levels of psilocin appearing between 80 and 105 min and psilocin half-life ranging between 2.25 h for 0.2 mg/kg and 2.7 h for 0.22 mg/kg.

4.7 α-Ethyltryptamine (Figure 4.29)

Also known as etryptamine and Monase, AET was first marketed in 1961 as an antidepressant under the brand name Monase. It is classified as a reversible MAO-A inhibitor (Fredriksson et al., 2000) but it also causes neuronal 5-HT release (Dulawa et al., 1998).

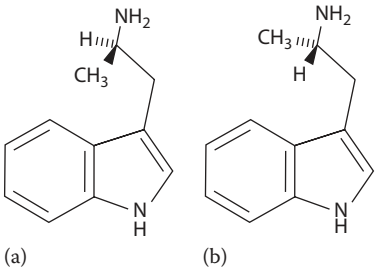


Figure 4.29 α-Ethyltryptamine (b) and α-methyltryptamine (a) are both MAO inhibitors, but they both release 5HT, NE, and dopamine but it appears to act more like indolic psychedelics, explaining the reputation of both drugs as *entactogens*.

Clinically unconfirmed laboratory studies have shown that AET can produce MDMA-like effects, in spite of the very substantial differences in molecular structure (Glennon, 1993).

Although it was apparently effective as an antidepressant, AET was withdrawn from the market when evidence of neurotoxicity was detected. Illicit use, or at least toxicity resulting from illicit use, is extraordinarily rare. The literature describes only two deaths. One involved a 19-year-old woman who took two tablets (approximately 700 mg) of what she thought was MDMA. Within a few hours she became disoriented, vomited, and collapsed. The principal autopsy findings were pulmonary edema and terminal aspiration. Epicardial petechiae were noted but almost certainly were the result of attempted resuscitation. The only drug detected was AET. The concentration in heart blood was 5.6 mg/L, with 2.4 mg/L in the vitreous, 18 mg/kg in the liver, 24 mg/kg in the kidneys, and 22 mg/L in bile (Morano et al., 1993). A second report describes a patient with a suicidal overdose. The quantity ingested was believed to be approximately 700 mg. The cause of death was malignant hyperthermia, and the postmortem blood concentration was 1.1 mg/L (Daldrup et al., 1986).

4.8 Lysergic Acid Diethylamide

4.8.1 Introduction

LSD-25, or 9,10-didehydro-*N*,*N*-diethyl-6-methyl ergoline-8β-carboxamide is an ergot alkaloid (see Figure 4.26). The term *ergot* refers to a fungal disease that affects both wild and cultivated grasses. Infection with one of the *Claviceps* species leads to the formation of hard, seedlike nodules instead of the normal seeds produced by the plants. In the Middle Ages, these nodules were referred to as *ergots*, but they are properly referred to as sclerotia. Contained within the sclerotia are a group of indole alkaloids, collectively known as ergot alkaloids. Ergotism is rarely encountered today. It results from ingestion of the sclerotia or the chronic use of ergotamine-containing medications. The main symptom is intense vasospasm (Zavaleta et al., 2001). The most important of the alkaloids is *d*-lysergic acid, but LSD is just one of many *d*-lysergic acid derivatives. The hallucinogenic agent lysergic acid amide, found in the morning glory plant (*Ipomoea violacea*), is another. All of the ergot alkaloids act, with varying degrees of specificity, at α-adrenergic, dopaminergic, and serotonergic receptor sites (Dewick, 1998).

4.8.2 History

Albert Hoffman synthesized LSD in 1938. He was then working as a research chemist in the laboratory of Sandoz Pharmaceuticals in Basel, Switzerland, studying the chemistry of the ergot alkaloids. He had already isolated lysergic acid from ergot and was in the process of combining it with various amides via peptide linkages. Hoffman's goal was to synthesize new compounds that lacked the toxic side effects of ergot but still might have positive effects on the circulation. He succeeded in that goal when he synthesized Methergine® (methylergonovine), which is still used today to control postpartum hemorrhage. During the course of his experiments, Hoffman had synthesized a series of compounds related to acid. The 25th lysergic compound that he produced was LSD, now known as LSD-25. When Hoffman first tested LSD-25 on animals, the results were disappointing and he did no further research with LSD-25 for 5 years. However, in April of 1943, he began working with it again. He accidentally ingested some, and that accident led to the dawn of the modern *psychedelic* age (Hoffman, 1984; Ulrich and Patten, 1991). Sandoz eventually marketed LSD as a product called Delysid. Psychiatrists were told to try it themselves, and they found out firsthand what the subjective experiences of a schizophrenic would be like!

LSD was never a commercial success but its availability fostered research into the chemical origins of mental illness. None of the theories proposed during the 1950s and 1960s proved to be correct, but they did lead to more modern research into the neurochemistry of schizophrenia, though a definitive answer to the question "What causes schizophrenia?" remains wanting. Genome-wide approaches have revolutionized the field of genetic mapping of schizophrenia, and the aggregate genetic data increasingly support a combination of (1) rare and common genetic variations in schizophrenia, (2) a major role for polygenic inheritance and a genetic overlap (pleiotropy) of schizophrenia, and (3) other psychiatric disorders such as bipolar disorder and autism.

The early theories also led the Central Intelligence Agency (CIA) to conduct some rather bizarre experiments of its own in the field of mind control. The CIA mounted a special operation called MK-ULTRA in which prostitutes were used to lure businessmen to brothels where they were surreptitiously dosed with LSD and their behavior observed. Although all the data about this episode are yet to be disclosed, it appears that no useful scientific information was ever generated and the experiments were abandoned.

The psychedelic age began in the early 1960s when the late Timothy Leary, then a professor at Harvard, undertook his research with psilocybin. He eventually began to experiment with LSD and was so transformed by his experiences that he stopped experimenting with psilocybin in order to concentrate on the effects of LSD. He gave LSD to some of his students. Predictably, he was forced to leave Harvard in 1962. However, even before he was fired from Harvard, Leary's anthem—Tune in, turn on, drop out—had been adopted by the media, and the psychedelic age was launched.

LSD became a scheduled drug in 1965, and at nearly the same time, some dubious and unconfirmed studies about it were published. These studies purported to show that LSD led to chromosomal damage. Decreased availability and fears about toxicity led to a rapid decline in LSD use (Ulrich and Patten, 1991). In the late 1990s, interest in LSD seemed to renew, but reports of toxicity continued to remain extremely rare. The emergency room component of the DAWN report contains 2006 LSD mentions during the first half of the year 2000 and 2028 in 2009 (compared with 422,896 cocaine-related visits in the same year). According to the UN's 2011 World Drug Report, seizures of LSD, which in terms of

volume terms are hardly noticeable, have shown a downward trend over the 2005–2009 period. Europe accounts for 80% of all LSD seizures made worldwide. Estimating the true frequency of use is no easy matter. Even when large doses of LSD are taken, the drug is difficult to detect. The standard dose of LSD today is much lower than during the psychedelic era, and only very small amounts of LSD appear in the urine (concentrations rarely exceed 2–3 ng/mL). Because LSD is not included on standard immunoassay screening panels, use estimates must rely on self-reporting, a notoriously unreliable methodology.

4.8.3 Incidence and Epidemiology

The market for LSD is shrinking. There has been a 75% decline in use of LSD among high school students in the United States over the period 1996–2013 (UNODC, 2014), but the decline may just reflect the increasing difficulties drug makers face when trying to buy the precursor chemicals. Elsewhere, there appears to be a slight increase in use, but when samples alleged to be LSD have been analyzed, they have turned out to be synthetic phenethylamines, 25B-NBOMe and 25C-NBOMe in particular (UNODC, 2014).

4.8.4 Illicit Production

Production of LSD demands considerably more skill than is required for methamphetamine, but it can be made in small, clandestine laboratories. Synthesis is possible from any one of a number of lysergic acid derivatives, including morning glory seeds or synthetic lysergic acid. Detailed instructions on how to harvest and grow ergot (*Claviceps purpurea*) can be downloaded from the Internet. The synthetic process involves potentially explosive solvents, and the investigation of possible clandestine LSD laboratories is a practice best left to individuals with specific training.

Only the *d* isomer of LSD is psychoactive, but it undergoes isomerization at the C8 position. At equilibrium, 90% will be in the *d* form and 10% will be present as iso-LSD (Salamone et al., 1997). Iso-LSD is usually found as a contaminant in clandestinely produced LSD. Also, because *d*-LSD is almost completely metabolized, urine may contain more iso-LSD than *d*-LSD.

Once synthesized, the standard practice is to add the LSD to absorbent blotter paper and then divide the paper into small squares, with each square constituting an individual dose, although it can also be sold in bulk, usually as a purple-colored powder, or in capsules (Figure 4.30). The average LSD content per square in the year 2000 was between 20 and 80 mg (Nelson and Foltz, 1992). A recurring urban myth has it that children may be exposed to LSD when they apply water-soluble tattoos that are given at Halloween. In fact, no such episode of poisoning has ever been documented.

4.8.5 Metabolism

Absorption is rapid and complete, and the drug is extensively metabolized in the liver (Lim et al., 1988) with only very small amounts ever appearing in the urine. Humans produce at least five different metabolites formed after initial dealkylation. These include *N*-demethyl LSD (which is sometimes referred to as nor-LSD), nor-iso-LSD, and lysergic acid ethylamide. An alternative pathway involves oxidation and hydroxylation, leading to the formation of 2-oxo-3-hydroxy-LSD, trioxylated-LSD, lysergic acid ethyl-2-hydroxyethylamine,

Figure 4.30 *Blotter acid*. This means distribution is not as popular as it was in the past. Blotter paper is soaked in an LSD-containing solution. The problem with this approach is that the LSD will migrate to the edges, so consumers who purchase tabs from the middle of the paper get too little, and those who purchase the edges get too much. (From the website of the DEA.)

13- and 14-hydroxy-LSD, and their glucuronide conjugates. Experiments in animals have disclosed other metabolites as well, but it appears that man does not produce them. Concentrations of the main metabolite, 2-oxo-3-hydroxy-LSD, in urine are much higher than in plasma, and analysis for this compound can widen the window of detection (which should be on the order of 40 h) substantially (Canezin et al., 2001).

4.8.6 Blood and Tissue Concentrations

In the only recent study where substantial doses of LSD (4 mg/kg) were given to volunteers, plasma concentrations of LSD peaked at just under 0.8 ng/mL approximately 1 h after administration, then fell to zero after 24 h (Reuschel et al., 1999a, b). In one study of 14 emergency room patients with suspected LSD intoxication, plasma LSD concentrations, measured between 2 and 11 h after ingestion, ranged from 0.2 to 7.7 ng/mL (McCarron et al., 1990). In a more recent study of two symptomatic patients seeking emergency room treatment, 4 h after ingestion, one patient had urine LSD and iso-LSD concentrations of 1.3 and 0.82 µg/L, respectively. In plasma they were 0.31 and 0.27 µg/L. Samples were taken 11 h after ingestion in the second patient, and the corresponding values were 0.24 and 0.6 µg/L in urine, but plasma concentrations were not reported (Canezin et al., 2001). One postmortem study has been published (Favretto et al., 2007).

4.8.7 Testing

Because plasma concentrations are so low, detection has in the past been limited to urine, and even there LSD and its metabolites are not detected easily without the use of highly sensitive methods. Furthermore, LSD is unstable in urine when samples are exposed to temperatures greater than 0°C or to light with losses occurring after the first day (Skopp et al., 2002). LSD metabolites can be detected in urine using immunoassays with a cutoff of 500 pg/mL (Wiegand et al., 2002).

Newer chromatographic techniques rely on tandem mass spectrometry. For example, solid-phase extraction and LC–MS/MS can be used for the simultaneous determination of LSD and a number of other drugs of abuse including cocaine metabolites, opiates, and amphetamines in urine (Concheiro et al., 2007). An LC–MS/MS method in blood has a limit of detection for LSD of 5 pg/mL and is also capable of detecting a number of metabolites including nor-LSD, iso-LSD, and 2-oxo-3-hydroxy-LSD (Chung et al., 2009).

4.8.8 Clinical Syndromes

LSD and mescaline (a different class of hallucinogen) share a common mechanism of action in that their ability to cause hallucinations correlates directly with their ability to bind to 5-HT_2 receptors (Aghajanian and Marek, 2000). Changes in heart rate, respiration, and blood pressure occur, but these may just be secondary to anxiety induced by perceptual changes (Klepfisz and Racy, 1973). Panic attacks are said to be relatively common, but not frank psychotic episodes (Blaho et al., 1997). *Acid flashbacks*, though widely publicized, also appear to be uncommon. When formal neuropsychological testing is performed, few, if any, sequelae can be attributed to LSD use (or to the use of any other hallucinogen, for that matter) (Halpern and Pope, 2003).

In the past, LSD was almost exclusively sold impregnated in blotter paper, but pills and tablets are more likely to be seen today. The likelihood of massive overdose is small, although adulteration with other drugs may lead to confusing symptoms. Massive LSD overdose results in vomiting and collapse along with signs of sympathetic hyperactivity, hyperthermia, coma, and respiratory arrest. Hyperthermia and mild generalized bleeding may also occur due to platelet dysfunction (Klock et al., 1975). Hyperthermia has been reported in other LSD users who have not taken massive doses (Bakheit et al., 1990).

As recently as 1990, it was generally believed that no death had ever been caused by the direct action of LSD; however, several very probable cases have been reported and more than a few have required medical care. In 1977, Griggs and Ward reported the death of a 34-year-old man who had been behaving erratically. He was found 1 month later with extremely high concentrations of LSD in his liver (there would have been no blood left to sample after 1 month) (Griggs and Ward, 1977). In 1975, "eight patients were seen within 15 min of intranasal self-administration of large amounts of pure D-LSD tartrate powder. Emesis and collapse occurred along with sign of sympathetic overactivity, hyperthermia, coma, and respiratory arrest. Mild generalized bleeding occurred in several patients and evidence of platelet dysfunction was present in all. Serum and gastric concentrations of LSD tartrate ranged from 2.1 to 26 ng/ml and 1000 to 7000 ng/100 ml, respectively. With supportive care, all patients recovered" (Klock et al., 1975). However, the situation has changed drastically since LSD was so popular and now, more often than not, one of the designer amphetamines such as 25I-NBOMe is likely to be detected.

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Opiates and Opioids 5

5.1 Worldwide Opium Consumption

The United Nations (UN) World Drug Report for 2014 reported that global drug use was stabilizing, though not the number of deaths occurring as a consequence of drug abuse. For the third consecutive year, Afghanistan remained the world's largest cultivator of the opium poppy with the area under cultivation having increased (Smith et al., 2014) from 154,000 ha in 2012 to 209,000 ha in 2013. Myanmar increased opium poppy cultivation, though the increase was smaller than in the previous year. The burden of disease from opioid dependence increased by 74% between 1990 and 2010. According to the United Nations Office on Drugs and Crime (UNODC) data, the prevalence of opioid use has been increasing globally over the past 5 years, chiefly because of the misuse of prescription opioids, even though the prevalence of heroin and opium use has been stable at the global level for some time; as of 2012 rates were continuing to increase in the United States. In fact, there is evidence of declining use in Europe and other regions. A total of 43,000 deaths were attributed to opioid dependence in 2010 (UNODC, 2014). Put another way, little or no progress has been made toward the goal of reducing opiate abuse. Because heroin must be injected, its widespread use is intimately connected with the spread of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) and hepatitis C.

In 2013 potential opium production was estimated at 6883 tons, roughly the same as it was from 2008 to 2011. Opium production in Afghanistan still accounts for 80% of the global opium production estimated at 5500 tons, translating to total production of 560 tons, which has been consistent for the last several years (UNODC, 2014). The use of synthetic opioids has become increasingly popular in the United States, and black market prices for this drug have risen. The increases may explain why officials of the U.S. Drug Enforcement Agency (DEA) have noted that prescription opioid abusers are switching to heroin, which is cheaper than the prescription drug and easier to obtain. Ironically, the exact opposite is being observed in many parts of Europe.

The use of opioids like OxyContin® is increasing worldwide. The UN estimates that there are between 29 and 38 million opiate users globally, giving a prevalence of 0.7%. The greatest increases within the past 2 years have occurred in the United States. However, there is no way to estimate with certainty the extent of these increases as the United States does not closely track such data. In fact the U.S. Department of Justice actually closed its National Drug Intelligence Center in 2012, leaving the UN as the only source of drug intelligence on a worldwide basis. The UN estimates that a total of 183,000 drug-related deaths occurred worldwide (range estimated between 95,000 and 226,000).

At UNODC, and its Joint United Nations Programme on HIV/AIDS (UNAIDS), the World Bank, and the World Health Organization (WHO) estimated worldwide 12.7 million (range, 8.9–22.4 million) self-administer drugs intravenously, corresponding

to a prevalence of 0.27% (range, 0.19%–0.48%) of all individuals. The sharing of used injecting equipment makes these people vulnerable to HIV and hepatitis C. According to the collected figures of the organizations listed earlier, it is estimated that an average of 13.1% of all illicit drug injectors are living with HIV. That would amount to 1.7 million persons (range, 0.9–4.8 million) with HIV. The prevalence in Eastern Europe is thought to be substantially higher (23%–28%). More than half of the people who inject drugs are also infected with hepatitis C. Effective drugs that can cure hepatitis C are now on the market, but the current cost is U.S. $80,000/person, which means that the cost to cure this one complication of drug abuse would be hundreds of millions of dollars.

5.2 Epidemiology

The United States conducts an annual survey, the National Survey on Drug Use and Health (NSDUH). The most recent estimates date from 2012 (Office of Applied Studies, 2012) and may or may not still be relevant. NSDUH contains information on nine categories of illicit use: marijuana, cocaine, heroin, hallucinogens, inhalants, and the nonmedical use of prescription-type pain relievers, tranquilizers, stimulants, and sedatives. In 2012, an estimated 23.9 million Americans aged 12 or older were current (past month) illicit drug users, meaning they had used an illicit drug during the month prior to the survey interview. This estimate represents 9.2% of the population aged 12 or older. Illicit drugs include marijuana/hashish, cocaine (including crack), heroin, hallucinogens, inhalants, or prescription-type psychotherapeutics (pain relievers, tranquilizers, stimulants, and sedatives) used nonmedically. Of these, NSDUH estimates that the number of past year heroin users increased during the period 2007–2012, rising from 373,000 to 669,000. In 2012 there were 156,000 persons aged 12 or older who used heroin for the first time within the past year, which was similar to the estimates from 2007 to 2011. However, this was an increase from the annual numbers of initiates during 2003 (92,000) and 2006 (90,000). The average age at first use among recent initiates aged 12–49 was 21.3 years, significantly lower than the 2009 estimate (25.5 years). The number of persons who had heroin dependence or had abused heroin increased from 214,000 in 2002 to 359,000 in 2010 (Office of Applied Studies, 2012).

After phencyclidine (PCP) (and the newly introduced beta-keto amphetamines and cathinone derivatives, for which no reliable estimates exist), heroin is the least common cause of drug abuse in the United States, amounting to only 0.2% of all recognized cases, with only a few hundred thousand initiates each month (versus more than 3–5 million monthly episodes of cocaine abuse). According to the 2013 World Drug Report, cannabis was the most popular drug, used by an estimated 177 million people around the world. The number of persons aged 12 and over abusing opioids was 33 million, while only 16 million abused opiates (heroin). The report also estimates there were 17 million cocaine users and 34 million users of amphetamine-type drugs. The lowest on the list was ecstasy; it is not clear why the UN chooses to separate 3,4-methylenedioxymethamphetamine (MDMA) from the other amphetamines, but it has been their tradition for some years (UNODC, 2014).

While the number of heroin abusers remains relatively static, the number abusing diverted prescription opioids continues to increase. Data taken from the most recent Drug Threat Assessment (a report released annually but based primarily on NSDUH data) show

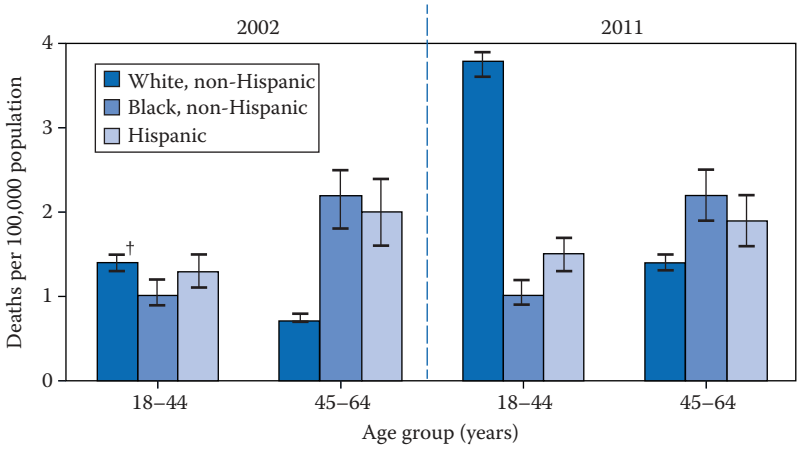


Figure 5.1 The rates of drug poisoning deaths involving heroin in the United States between 2002 and 2011. In the decade from 2002 to 2011, the number of drug poisoning deaths involving heroin doubled, from 2089 in 2002 to 4397 in 2011. In 2002, non-Hispanic blacks aged 45–64 years and Hispanics aged 45–64 years had the highest rates of drug poisoning deaths involving heroin (2.2 and 2 deaths/100,000, respectively). In comparison, in 2011, non-Hispanic whites aged 18–44 years had the highest rate. From 2002 to 2011, the rate for non-Hispanic whites more than doubled for the 18–44 years age group (from 1.4 to 3.8 deaths/100,000) and doubled for the 45–64 years age group (from 0.7 to 1.4/100,000). The rates for both age groups of Hispanics and non-Hispanic blacks did not significantly change during the decade. (From CDC, *Morbid Mort. Wkly.*, 63(27), 595, July 11, 2014b.)

that opioid pain relievers (OPRs) are the most widely abused of the opioids (United States Department of Justice, 2013). The report claims that nonmedical use of prescription drugs such as oxycodone (up by 26.6%) and hydrocodone (14.11%) increased 112% from 2006 to 2010, rising from 84,671 to 179,787 (United States Department of Justice, 2013).

A similar pattern prevails in Oceania, where prescription opioid abuse vastly exceeds heroin abuse. Elsewhere in the world, heroin remains the drug of choice. Drug-related deaths account for between 0% and 1.3% of all-cause mortality at the global level among persons aged 15–64, but they vary considerably by region. Accounting for approximately 1 in every 20 deaths among persons aged 15–64, drug-related deaths are highest in North America (Figure 5.1) and Oceania. In Asia, they account for approximately 1 in 100 deaths, in Europe 1 in 110, in Africa 1 in 150, and in South America approximately 1 in every 200. There is a higher rate of drug-related deaths in North America and Oceania because those regions have a higher number of problem drug users and better monitoring and reporting of drug-related deaths.

5.3 Classifying Narcotic and Nonnarcotic Agents

There are several ways to classify opiates. Earlier schemes grouped them according to their origins: naturally occurring opiates such as morphine and codeine, morphine-based semisynthetic opiates such as heroin and hydromorphone, thebaine-based (one of the components of opium) semisynthetic opiates such as oxymorphone and oxycodone, and purely synthetic opiates such as methadone, meperidine, and pentazocine. This type of classification offers little help in understanding mechanisms of opiate toxicity.

Another, perhaps more helpful, way is to divide these drugs into opiates and opioids. The term *opiate* is reserved for peptide compounds derived from the morphine molecule. These drugs specifically bind to opioid receptors. The term *opioid* is used to describe any nonpeptide agent that can also bind at the opiate receptor site. Members of this class include morphinans, typified by butorphanol, the benzomorphans such as pentazocine, the 3,5-diphenylamines including methadone, phenylpiperidines, and, especially, meperidine. There are two principal considerations in determining which opiate or opioid to administer for the treatment of peripheral pain. The first is to find an agent that produces the desired effect, at the lowest dose, when treating peripheral pain, but which, at the same time, when compared to alternative choices, crosses the blood–brain barrier (BBB) least readily. The hope is to produce fewer central and systemic side effects but still relieve the pain. A second consideration involves knowing which of the peripheral opioid receptors may also have therapeutic ramifications. The treatment of opioid-induced bowel dysfunction by methylnaltrexone is one example, and the treatment of pain from acute myocardial infarction would be another (Rachinger-Adam et al., 2011).

Opioids can also be grouped into exogenous and endogenous systems. The endogenous opioids bind to three G-protein–coupled receptors, namely, the mu, delta, and kappa, and their respective ligands beta-endorphin, enkephalin, and dynorphin, all of which are widely distributed throughout the central and peripheral nervous system (Kieffer and Evans, 2009). Each of the receptors has multiple subtypes: μ 1–3, δ 1–2/δ complexed/non-complexed, and κ 1–3. Until recently, it was generally accepted that each of the subtypes had specific functions (e.g., analgesia vs. respiratory depression), but the usefulness of these distinctions has come into question (Dietis et al., 2011).

5.3.1 Opiate Receptors

Exogenous opiates such as morphine, and endogenous pain-relieving molecules called enkephalins, bind to the same types of opioid receptors located throughout the body. That stimulation of peripherally located opioid receptors can cause bowel dysfunction and pruritus has been known for many years. More recently it was discovered that peripheral receptors, in addition to producing analgesia, also play a role in wound healing and cardioprotection. Human opiate receptors have been cloned and expressed in tissue culture and their behavior studied in *knockout* mice. The DNA sequences of these receptors, if not their exact mechanism of operation, are known, largely from work with mice bred to be missing (or contains exaggerated amounts of) parts or all of a given receptor. One especially important consequence of this study method is that receptors can now be identified and counted, even in postmortem material (Gabilondo et al., 1994; Wehner et al., 2000). As a result, we now know that μ-opioid receptor is upregulated in inflammatory conditions (Zollner et al., 2003). Inflammation triggers the synthesis and axonal transport of opioid receptors in dorsal root ganglia (DRG) with resultant μ-opioid receptor upregulation and increased μ-opioid agonist efficiency at peripheral nerve terminals (Jeanjean et al., 1995).

The three opiate receptors are approximately 70% homologous, meaning that their structures have in common 70% of their amino acids. Differences between receptors occur mainly at the N- and C-terminal ends. Morphine and all of the clinically significant opiates exert their effect at the μ receptor. Opiate receptors are connected to a long chain of amino acids folded in such a way that they loop in and out of the cell membrane. The chain connects to a receptor sitting on the outer cell surface; opiate receptors (and many others,

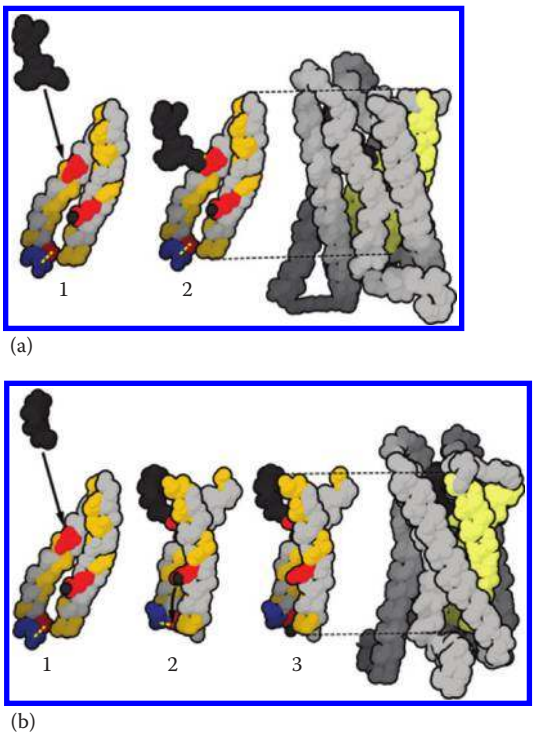


Figure 5.2 Morphine receptor and mechanism of (a) antagonist and (b) agonist binding.

such as adrenergic receptors) cross the cell membrane seven times and thus are described as having seven transmembrane domains (Chazot and Strange, 1992). No matter whether morphine or oxycodone or fentanyl binds with a μ-receptor, the seven transmembrane units become activated. Once that occurs a signaling cascade is initiated (also called second messenger pathways) and effector proteins activated, but only for as long as the opiate, or other ligand, remains connected to the receptor (Figure 5.2).

The μ-receptor is usually located on the presynaptic side of the synapse. These are mainly located in the periaqueductal gray region of the brain. High densities of μ-receptors are also found in the superficial dorsal horn of the spinal cord. Other areas rich in μ-receptors include the external plexiform layer of the olfactory bulb, the nucleus accumbens (an area deeply implicated in the process of addiction), some parts of the cerebral cortex, and some of the nuclei of the amygdala (Herz and Millan, 1990). μ-Receptors avidly bind enkephalins and beta-endorphin as well as morphine. The existence of different single-nucleotide polymorphisms (SNPs) in the coding region of the μ-opioid receptor gene has been known for more than a decade. The most prevalent SNP is a nucleotide substitution at position 118 (A118G), predicting an amino acid change at a putative *N*-glycosylation site. This SNP displays an allelic frequency of approximately 10% in the U.S. population (Bond et al., 1998). The presence of these SNPs partially determines an individual's response to pain medications and may even relate to the likelihood of the risk of addiction (Fukuda et al., 2010).

Morphine causes pupils to constrict because it excites the parasympathetic nerves supplying the pupil. Opiates cause respiratory depression because they activate μ-receptors in brain stem respiratory centers. When μ- and δ-receptors in the respiratory center are stimulated, they become less responsive to carbon dioxide; respiration is decreased and may even

stop. At the same time, μ-stimulation also depresses respiratory centers located in the pons, further inhibiting the respiratory drive. While opiate-induced respiratory disturbances are mainly due to agonist activation of μ- and δ-subtypes of receptors, endogenous dopaminergic modulation in the central nervous system (CNS) and carotid bodies enhances CO_2-dependent respiratory drive and depresses hypoxic drive. Within the CNS, synthetic agonists with selectivity for D_1 and D_4 types of receptor slow respiratory rhythm, whereas D_2-selective agonists modulate acute and chronic responses to hypoxia. D_1-receptor agonists also act centrally to increase respiratory responsiveness to CO_2 and counteract opiate blunting of CO_2-dependent respiratory drive and depression of breathing (Lalley, 2008).

Most opiate-related deaths are due to suppression of respiratory drive. Some of morphine's other side effects, such as nausea and vomiting, are also the result of μ-receptor stimulation, but these receptors are located in the chemoreceptor vomiting trigger zone of the medulla. Morphine compounds that bind the μ-receptor are also used to treat diarrhea. Morphine and heroin (which is rapidly converted to morphine once within the body) are both powerful cough suppressants (Todaka et al., 2000). Indeed, heroin (Figure 5.3) was originally sold by Bayer as a cough suppressant (Karch, 1989).

Dynorphin is one of a class of peptides produced by many different types of neurons, and it is also classified as an endogenous opioid peptide. Dynorphin functions primarily as a κ-opioid receptor agonist, meaning that it acts mainly at κ-opioid receptors, via which it modulates the pain response. It is a substantially more potent pain reliever than morphine (Han and Xie, 1984). Yet, for reasons that are not understood, it can cause pain by interaction with bradykinin receptors (Lai et al., 2006a). In experimental animals cocaine causes elevated concentrations of dynorphin to increase further, as do stress and depression in humans (Nestler and Aghajanian, 1997; Land et al., 2008). The same molecule has also been implicated in appetite control, circadian rhythm, and temperature control.

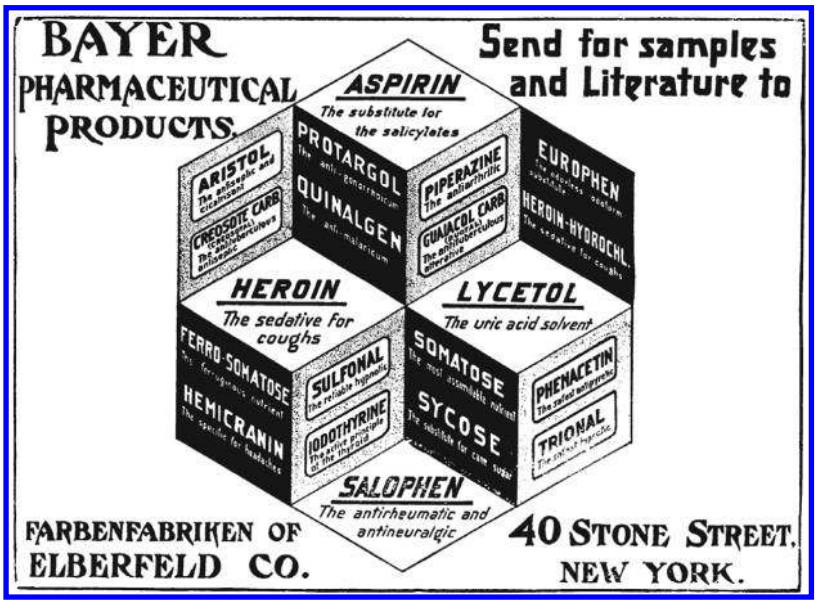


Figure 5.3 Heroin was first marketed as a cough suppressant and recommended for the treatment of tuberculosis. Bayer began selling heroin in 1898. The name heroin derives from the German for "great" or "heroic." (Courtesy of National Library of Medicine.)

5.3.2 Opiates and G-Coupled Proteins

G-coupled proteins are special proteins associated with opiate and other types of receptors. Their main purpose is the transmission of signals from outside cells to within them. Disruption of this signaling system can lead to disease such as diabetes, blindness, allergy, depression, cardiovascular defects, and certain forms of cancer. It is estimated that more than half of the drugs now in use act by exerting an effect on a G-coupled protein (Wu, 2010). GTP is the guanosine analog of ATP. When a signal (such as morphine binding to a cell wall receptor) reaches a G protein, the protein exchanges GDP for GTP causing some specific action within the cell to occur. Another type of G protein, known as *small G protein*, is believed to regulate many activities within the cell (i.e., control the cell components such as the endoplasmic reticulum and Golgi apparatus) (Wennerberg et al., 2005). Still a third set of G proteins connects the receptors directly to ion channels. In total, the family of G proteins is believed to relay signals from more than 1000 different receptors and, indirectly, control the activity of ion channels and even enzymes.

The portions of receptors that penetrate the cell membrane are referred to as helices. Different types of receptors penetrate the outer membrane variable numbers of times. As a rule, most of the compounds involved in the process of addiction (and the pathologic changes that result from them) involve the seven transmembrane domains mentioned earlier. The list of compounds includes epinephrine (adrenaline), glucagon, 5-HT (serotonin), vasopressin, adrenocorticotropic hormone (ACTH), and adenosine, among many others. In general, small ligands bind to the amino acid residues in the membrane, whereas large polypeptide and protein ligands bind to the extracellular domains, which are much larger in size and better able to combine with large protein molecules.

5.3.3 Opiate–Receptor Interactions and Naloxone Reversal

The effectiveness of any pain reliever (and its addiction potential) is determined by interactions between the drug and the receptors. Obviously, not all opiates have the same molecular structure. Perhaps less well known is that neither do opiate receptors. Not only are there different generic types of receptors (δ, κ, μ), the receptors themselves are subject to mutations, which, in turn, affect the goodness of fit between opiate and receptor. In other words the association–dissociation kinetics determines the drug's effect. It also controls how well and how long narcotic antagonists exert their effect. Just how long any particular opiate occupies a given opiate receptor is different in every case, and this difference is expressed as an association–dissociation constant. Some opiates disassociate from receptors faster than others. In addition, after ingestion, some drugs take longer to arrive at their receptor, which may have as much to do with speed and duration of onset as receptor fit itself.

Excessive stimulation of the opiate receptor, of course, results in respiratory depression, reversed by treatment with naloxone, a noncompetitive antagonist for the opiate receptor. The ability of naloxone to effectively reverse opioid-induced respiratory depression depends on many factors, including the pharmacologic properties of the opioid that needs reversal and its interaction with naloxone (Olofsen et al., 2010). There is, for example, a three- to fourfold difference in naloxone's ability to reverse the effects of morphine and morphine-6-glucuronide (M6G), its active metabolite. The most likely explanation for naloxone's higher potency against M6G than morphine, and lower potency against buprenorphine, is that M6G may increase naloxone affinity for the target receptor.

The rate (speed) of naloxone reversal depends on the opioid agonist receptor association–dissociation kinetics: lower values of k_{on} and k_{off} make reversal more difficult, and consequently the reversal process is slower. The most notorious example of this effect is seen with buprenorphine, where very large doses of naloxone may be required to achieve the desired effect. Some have even concluded that because of the slow receptor association–dissociation kinetics of buprenorphine (Escher et al., 2007) and the fast elimination kinetics of naloxone, naloxone is best administered as a continuous infusion for reversal of buprenorphine-induced respiratory depression (Yassen et al., 2007). The truly important forensic issue is that an increase in naloxone dose will increase the duration of reversal but the speed of reversal remains unchanged. Morphine, for example, has an "on–off" time (the length of time it occupies the opiate receptor) of 0.8 s, while M6G persists for nearly three times as long (2.72 s). Studies in human volunteers show that the maximal effect of morphine-induced respiratory depression does not occur until nearly 30 min has elapsed, and that maximal reversal (after 200 μg naloxone) requires nearly 12 min (Olofsen et al., 2010). The times for buprenorphine are longer still, where the use of a combined biophase equilibration–receptor association–dissociation pharmacodynamic model makes clear the competitive interaction between buprenorphine and naloxone at the opioid μ-receptor. For buprenorphine, even in the absence of naloxone, the rate constants of receptor association (k_{on}) and dissociation (k_{off}) are 0.203 mL/ng/min and 0.0172 min, respectively. The half-life ($T_{1/2}$) of biophase equilibration was 173 min. These estimates of the pharmacodynamic parameters are similar to values obtained in the absence of naloxone coadministration. For naloxone, the half-life of biophase distribution was 6.5 min—put another way, more than 6 min must elapse before naloxone can even reach its target receptor (Yassen et al., 2007).

5.4 History

5.4.1 Origins in Antiquity

Drawings of opium poppies antedate any written mentions in the Greek literature by at least 1000 years (Kritikos and Papadaski, 1967). Homer and Hesiod discussed the medicinal merits of poppies, and writings from the classical period frequently dwell on the same subject. In Greece, the poppy was called *opion*, a term derived from the word *juice* (*opos*). When translated into Latin, *opion* becomes opium. For the ancients, the poppy symbolized sleep, occasionally everlasting. The cup given to Socrates contained the standard solution used at the time for euthanasia and suicide: a mixture of hemlock and opium. Opium was known but used sparingly in Europe during the Middle Ages, possibly because medieval surgeons seemed to have been largely indifferent to the suffering of their patients (Kramer, 1979).

5.4.2 Introduction to Europe and Asia

Opium's popularity increased during the Renaissance. Much of the popularity had to do with the success of the efforts made by Philippus Theophrastus Aureolus Bombastus von Hohenheim, a.k.a. Paracelsus (1493–1541). Paracelsus recognized that no matter what the cause of a disease, sleep and pain relief were part of the cure. Following that precept, Paracelsus medicated his patients with formulas that contained opium. He prescribed opium in a host of different formulations, calling one of them "laudanum" (from the Latin for "something to

be praised"). Laudanum was comprised of one-fourth opium, to which was added henbane juice, crushed pearls, and coral, "bone of the heart of a stag, bozar stone, amber, musk, and essential oils." Paracelsus also used opium in combination with orange and lemon juice, frog sperm, cinnamon, cloves, ambergris, and saffron (Macht, 1916).

More streamlined versions of laudanum were used well into the nineteenth century (Lewin, 1931). In the same way that Freud later enthusiastically recommended cocaine as a wonder drug (Freud, 1884), Sydenham (1624–1689) argued that opium was the drug of choice for a range of conditions, not all of them painful (Sydenham, 1848). Thomas Dover, a ship's doctor and one of Sydenham's students, will go down in history for two different and unrelated reasons: he rescued the real Robinson Crusoe, and he created a powdered opium formulation that became an immensely popular home remedy. Dover's powder was still being sold in stores in the early 1900s. These developments may explain why medical writers began discussing the issue of opiate toxicity in the early 1700s.

In their groundbreaking book on addiction, Terry and Pellens (1928) quote a naïve English physician who claimed to have separated opium's *noxious quality* from its *palliative* and *curative* actions, thus avoiding the complications associated with excessive opium use. Physicians overrelied on opium because it was one of the few drugs they could prescribe that worked; it relieved pain, calmed the stomach, and suppressed coughs. Until the twentieth century, few other drugs were as effective. Because opium was widely available and widely used, it was inevitable that many people would become addicted (Haller, 1989).

In a letter to the editor of *Trommsdorffs Journal der Pharmacie* (Vol. 13, 1805), a young chemist, Friedrich Sertürner, reported on the isolation of a substance from opium that was alkaline in nature. In 1806, Sertürner moved to Einbeck where he first worked as assistant to the tenant of the magistrate's pharmacy and published yet another paper on his discovery (Serturner, 1806). In Einbeck, Sertürner continued his research work on morphine and published two papers describing his results. In one of these (1817), he introduced observations made with the drug in humans and for the first time called it "morphine." The French chemist Gay-Lussac read the paper and had it translated into French. Sertürner published a report announcing that he had isolated an alkaline base in opium called *morphium*. He continued his research on *morphium* for many years, frequently using himself as a subject; at one point he nearly died of an overdose. His discovery of morphine had vital clinical importance (Weiser, 1956), but it also marked a sea change in the way researchers thought about the chemicals contained in plants and the way in which they worked. Prior to the discovery of morphine, it was universally believed that plants could only synthesize products that were acid or, at most, neutral. It was believed that only metallic compounds could be alkaline. Sertürner's discovery changed that. In relatively rapid succession, hundreds of other potent plant alkaloids, including quinine and cocaine, were isolated (Macht, 1916). Commercial morphine production began not long after morphine was first isolated. The founder of England's Royal Pharmaceutical Society, Thomas Morson, started refining and selling morphine in 1821. Shortly after, the great pharmaceutical company, Merck of Darmstad, began wholesale production (Berridge, 1987).

Addiction and abuse had become major problems by the dawn of the nineteenth century, although there is some evidence to suggest that morphine addiction (as opposed to opium eating) may not have been all that widespread (Kramer, 1979). Patent medications such as Dover's powder and other *cordials*, *carminatives*, or *soothing syrups* were nothing more than tincture of opium combined with flavorings and ample amounts of alcohol (Figures 5.4 and 5.5). Case reports describing *morphia* toxicity began to be published with

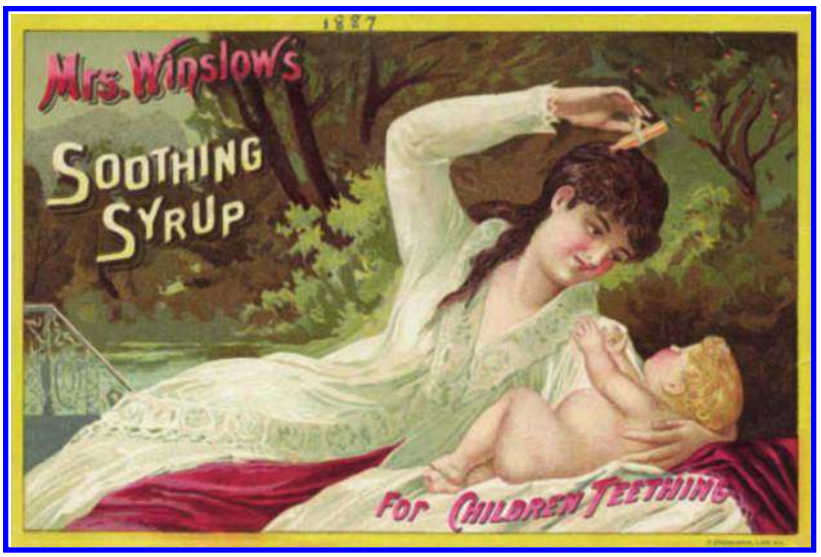


Figure 5.4 Mrs. Winslow Soothing Syrup for children teething. This product was popular before the passage of the Food and Drug Act of 1906. Infants cutting teeth generally are crying and irritable, but not after a dose of morphine. It was also said to be very good for treating colic. (Courtesy of Dr. Michael Bozarth.)

some regularity by the late 1830s. The best-known addict of that period was De Quincy. He had first used opium to treat a toothache, but he rapidly developed a formidable habit. At one point, he was consuming more than 20 g (not grains)/day (De Quincy, 1821). While he was only one of many addicts to be found within London's artistic community, he was the most vocal advocate for opium, having written, among other things, "happiness might now be bought for a penny, and carried in the waistcoat pocket." De Quincy's *Confessions of an English Opium Eater* was first published in 1821, and a revised, considerably expanded, second edition was published in 1856. That same year, Elizabeth Barrett Browning published her acclaimed narrative poem, *Aurora Leigh*. Although Browning was also addicted, and the poem was highly autobiographical, she never argued that much good came from the habit (Bishop, 1994). This probably explains why De Quincy's name is synonymous with drug use and Browning's is not.

Arab traders introduced opium into China during the Tang Dynasty (AD 618–907). At first, the Chinese used opium only for medicinal purposes. The *Pen Tsao Kang Mu*, a *materia medica* published in 1590, nearly 1000 years after opium was first introduced into China, makes absolutely no mention of addiction or abuse (Way, 1982). Opium was only taken orally, and then only for treatment of pain and diarrhea. Opium smoking, which probably originated in Java, began nearly a millennium later. The first mentions of opium smoking in China are from the sixteenth century, occurring at just about the same time that the Portuguese were introducing tobacco to the Chinese.

Over the next two centuries, the popularity of opium smoking steadily increased. In 1880, for reasons having more to do with a balance of trade deficit than any concerns with abuse, Emperor Chin Ching banned opium importation. The East India Company ignored the ban and continued to smuggle large amounts of opium into China. In 1839, the Chinese government finally decided to take active measures against opium importation. The measures prompted England to declare a war that China quickly lost. Customs figures

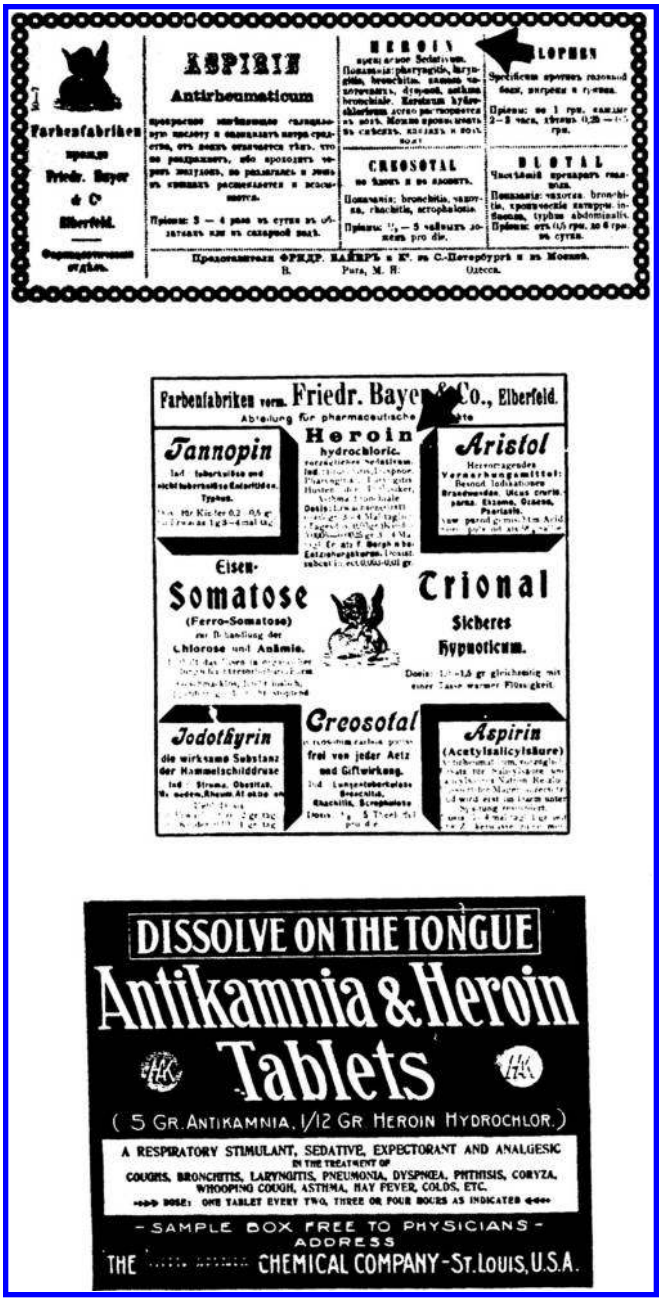


Figure 5.5 Heroin. Because of their effectiveness, heroin and heroin-containing products were not difficult to market. Many different formulations were sold in Europe and in the United States. (From Kritikos, P. and Papadaki, S., *Bull. Narc.*, 19(4), 5–10, 1967.)

from 1881 show that opium imports into China were in excess of 6 million kg per year, enough to supply one million smokers. In spite of numerous conventions and treaties, addiction remained a major problem in China until Mao Tse-tung suppressed the habit in the early 1960s.

Striking historical parallels in the evolution of opium and cocaine abuse are apparent. Thousands of years of coca leaf chewing in South America caused few social and no

detectable medical problems for the Incas. However, as soon as purified cocaine became widely available in Europe, the amount of cocaine used increased greatly. As the amount used increased, so did toxicity (Karch, 1989). Taking small amounts of opium orally was medically effective and, at worst, a benign indulgence. Much of orally administered opium is inactivated on its first pass through the liver, so this route of ingestion has some built-in safeguards. Smoking opium is another matter entirely. When smoked, much more morphine gets into the body via the lungs, blood levels rise more quickly, and no *first-pass* effect occurs. The net result is that when opium is smoked, the dosage is effectively multiplied. Not surprisingly, serious toxicity and addiction result.

Chinese laborers are said to have introduced opium smoking into the United States, but opium was already popular in America long before the Chinese immigration. In 1844, the New York City coroner held 6 inquests regarding opium-related deaths and 23 inquests on deaths related to laudanum (Woodman and Tidy, 1877). According to the U.S. Government figures, over 5 million tons of opium was imported into the United States from 1850 to 1877. This figure does not take into account opium smuggled in to avoid taxation, or any of the opium cultivated domestically. Opium was produced in California, Arizona, and the New England states (Brecher, 1972). Like their European counterparts, American physicians would have been unable to practice without opium. A survey done in Boston in 1888 disclosed that, of 10,000 prescriptions dispensed by 35 pharmacies, 15% contained opium and 78% contained opiates (Brecher, 1972). Whatever the problems associated with opium abuse, they very likely would have been manageable had the hypodermic syringe not become available in the 1870s, and had heroin not been introduced at the turn of the century.

5.4.3 Invention of the Hypodermic Syringe

In 1855 a Scottish physician, Alexander Wood (Figure 5.6), published an account of his experiments injecting humans with opium (Brecher, 1972). He injected tincture of opium, and although his original intent was to achieve something akin to a nerve block, he quickly realized that injected morphine was carried throughout the body. In the course of his experiments, Wood also managed to addict his wife to intramuscular morphine. She probably was the first woman to die of an injected narcotic overdose (Terry and Pellens, 1928). Wood may have received most of the credit, but the idea of injecting people with narcotics had been around for hundreds of years before Wood was born. Christopher Wren, the famous architect and professor of astronomy at Gresham College, Oxford, was also a physician. According to the history of the Royal Society, Wren injected dogs with intravenous opium in 1656. Using a quill attached to a small bladder, he injected lean animals with easily visible veins. No fatalities resulted. Wren was so encouraged by his preliminary studies that the following year he tried the same experiment on a man. An ambassador to the Court of St. James volunteered the services of a *delinquent servant*. The volunteer was injected with an emetic, which made him faint. Other experiments were even less successful, and this area of research was ignored for nearly the next 100 years (Terry and Pellens, 1928).

Wood's publication prompted others to experiment with injecting many different drugs, but narcotics attracted the most interest, and narcotic injection soon became standard practice. Hypodermic syringes were said to have been in great demand and short supply during the U.S. Civil War (Figure 5.7) (Billings, 1905), although the shortage could not have been all that severe, as many of the veterans became addicts. Addiction was slower to

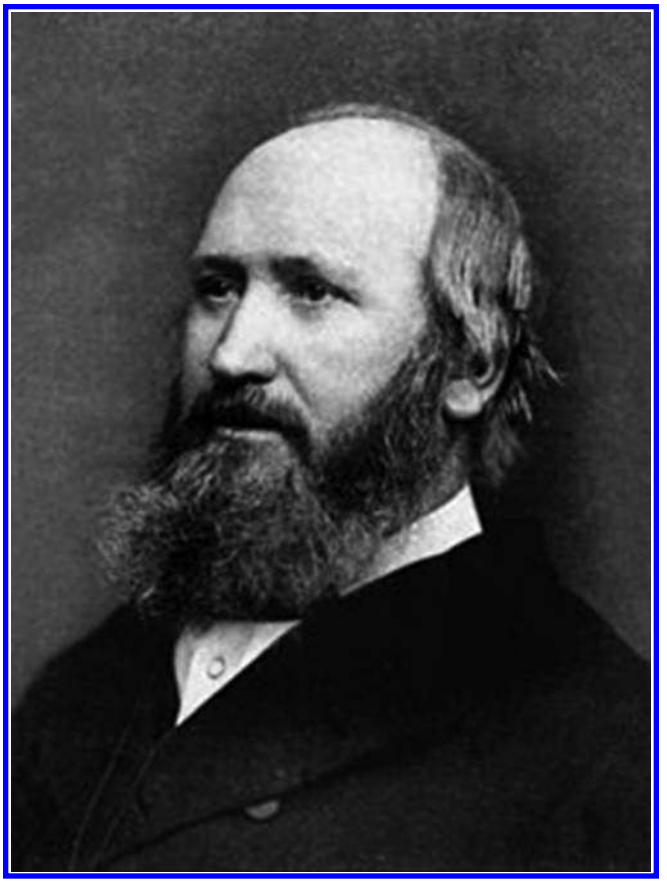


Figure 5.6 Alexander Wood, inventor of the hypodermic syringe.

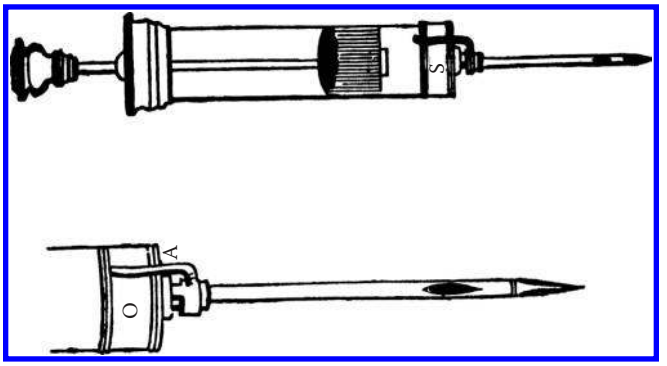


Figure 5.7 Hypodermic syringes. Commercial production of syringes began just before the Civil War. Initially, opiates were injected only subcutaneously. The intravenous injection of morphine and heroin did not become common practice until the 1920s. (Courtesy of National Library of Medicine.)

evolve as a problem in America than in Europe, but by the 1870s *morphinism* was rampant in both the Old and New Worlds. The lag time may have been partially due to the fact that hypodermic injection did not catch on as quickly in the United States as in Europe.

Even though addiction was common, neither the mechanism of opiate action nor the process of addiction was even remotely understood. It was widely thought, for instance, that using morphine injections, as opposed to *eating opium*, minimized the probability of addiction (Anstie, 1868; Howard-Jones, 1972). Treatment modalities for addiction were simplistic to the extreme. Freud's paper *Über Coca*, published in 1884, reflects the thinking of many during that period. Because the effects of cocaine seemed to be so opposite to those of morphine, Freud concluded that cocaine would be a logical treatment for *morphinism*. Some prominent physicians, including Erlenmeyer (1885), disagreed, but Freud's notions were widely accepted, and a large group of patients became addicted simultaneously to cocaine and morphine. It is only quite recently, since the discovery of opiate receptors and neurotransmitters, that rational approaches to narcotics addiction have been formulated.

5.4.4 Synthesis of Heroin

The other key development in the history of narcotic addiction was the synthesis of heroin. In 1874, C.R. Wright, a researcher at St. Mary's Hospital in London, boiled anhydrous morphine with acetic anhydride and produced a series of acetylated morphine derivatives (Eddy, 1953). One of the derivatives was diacetyl morphine (although the nomenclature was different at the time). He sent samples to an associate at Owens College, London, who assayed the substance for biological activity. The ability of the drug to decrease respiratory rate and blood pressure quickly became obvious. For reasons that are not clear, the discovery created very little interest. In 1898, Strube published a paper outlining his favorable results when he had used heroin to treat patients with tuberculosis. He found that the drug effectively relieved severe coughs and allowed patients to sleep. Perhaps more important, he claimed to have observed no ill effects (Strube, 1898). The Bayer Company in Elberfeld, Germany, began commercial production of heroin in 1898.

Bayer had been in the business of making pharmaceuticals since 1889, but not exactly making great sums of money. The situation changed almost overnight when they began to supply the really profitable market for alkaloids (morphine, quinine, and cocaine). Previously this market had been dominated by other, larger companies such as Merck, Knoll, and Boehringer. Bayer's lead chemist, Felix Hoffman, synthesized heroin on August 21, 1897, just 2 weeks after he produced aspirin! Bayer pharmacologists began experimenting with both codeine and heroin, carrying out a number of tests on themselves, animals, and their employees. The Bayer chemists concluded, quite mistakenly, that heroin produced less respiratory depression than codeine (in fact, codeine only has activity as a pain reliever because it is metabolized to morphine, a heroin breakdown product). Based mainly on Strube's observations, Bayer began production, marketing heroin as a safer, more potent cough suppressant (see Figure 5.3) (deRidder, 1994).

Whatever the medical profession believed about heroin, it was warmly received by the underground. By 1920, heroin addiction was such a problem that the House of Delegates of the American Medical Association (AMA) voted to prohibit heroin importation, manufacture, and sale. Legitimate heroin production in the United States ceased after 1924, although low levels of illegal imports persisted. Interestingly, it seems that no one thought to inject heroin intravenously until the early 1920s. The dating is suggested by the fact

that the first report describing typical track marks was not published until 1929 (Biggam, 1929). The outlawing of production, along with international treaties and conventions, but most especially the advent of World War II, led to sharp reductions in clandestine imports. In 1950, fewer than 40 heroin seizures were reported within the United States.

Interest in heroin resurfaced with the advent of the Vietnam War but was temporarily eclipsed by the medical community's general disinterest in sedative hypnotics and the superimposed cocaine pandemic. Heroin use, at least when judged by the amount of illicit heroin now being confiscated, is again increasing, at a remarkable rate, though the most striking increases seem to be in Europe. In the United States, the number of heroin addicts increased from 214,000 in 2002 to 359,000 in 2010 (Office of Applied Studies, 2012).

5.4.5 First Pathology Studies

The first autopsy demonstrating both cerebral and pulmonary congestion (the classic hallmarks of narcotic overdose) was that of a New Yorker who died of laudanum poisoning. A Dr. Lee, first name unknown, described the case in 1852 (Woodman and Tidy, 1877). The autopsy findings in a second case of narcotic overdose were described in a second paper published in 1862, but it may not have been entirely accurate. A young woman drank "gin mixed with a shilling's worth of laudanum." She quickly became comatose and intense meiosis was noted. Autopsy disclosed cerebral congestion; however, the lungs were said to be unremarkable (Slayter, 1862), and pulmonary congestion is now known to be a marker for narcotic overdose. A forensics text from 1877 finally mentions that "congestion of the lungs and of the vessels of the brain" are typically seen in opiate-related deaths but cautioned that that particular finding at autopsy was "neither certain nor characteristic" (Woodman and Tidy, 1877). Understanding of the problem advanced very little until Helpern and Rho published their paper *Deaths from narcotism in New York City* in 1966. In addition to carefully describing the epidemiology of the addiction, the authors systematically described all of the signs that have come to be classically associated with narcotism, including pulmonary edema, portal adenopathy, and track marks. During the years since their publication, opiate receptors have been discovered, and other disorders such as heroin-associated nephropathy (HAN) (Rao et al., 1974) and leukoencephalopathy (Wolters et al., 1982) have all been described. Nonetheless, our basic understanding of the pathologic changes produced by narcotic abuse has advanced very little.

5.5 Cultivation and Manufacture

5.5.1 Botany

The Papaveraceae family is comprised of 42 genera and approximately 650 distinct species. Just how they should be divided is a matter of some dispute, and at least six different classification schemes have been proposed. *Papaver somniferum* is the most commonly cultivated "opium" poppy, but the wild growing *Papaver setigerum* also contains significant amounts of morphine. Over the years many hybrids have been developed, and describing a generic "poppy" is difficult, if not impossible. Flowers may be single or double, with variation in both shape and color. Blossoms may be white, red, pink, purple, crimson, or one of the many shades in between (Figure 5.8). The capsules, from which

Figure 5.8 Red opium poppy in full bloom. (Courtesy of DEA, Washington, DC.)

the juice is extracted, also vary in shape and alkaloid content. A plant can have two, three, or more capsules. Height is also variable and may range from 30 to 150 cm or more (Kapoor, 1995).

The poppy is an annual plant, with a 3–5-month life cycle. This means that, even though the poppy can be cultivated almost anywhere, only one crop per year can be grown in areas with distinct hot and cold, or wet and dry seasons. Poppies cannot be grown in areas subject to frost. The more moderate climate to be found in many parts of Latin America permits year-round cultivation, an advantage that has not gone unnoticed by heroin producers.

When grown in humid regions, the poppy is vulnerable to infection by fungi and plant parasites. Poppies grow well in average soil, but the soil requires treatment with manure or chemical fertilizers. Plants take 2–3 weeks to germinate and 2 months to fully develop. After a field has been weeded and thinned, as many as 15 plants can be grown in a square meter. After the plant flowers and the petals have fallen off, the capsule continues to ripen for another 2 weeks, at which time the opium-containing latex can be harvested. The entire cycle takes less than 3 months.

Traditional harvesting is a two-step process. First the capsule is incised, allowing the sap to run out and then solidify (Figure 5.9). Twelve hours after the capsule has been incised, the latex is harvested. Incising the capsule is a delicate operation: if the incision is too deep, the latex will run down the inside of the plant and be lost to harvest. Farmers prefer to do the incising at sunrise or sunset. That allows the latex to exude and solidify for 8–14 h. The caked latex can then be scraped off the capsule using a dull blade; however, by the mid-1970s, traditional harvesting techniques had been largely replaced by the use of opium "straw," where instead of collecting resin from the sides of the capsule, the entire plant is dried and processed. As the number of fields planted has increased, there seems to have been a revision to older techniques, particularly in Afghanistan, which is now the world's principal opium producer.

Figure 5.9 An incised poppy bulb. A sharp razor-like knife is used to incise the bulb, allowing the latex to leak out. Once it is dried, it is scraped off the bulb.

The opium yield per acre depends on many variables. Historically, the yield in the Mediterranean is said to be 10 kg of opium/ha. The most recent surveys show that yields in Afghanistan are just over 39 kg of opium/ha.

Yields in the newer fields being established in South America and the Central Asian republics have yet to be determined, but it seems likely that they are intermediate between the high yields reported from India and the much lower yields reported from Mediterranean countries.

Over 20 different alkaloids have been identified in opium, but only 3 are of any significance: morphine, codeine, and thebaine. Thebaine has almost no morphine-like activity of its own, but it can be used to manufacture other narcotic agents. Hundreds of semisynthetic derivatives, referred to as Bentley compounds, have been synthesized from thebaine, and many of these do have narcotic effects. A few of the derivatives, such as etorphine, have 1000 times the activity of morphine. The increasingly popular heroin substitute, buprenorphine, is also synthesized directly from thebaine (Elkader and Sproule, 2005). Noscapine, which was once widely used as an antitussive, has emerged as a possible antineoplastic drug and is now in clinical trials (Aneja et al., 2007).

Morphine is the principal alkaloid found in opium. It constitutes between 8% and 19% of air-dried opium. Reported ranges for codeine content are from 1.25% to 3.4% (Anon., 1963). Poppy straw, depending on the country of origin, may have a morphine content of anywhere from 0.3% to 1.3%, all located mainly in the mature capsules of the plant and in the upper part of the stem (INCB, 1998).

Poppy seeds sold for cooking and baking purposes may contain very substantial amounts of morphine and codeine. In one study, the morphine content was found to be anywhere from 7.3 to 60.1 mg/g of seed, while the codeine content ranged from 6.1 to 29.8 mg/g (Pelders and Ros, 1996). Even commercial poppy-seed fillings, used to make pastries, have high alkaloid contents. Concentrations in the range of 17.4–18.6 mg/g (morphine)

and 2.3–2.5 mg/g (codeine) have been reported in different lots of the filling. Urinary morphine concentrations as high as 4.5 μg/L have been reported after eating these fillings, and large amounts of morphine may persist in the urine for several days after ingestion (Mule and Casella, 1988). This has posed difficulties for workplace drug testing programs where urine is screened for morphine. The initial screening cutoff of 300 ng/mL was raised to 2000 ng/mL in the 1990s; however, recent studies show that even that cutoff is too low, given that the quantity of poppy seeds contained in two bagels will cause urine to test positive for more than 24 h (Rohrig and Moore, 2003).

Recently, better controlled studies have been published. Volunteers were given two 45 g oral doses of poppy seeds 8 h apart, each containing 15.7 mg morphine and 3 mg codeine, and urine was collected ad libitum up to 32 h after the first dose. Morphine concentrations ranged from <300 to 7522 μg/L with a median peak concentration of 5239 μg/L. The median first morphine-positive urine sample at 2000 μg/L cutoff concentration occurred at 6.6 h (1.2–12.1), with the last positive from 2.6 to 18 h after the second dose. No specimens were positive for codeine at a cutoff concentration of 2000 μg/L, but 20.2% exceeded 300 μg/L, with peak concentrations of 658 μg/L (284–540 μg/L) (Smith et al., 2014).

Another way to avoid this problem is to test for acetylcodeine, which is only found in acetylated opium or heroin, not poppy seeds. The problem is that this compound has a relatively short half-life (Trafkowski et al., 2006). The same holds true for 6-acetylmorphine (6-AM), a unique heroin metabolite; however, its window of detection in urine is only a matter of hours.

5.5.2 Opium Production

From the end of World War II until the late 1980s, opium production was confined to two primary areas: Southeast and Southwest Asia. Since then production and output have shifted in unpredictable ways from country to country. Production in Asia's notorious Golden Triangle, once the world's center for narcotic production, is decreasing and production in Myanmar (formerly known as Burma) seems to be increasing (UNODC, 2014). Production in Afghanistan continues to increase at a frightening rate (Figures 5.10 through 5.13).

According to statistics accumulated by the UN, Afghan poppy production has increased for the third consecutive year, with the area under cultivation having increased from 154,000 ha in 2012 to 209,000 ha in 2013. In 2013, the estimated global production of heroin rebounded to levels last seen in 2008 and 2011. The global area of illicit opium cultivation in 2013 is estimated at 296,720 ha—the largest area since 1998, when estimates first became available. There is also evidence that the market for Afghan heroin is expanding, especially into new markets in Oceania and Southeast Asia, areas that had previously been supplied by the "golden triangle" countries (DEA, 2000; UNODC, 2014).

5.5.3 Heroin Manufacture

Heroin can be manufactured directly from opium, from semipurified morphine, or from poppy straw (Figures 5.14 and 5.15). The route utilized depends mostly on the availability of the precursors (INCB, 2006). Morphine and opium are both sold on the illicit market, and the availability of one or the other depends largely on local conditions, including the availability of chemical precursors, especially acetic anhydride (1250 L were seized in Afghanistan in 2008, while virtually none was confiscated in neighboring countries) (DEA, 2010; INCB, 2014).

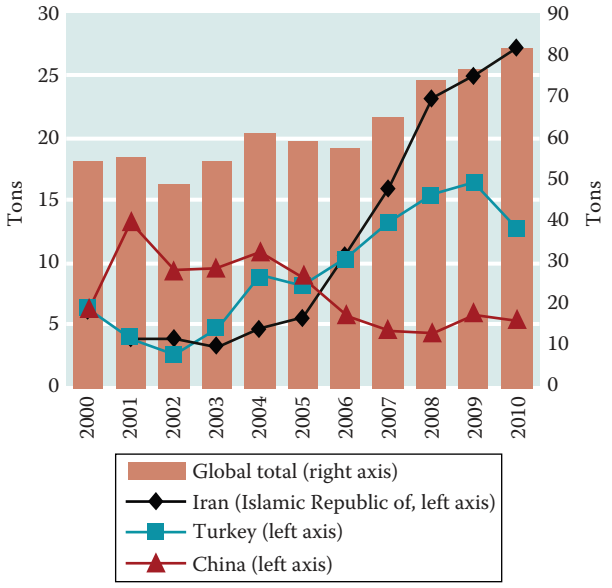


Figure 5.10 Heroin seizures worldwide and in selected countries (tons), 2000–2010. (Courtesy of UNODC, Vienna, Austria; Annual report questionnaire supplemented by other official sources.)

Figure 5.11 This single seizure, made 200 miles south east of Mogadishu netted 538.68 kg of heroin. The street value would have been in excess of $100 million dollars. (From Combined Maritime Forces, an anti-drug, anti-terrorism multi-national partnership, http://combinedmaritimeforces.com/2013/12/22/cmf-makes-its-largest-heroin-seizure-worth-us107-million, last accessed May 18, 2014.)

Until production recently exploded in Afghanistan, for many decades opium straw had been increasingly used as the starting material for opiate production. Extract derived from poppy straw has a morphine content that ranges from 40% to 80%.

The clandestine separation of morphine from crude opium involves three separate steps. A kilogram of opium is dissolved in 2 L of water along with 200 g of lime, and the resultant solution is poured through a coarse filter. Then, 250 g of ammonium chloride is added to the filtrate, causing the morphine base to slowly precipitate out. The morphine is

Figure 5.12 Bags of opium on the streets in Kandahar. (From U.S. Army, www.arm.mil, last accessed September 11, 2012.)

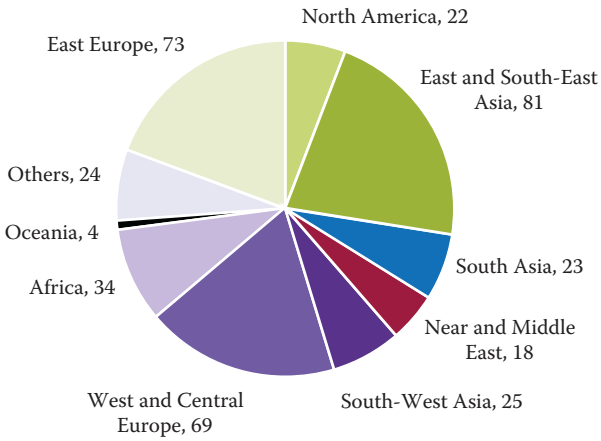


Figure 5.13 Heroin consumption by region. (Courtesy of UNODC, Vienna, Austria.)

collected on a fine cloth filter and then washed with water. The crude morphine is mixed with charcoal, and with either hydrochloric or sulfuric acid. The mixture is filtered, and ammonium hydroxide is added to the filtrate, causing purified morphine to precipitate out. The precipitate is collected by filtration and allowed to dry in room air.

In the second phase of production, the dried morphine is added to acetic anhydride. Under the terms of an International Convention signed in 1988, sales of acetic anhydride are controlled by International Law. All exports must be reported to the UN, which operates a monitoring program known as Operation Topaz (a similar operation to monitor sales of potassium permanganate, used in the production of cocaine, is known as Operation Purple) (U.N., 2005). Afghan seizures of acetic anhydride continue to rise, even though that country has absolutely no legitimate need for that chemical. Throughout the world, multiton seizures of this compound are not uncommon. In one

Figure 5.14 Asian refined heroin. (Photograph from the website of the DEA.)

Figure 5.15 *Black tar heroin*. The same term is applied to heroin of Mexican and Iranian origin. It is black because it is semi-refined. Microscopic examination will disclose plant and animal parts that may explain why skin infection among abusers is so common. (Photograph from the website of the DEA.)

case, reported to the International Narcotics Control Board (INCB), 46 tons of acetic anhydride were smuggled across the border from China into Pakistan. Illicit heroin production can be, to some extent, gauged by demand for acetic anhydride, the key agent in conversion from morphine to heroin. Because the uses of acetic anhydride are so well known and because sales are tracked by governmental authorities, alternative agents have been used. In the past, ethylidene diacetate was used by Southeast Asian producers. More recently, it has been reported that acetyl chloride was being substituted for acetic anhydride in Southeast Asian clandestine labs (INCB, 2006), though there is little evidence of this trend spreading.

Still another surrogate for heroin production is the price of acetic anhydride in the illicit market. Finished heroin cannot be produced without it, and there are no uses in Afghanistan for acetic anhydride except to make heroin. In Afghanistan, roughly one liter of acetic anhydride, the amount needed to convert 1 kg of morphine to heroin, costs U.S. $230, making the cost U.S. $340 for 1 kg of heroin (UNODC, 2014).

The dried morphine mixture is then refluxed with acidic anhydride at a constant temperature for 5 h. After the mixture has been allowed to cool, it is neutralized with sodium carbonate. The crude heroin that precipitates out is filtered and washed with water. In the final stage of production, heroin is purified by redissolving it in boiling water containing citric acid and charcoal. The mixture is filtered and purified, and heroin is precipitated by the addition of sodium carbonate. If the lab wants to produce the hydrochloride form of heroin instead of heroin base, the heroin is redissolved in acetone, and hydrochloric acid is added to the solution.

Depending on market demand, clandestine chemists sometimes synthesize morphine instead of heroin. Production begins by dissolving 1 kg of opium in 2 L of water and adding 200 g of slaked lime, 500 mL of alcohol, and 500 mL of ether. The resultant solution is then filtered through a cloth, leaving crude morphine on the cloth. This material is further purified and decolorized by refluxing it with 2 L of dilute sulfuric acid and 250 g of charcoal for about half an hour. This solution is then filtered, and ammonium hydroxide is added to the filtrate. The off-white, semipurified morphine that precipitates out is then air dried, and the hardened dried morphine granules are rubbed against a hard surface to produce a powder (Narayanaswami, 1985).

Until very recently, the large-scale conversion of morphine to heroin was not possible without the use of acetic anhydride or some chemical very much like it. However, a way to convert morphine to heroin at room temperature, using a mixture of trifluoroacetic anhydride and acetic acid, has been introduced. This method provides yields (close to 90%) that are almost comparable to those attained with acetic anhydride. Presumably a yield of nearly 90% would still be considered acceptable to illicit producers (Odell et al., 2006).

5.5.4 Sample Analysis

The ratio of heroin to acetylcodeine in illicit heroin is nearly the same as the ratio of morphine to acetylcodeine in the illicit morphine that was used to produce the heroin in the first place. The relationship is so constant that studies have shown that the ratio can be used to identify the sample's country of origin. The ratio is fairly high for samples emanating from Afghanistan (20.9:1) and quite low for specimens coming from China (6.38:1) (Narayanaswami, 1985). Traditionally, these differences, along with the detection of trace chemicals introduced during illicit process and transport, have been used to "profile" heroin samples, allowing enforcement officials to trace routes of distribution, and even laboratories (O'Neil and Pitts, 1992). The U.S. Drug Enforcement Administration has shown that impurities introduced during the heroin manufacturing process can be used to differentiate heroin produced in different source countries/regions. This information may also be used to compare analyses of heroin samples. The illicit manufacture of heroin results in the formation of trace level acidic and neutral impurities that arise from the reaction of acetic anhydride with morphine and other alkaloids found in opium. The presence or absence and relative amounts of these impurities in illicit heroin are a direct result of the manufacturing process used and, to a lesser extent, the origin of the opium itself. Generally, these impurities are related to, or arise from, a variety of compounds including narcotine, norlaudanosine, thebaine, and the tetrahydrobenzylisoquinolines (Morello et al., 2010). The routine isolation, separation, and quantitation of acidic and neutral manufacturing

impurities are now possible, allowing the origin of semirefined and refined heroin to be identified on a regular basis.

Alternatively it is now possible to profile the inorganic elements found in the sample. It has been demonstrated in controlled studies that profiling the 19 inorganic elements (Ag, As, Ba, Cd, Co, Cr, Cu, Mn, Mo, Ni, P, Pb, Se, Sb, Th, Tl, U, V, and Zn) generally found in heroin can be performed by using inductively coupled plasma mass spectrometry (ICP-MS). After Wilcoxon–Mann–Whitney test and correlation analysis, 10 element contents (P, V, Cr, Ni, Cu, Zn, As, Se, Pb, U) and 7 element ratios (U/Ba, Ba/Pb, Cd/Mn, Co/Ni, V/Cr, P/V, Cd/V) have been identified that can allow point of origin determination (Liu et al., 2014).

5.6 Heroin Diluents and Adulterants

Substances carried over from the original plant or from opium are referred to as *adulterants*. Substances added with the intent of altering the character of the heroin in some way are also called adulterants. Included in this latter group are compounds such as quinine, caffeine, and diphenhydramine and more exotic agents such as levamisole (an anthelmintic), a dangerous injectable anesthetic known as xylazine, and most recently alpha-methyl-fentanyl. Xylazine is a veterinary medication used for sedation, anesthesia, muscle relaxation, and analgesia in large mammals. It is actually an analog of the antihypertensive drug clonidine and is an agonist at the α_2-adrenergic receptors. Like all other α_2-agonists, xylazine has adverse effects, which include bradycardia, conduction disturbances, and myocardial depression (Wong et al., 2008). However, reports of adulteration with xylazine and alpha-methyl-fentanyl remain extremely uncommon.

The term *diluent* is reserved for those substances devoid of physiologic effects that are added to increase the bulk of the final product. In the past, heroin produced in different regions could be characterized by the adulterants and diluents that had been added. However, there is very little current information on what substances are being added, and older compilations of popular adulterants and diluents (as in past issues of this book) may no longer be relevant.

The type of material used as diluents varies from region to region and from time to time, depending on local conditions and on the preferences of the illicit manufacturer. In the early 1990s French chemists observed drastic shifts were occurring in the composition of heroin seizures. In the late 1980s, caffeine and mannitol were the diluents most frequently encountered by French officials, but by 1991 they had been almost entirely replaced by paracetamol (Chaudron-Thozet et al., 1992). Diluents are likely to have changed in the last decade, but no current survey data are available. Lactate and mannitol are still widely used, and periodically there are reports of bizarre compounds such as scopolamine (Dillmann, 1997), atropine (Perrone et al., 1999), and clenbuterol (Werder et al., 2006) causing outbreaks of toxicity. There is no satisfactory explanation for the use of these drugs other than, perhaps, they were available. In 2006 alpha-methyl-fentanyl began to appear in heroin sold in some parts of the United States (Wong et al., 2008).

A very large number of different agents found in illicit heroin have been added either as adulterants or as bulk excipients (Table 5.1). Fortunately, neither levamisole nor anything as likely to be toxic has been found in heroin but the situation is fluid and subject to change.

Table 5.1 Recognized Heroin Adulterants and Their Properties

Adulterant	Reason Added	Public Health Risks	Symptoms
Lactose	Bulking agent	Inactive	None
Lead	Manufacturing contaminant	Only mild effects at low doses	Abdominal weakness
Caffeine	Heroin vaporizes at lower temperature	Minimal	Insomnia anxiety
Procaine	Relieves injection pain	CNS toxicity at high doses	CNS nausea vomiting
Acetaminophen	Price, bulking agent	Minimal at low doses	GI side effects
Strychnine	Enhances stimulatory effects of heroin	Minimal at low doses	Spasm and convulsions
Phenobarbital	Enhances stimulatory effects of heroin	Large doses may be life threatening	Large doses may be lethal
Quinine	Mimics feelings felt by heroin injectors	Diverse adverse reactions	Renal failure, analeptic
Clenbuterol	Not known	Minimal at low doses	CNS effects at large doses
Scopolamine	Hard to detect bulking agent	Drowsiness at low doses	CNS depression

Source: The information contained in this table is adapted from Cole, C. et al., *Drug Test Anal.*, 3(2), 89, 2010. Detailed references for each of the drugs are included with the paper and should be consulted directly.

5.7 Prices and Purity (Figure 5.16)

Heroin consumption in the United States has hovered in the 20–25 tons range for the last decade, but prices have fallen dramatically. As can be seen from Table 5.2, peak prices have fallen from almost U.S. $1900/g, for 9% pure heroin in 1981, to U.S. $250/g for heroin that is 31% pure (Institute for Defense Analysis, 2008). It is speculated that

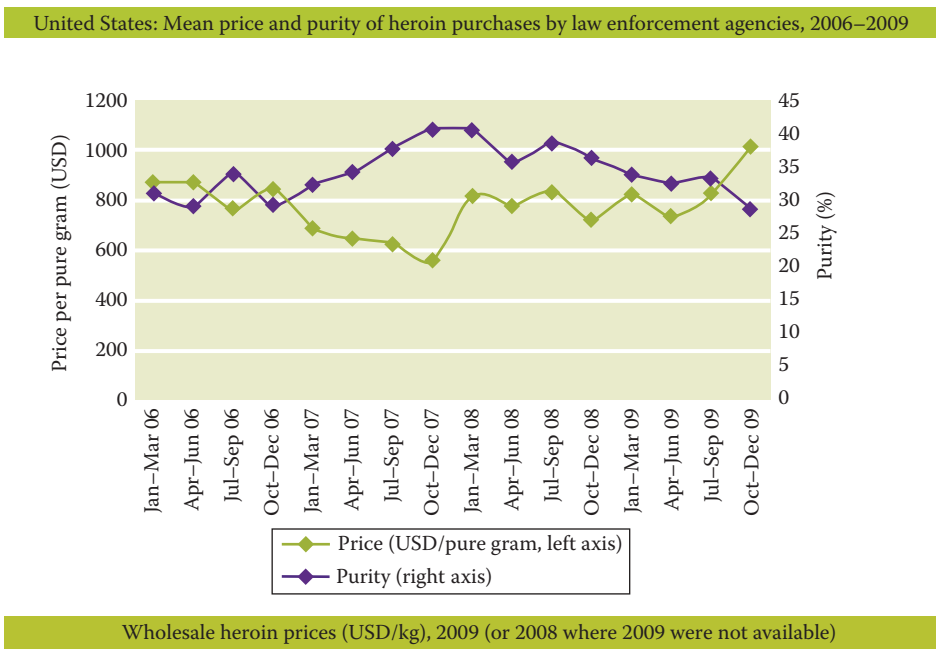


Figure 5.16 Mean price and purity of heroin purchased by law enforcement agencies, 2006–2009. No more recent information is available. (From U.S. Department of Justice, National Drug Threat Assessment, 2011, Product No. 2011-Q0317-001.)

Table 5.2 Average Price and Purity of Heroin in the United States, 1981–2009

Year	Purchases of 1 g or Less[a]		Purchases Greater Than 1 g, up to 10 g[b]		Seizures and Purchases Greater Than 10 g[a]
	Price per Pure Gram ($)	Purity (%)	Price per Pure Gram ($)	Purity (%)	Purity (%)
1981	1934.27	11	1839.97	9	12
1982	1693.51	18	1336.68	18	27
1983	1593.67	15	1689.98	12	28
1984	1505.40	22	1641.44	16	31
1985	1484.16	22	1341.81	24	38
1986	1524.96	26	1226.06	25	38
1987	1321.19	23	1255.71	21	33
1988	1123.32	29	995.23	30	43
1989	1000.47	32	808.57	31	52
1990	1025.13	22	1033.39	25	36
1991	942.42	28	955.14	27	40
1992	817.52	37	706.26	38	53
1993	679.97	41	513.70	41	59
1994	678.75	41	480.22	41	56
1995	601.09	43	447.78	41	55
1996	566.58	38	427.41	37	49
1997	534.60	44	370.62	40	51
1998	471.93	44	332.74	41	54
1999	469.98	42	303.84	40	56
2000	462.25	42	306.38	40	58
2001	437.87	38	279.06	37	55
2002	412.37	39	276.04	40	49
2003	411.37	37	270.39	34	45
2004	420.04	34	301.46	30	40
2005	387.55	36	259.43	32	44
2006	393.41	34	270.17	28	40
2007	376.20	36	230.46	32	42
2008	362.11	34	219.48	35	46
2009	394.81	32	250.85	31	38

Source: Anon, January 1981–December 2007: The price and purity of illicit drugs: 1981–2007, Institute for Defense Analyses, Paper P-4369, October 2008; January 2008–March 2010: Application of same methodology, unpublished, October 2010.

Note: Data not available.

[a] Quantities purchased at the "retail" level.

[b] Quantities purchased at the "dealer" level.

the price decline may be the result of local production and the proximity of consumers to the manufacturers. Over the period 2005–2008, heroin retail prices in key European markets, when adjusted for purity and inflation, displayed a marked sensitivity to the proximity of wholesale major gateways. One notable exception was the United Kingdom, where the adjusted price remained largely stable. In 2004, depending on the geographic location, street-level heroin purity within the United States varied hardly at all from the

purity levels recorded in the early 1990s, and still remains, contrary to the assertions of the White House Office on Drug Control (Institute for Defense Analysis, 2008) at, or about, the 70% level (Cunningham et al., 2010).

5.8 Morphine and Heroin (Tables 5.3 and 5.4)

5.8.1 General Considerations

Heroin is a synthetic prodrug produced from opium by Sertürner in 1805 (Sertürner, 1806), but more than 120 years passed before its chemical structure was characterized by Sir Robert Richardson in 1927 (Schopf, 1927), and total synthesis was only accomplished in 1952 (Gates and Tschudi, 1952). The time lag between the discovery of morphine and

Table 5.3 Physiochemical Properties and Pharmacokinetics of Morphine

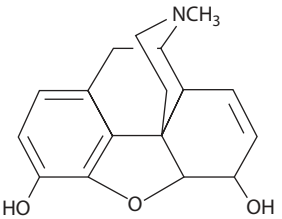
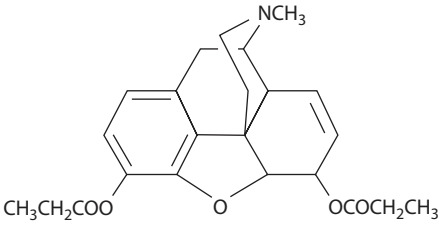
Chemical names	(5α,6α)-7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol
Physiochemical properties, structure, and form	Morphine is available as sulfate and hydrochloride salts that are water soluble. CAS: 57-27-2 (base) MW: 285.54 (base) p*K*a: 7.95 
Synonyms	Morphia, morphium, dolocontin, duromorph, nepenthe, Roxanol
Pharmacokinetic parameters	Bioavailability: PO: 20%–60% IM: 100% Subcutaneous: 100% Transnasal: 60% Transdermal: 65%–80% Rectal: 35%–90% V_d: 2–4 L/kg Protein binding: 35%
Common blood concentrations in drug users	Enormous variation depending on dose and route of administration. No clearly defined therapeutic range.
Blood terminal elimination half-life	1–6 h
Metabolism	Glucuronidation and *N*-demethylation 1. UGT2B7 forms M3G and M6G; UGT1A1, 1A3, 1A6, 1A9, and 1A10 contribute to M3G formation only (Stone et al., 2003). 2. CYP3A4 and CYP2C8 are mainly responsible for the conversion of morphine to normorphine (Projean et al., 2003).
Excretion	Urine: Morphine: 2%–10% M3G: 50%–60% M6G: 5%–10% Also excreted in feces and subject to enterohepatic recirculation
Postmortem artifacts	Blood concentrations increase up to 100% in peripheral blood.
Interactions	CNS depressants
Key papers	See morphine

Table 5.4 Physiochemical Properties and Pharmacokinetics of Heroin

Chemical names	(5α,6α)-7,8-Didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol diacetate (ester)
Physiochemical properties, structure, and form	Available as free base and hydrochloride salt; mostly water soluble CAS: 561-27-3 MW: 369.42 p*K*a: 7.6 
Synonyms	Diacetylmorphine, diamorphine, acetomorphine
Pharmacokinetic parameters	Bioavailability: PO: 20%–40% (Girardin et al., 2003) IM: Up to 420% (no esterases in muscle) SC: 100% Inhalation: 40%–75% Clearance: 930–1939 L/h depending on route V_d: 2–4 L/kg
Common blood concentrations in drug users	Too variable and degraded quickly to 6-acetylmorphine (6-AM) and morphine
Blood terminal elimination half-life	a. Heroin to 6-AM: 3.2–3.7 min b. 6-Monoacetylmorphine to morphine: 22–26 min
Metabolism	Hepatic human carboxylesterase 1 converts heroin to morphine, once the conversion to morphine is complete, as per morphine (Kamendulis et al., 1996; Redinbo et al., 2003). In addition the hydrolysis of 6-MAM to morphine and the glucuronidation of morphine to M3G and M6G (Thaulow et al., 2014)
Excretion	Heroin: 0% 6-Acetylmorphine: 0.5% Morphine: 5% Morphine glucuronides: 55%
Postmortem artifacts	As for morphine
Interactions	As for morphine

its chemical characterization is paralleled by the slow evolution in the understanding and knowledge of its metabolism and mechanism of action. Bayer first sold morphine in 1898, producing it by the acetylation of morphine's two hydroxyl groups. Heroin readily crosses the BBB where it is converted to morphine. Heroin itself has little or no activity at the μ-receptor. It only exerts an effect after it has been converted back to morphine. Injected heroin is hydrolyzed by serum and liver esterases into glucuronide-bound morphine metabolites (Figure 5.17). The first conversion, from heroin to 6-acetylmorphine, occurs in less than 8 min. The second conversion, to morphine, requires on average 22 min. Once the conversion is complete, the fate of heroin in the body is no different than that of morphine (Hasselstrom and Sawe, 1993). Heroin does not cross the nasal mucosa as readily as cocaine (Dale et al., 2002), and bioavailability via this route is poor, at least when compared to cocaine. However, metabolism does continue postmortem. Ethanol inhibits two steps in heroin metabolism: the hydrolysis of 6-monoacetyl morphine (6-MAM) to morphine and the glucuronidation of morphine to morphine-3-glucuronide (M3G) and M6G. This pharmacokinetic interaction could further complicate the outcome after

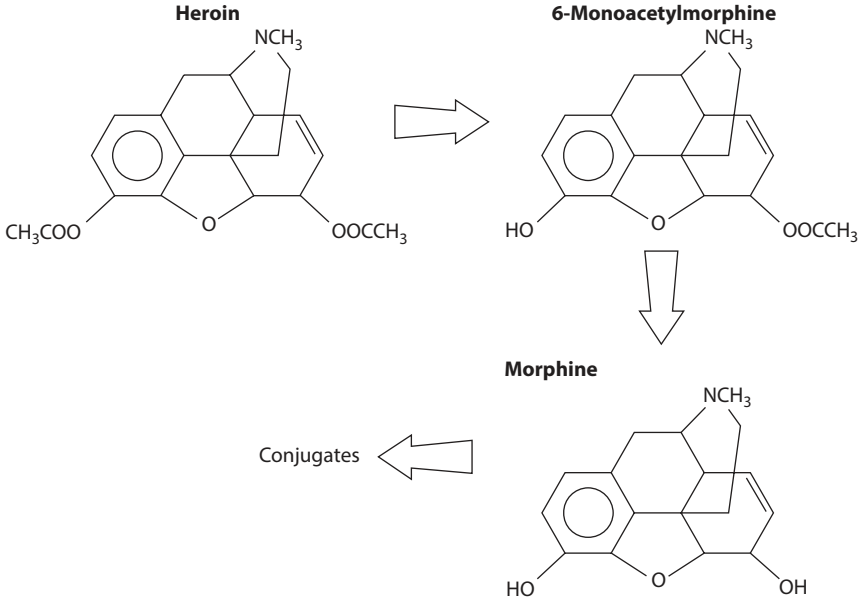


Figure 5.17 Human metabolism of heroin to morphine.

combined use of heroin and ethanol, in addition to the already well-known pharmacodynamic interactions (Thaulow et al., 2014).

Heroin's poor bioavailability probably explains why intravenous injection had, in the past, been the preferred route of administration by addicts in both the United States and Europe. Now that the purity of street heroin has increased so drastically, bioavailability is no longer a limiting factor. With the advent of harm reduction programs, the practice of injection has become increasingly stigmatized and the injection of heroin is becoming less popular (Westerling et al., 1982; Illum et al., 2002). According to the 2006 Drug Threat Assessment published by the DEA (DEA, 2006), which agrees with the more recent assessment suggested by the UN and even independent researchers, confiscated street heroin is between 60% and 70% pure. Heroin of this purity will readily cross the nasal mucosa, and so the practice of nasal insufflation is likely to remain popular. If it does, the prevalence of some infections associated with parenteral heroin abuse may decrease. Morphine's principal site of metabolism is the liver, but because the total body clearance of morphine is higher than hepatic flow, questions still remain about extrahepatic metabolism (Sawe et al., 1985).

Alternative pathways for the metabolism of morphine exist, though they have been minimally studied. These include conjugation with sulfonic acid, leading to morphine-6-sulfates and morphine-3-sulfates, acetylation to 3- and 6-acetylmorphines, oxidation to morphine-*N*-oxide, and demethylation to normorphine. Of the known morphine metabolites, the best studied are M6G, M3G, and normorphine. These are discussed in detail in Section 5.8.3.

It should be emphasized from the outset that the utility of measuring blood morphine concentrations, either in the living or the dead, is not very great. Tolerance occurs, and as is the case with cocaine, the morphine concentrations (indeed, the concentrations of all the opiates and opioids) in individuals where morphine is the cause of death overlap with concentrations found in other decedents where the presence of morphine is merely an incidental

finding. Addicts dying of overdose may have much lower blood concentrations than addicts being treated with heroin maintenance (Drummer et al., 2004). Indeed, today there are many heroin-replacement patients (Switzerland dispenses free heroin to addicts), and the plasma concentrations observed in these individuals were once presumed to be automatically lethal. Blood concentrations also overlap completely in the living and dead (Darke et al., 1997). Even in the living, relationships between impairment and pain relief on one hand and plasma opiate concentrations on the other have been very difficult to establish. Pharmacodynamic studies—no matter whether in normal volunteers or cancer or trauma patients—have failed to disclose any predictable relationship between morphine plasma concentrations and analgesic effects (Hoffman et al., 1997), although some evidence exists that, in cancer patients, M3G plasma concentrations do correlate with pain perceptions.

5.8.2 Morphine Metabolism

Morphine undergoes biphasic tissue distribution (Figure 5.18). The initial phase lasts only a few minutes during which morphine is rapidly distributed throughout those tissues receiving the highest blood flow: the lung, kidney, liver, spleen, and muscle (Brunk and Delle, 1974). During a secondary phase, morphine is quickly converted to its principal metabolite, M3G, and somewhat more slowly to smaller amounts of M6G. The transformation occurs almost entirely in the liver, takes several hours, and also leads to the production of small amounts of several other morphine metabolites. Less than 10% of a given dose of morphine is excreted in the urine as unchanged morphine.

The process of glucuronidation requires the production of free hydroxyl groups, catalyzed by several different enzymes, but mainly UGT2B7 (Coffman et al., 1997; Kirkwood et al., 1998). Morphine has two hydroxyl groups: one phenolic (position 3) and one hydroxyl (position 6). Depending on which hydroxyl group is involved in the reaction, morphine can be glucuronidated to form M3G or M6G. Aromatic hydroxyl groups are glucuronidated more easily than alicyclic hydroxy groups, which explains the pattern observed when intravenous morphine is given to healthy volunteers: the resultant M3G–morphine molar concentration ratio is roughly 6:1, whereas that of M6G–morphine is approximately 1:1.

The glucuronides have a much smaller volume of distribution (less than one-tenth) than that of the parent compound. This explains why near equal plasma concentrations of morphine and M6G can be observed, even though the amount of M6G in the body is only approximately 10% of that of morphine. Paying insufficient attention to the physical properties of morphine and its metabolites can lead to a confusing muddle when attempts are made at interpreting postmortem drug concentrations. Debate over the role of UGTB7 polymorphisms is ongoing and unresolved, further compounding the problem of interpretation.

Approximately 70% of the morphine administered is converted to one or other glucuronide (57% M3G, 10% M6G) (Hasselstrom and Sawe, 1993) and then excreted via the kidneys. Other morphine metabolites (such as morphine-3,6-diglucuronide, normorphine-3-glucuronide) are produced in very small amounts and appear to be devoid of physiologic effects (Yeh et al., 1979). Elimination of morphine is not altered by renal failure, but excretion of the morphine glucuronides is. The 6-glucuronide is psychoactive and may accumulate in individuals with renal failure, leading to prolonged coma. Orally administered morphine undergoes extensive first-pass metabolism. One consequence is that much more M6G is formed than if the morphine had been given intravenously.

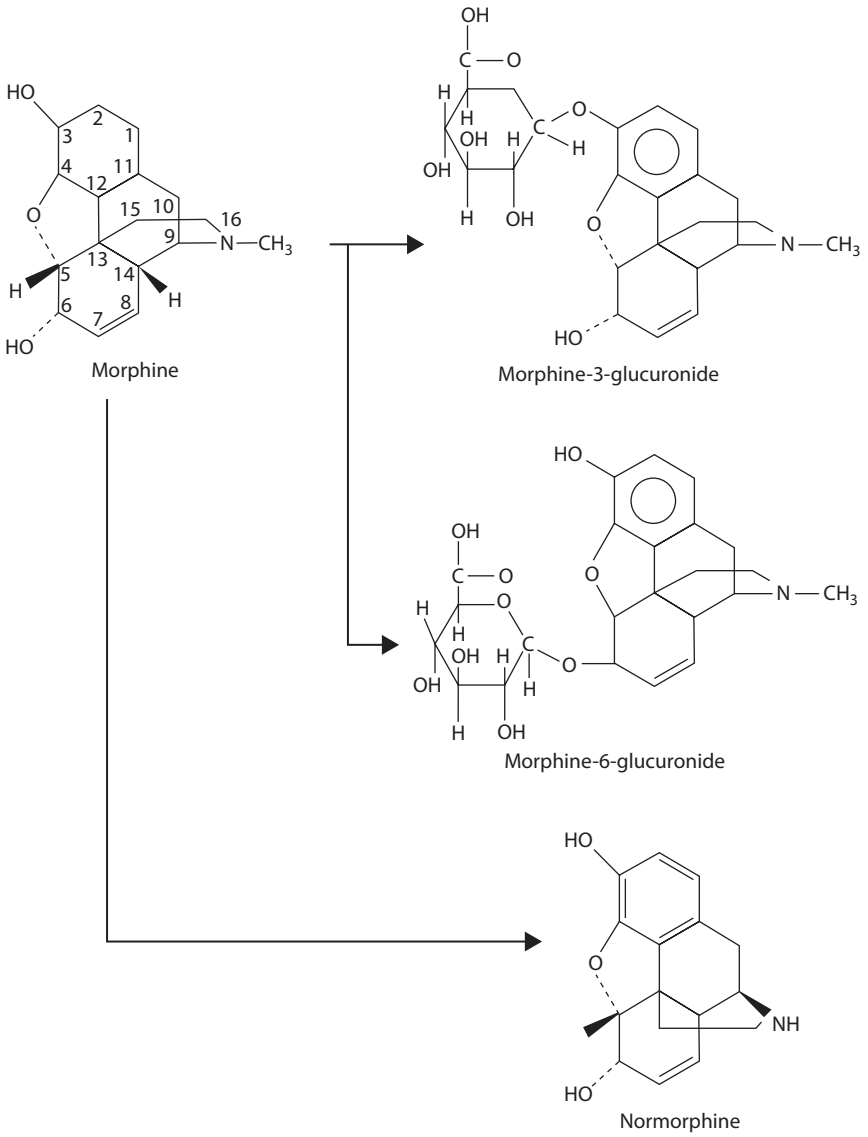


Figure 5.18 Human metabolism of morphine.

It has been proposed that increased production of M6G after oral dosing may explain the relative increase in potency of repeated oral doses of morphine relative to repeated doses given intravenously (Hanks et al., 1987). Morphine is not, as once thought, converted to codeine (though the reverse is certainly true); the detection of codeine in urine after morphine or heroin administration is explained by the presence of codeine impurities that are present even in pharmaceutical-grade morphine (Cone et al., 1991). During life, approximately half the morphine circulating in the plasma is protein bound (Osborne et al., 1990). Abnormalities of protein binding, such as occur in hepatic failure and/or malignancy, can alter the degree of protein binding. Indirectly this may lead to higher circulating levels of free morphine (Sawe, 1986).

Further complicating the issue is the fairly recent discovery of a group of molecules known as the organic cation transporters (OCTs) located in the sinusoidal endothelial cells

of the liver (Nies et al., 2009). OCTs are critical for the absorption, distribution, and elimination of many low molecular weight basic compounds, including endogenous amines. OCT1 is predominantly expressed in the sinusoidal membranes of the human liver. It is thought to mediate cellular uptake of morphine into hepatocytes. OCT1 is highly genetically polymorphic. Approximately 10% of Caucasians are compound homozygous carriers of one of the four common coding polymorphisms (Arg61Cys, Gly401Ser, Gly465Arg, and deletion of Met420) that result in reduced or lost OCT1 transporter activity (Saadatmand et al., 2012). Even healthy carriers of the loss-of-function *OCT1* genetic variants listed earlier will have increased plasma concentrations of morphine compared to healthy normals (Tzvetkov et al., 2012; Fukuda et al., 2013).

P-glycoprotein (P-gp)-mediated transport is the central mechanism responsible for the movement of morphine across the BBB. P-gp serves as an important transport protein, located in the apical membrane of endothelial cells. Using ATP hydrolysis for energy, P-gp exports molecules attempting to pass through the cell membrane from the outside to the inside. It serves the same function with many different compounds, not just morphine (Ebinger and Uhr, 2006). For example, the common antidiarrheal medication loperamide binds strongly to the opiate μ-receptor but produces no CNS symptoms because it is entirely bound to P-gp and cannot enter the brain.

The detection time of morphine in blood is 20 h after the intravenous administration of 12 or 20 mg of heroin to a test subject. After being smoked, the detection time appears to be much shorter. In individuals who smoked 10.5 mg of heroin, detection time varied between 22 min and 2 h (Jenkins et al., 1994). In the blood of chronic users, total morphine is detectable for 29.2 h on average and free morphine for 14.4 h (Reiter et al., 2001). After administration of 3–7 or 10.5–13.9 mg heroin intravenously, 6-acetylmorphine is detectable in urine up to 5.1 h (median 2.3) and 2.3–11.2 h (median 4.5), respectively, whereas total morphine remains detectable in urine for 7.4–31.9 h (median 15.7) and 10.7–53.5 h (median 34).

Two types of drug transporters circulate in the bloodstream: efflux and influx. Efflux transporters like P-gp belong to a family of membrane proteins that heavily influence drug concentrations inside cells. P-gp is the best characterized but there are many other members of this class. There is increasing evidence that genetic heterogeneity among members of this protein family may account for problems such as multidrug resistance, not to mention the effects of individual drugs such as morphine. Table 5.5 lists some of the drugs transported by P-gp.

Many distinct sequence variations in genes of this P-gp transporter family have been identified, though the clinical significance of most of these polymorphisms remains unclear. There is, however, no question that brain concentrations of morphine depend on the amount of free morphine circulating in the plasma and that the amount of free morphine circulating in the plasma is partly determined by the amount of P-gp available to bind with morphine. Because many other drugs are also P-gp bound, the potential exists for unanticipated drug reactions. For example, it has recently been suggested that upregulation of P-gp production leads to decreased delivery of antiseizure medications to the brain, resulting in refractory seizure disorders (Robey et al., 2008).

P-gp inhibitors can disrupt brain uptake and tissue disposition of morphine. Two of the best-known inhibitors are verapamil and cyclosporine (CyA) (Letrent et al., 1999). P-gp proteins normally complex with morphine, but they cannot complex with either M3G or M6G because both molecules are too polar. Once the two metabolites are formed in the

Table 5.5 Drugs Known to Bind or Interact with P-gp

Anticancer drugs
Docetaxel, doxorubicin, etoposide, imatinib, paclitaxel, teniposide, vinblastine, vincristine, doxorubicin, etoposide, dexamethasone, methylprednisolone, hormone conjugates
Immunosuppressants
Cyclosporine, sirolimus, tacrolimus
HIV protease inhibitors
Amprenavir, indinavir, nelfinavir, saquinavir, ritonavir
Antibiotics
Erythromycin, ofloxacin, ampicillin, cefoxitin, ceftriaxone, grepafloxacin
Beta blockers
Bunitrolol, carvedilol, celiprolol, tanilolol
Ca^{2+}-channel blockers
Diltiazem, verapamil
Cardiac drugs
Digoxin, digitoxin, quinidine
HMG-CoA inhibitors
Atorvastatin, lovastatin, pravastatin
H_1-Antihistamines
Fexofenadine, terfenadine, cimetidine
Antiemetics
Ondansetron
Diverse
Amitriptyline, colchicine, itraconazole, lansoprazole, loperamide, losartan, morphine, phenytoin, rifampin
Glucuronide, sulfate, and glutathione conjugates (e.g., leukotriene C_4)
Bilirubin and certain drug glycosides, such as flavonoids and saponins

Source: Adapted from Cascorbi, I., *Pharmacol. Ther.*, 112(2), 457, 2006.

liver, they are transported back into the circulation by forming complexes with another glycoprotein called multidrug resistance protein 1 (MDR1), which is normally found in hepatic sinusoidal membranes. The gene for MDR1 is polymorphic, and individuals who have an abnormal form of the protein may not be able to move the glucuronides out of the liver effectively (Zelcer et al., 2006).

Failure to transport may explain the occasional case in which a very high G3P hepatic concentration is seen in the face of low plasma levels—another reason why the possible role of genetic polymorphism should always be considered when attempts are made at the interpretation of postmortem drug concentrations. An issue of major concern is the role that P-gp and related efflux proteins play in the success or failure of highly active antiretroviral therapy (HAART). Effective combination antiretroviral (ARV) treatment includes the presence of a P-gp, polymorphisms of which may induce efflux transporters and/or cytochrome P450 3A4 (CYP3A4). This would result in subtherapeutic blood levels and therapeutic failure, entirely due to reduced absorption and/or increased metabolism of the ARVs. A similar prognosis is true for other ARVs and the situation becomes even more complex if the ARVs are used in conjunction with some abused drugs. Heroin (morphine) and nicotine enhance CYP3A4 and efflux protein production. The uncertain nature of the relationship between efflux transporters and cytochrome P450 (CYP) enzyme family members makes it difficult to predict the outcome of HAART as such, especially when HIV

patients are also drug abusers. HIV-positive pregnant women on HAART may develop a higher viral load as a consequence of such interactions, leading to increased mother-to-child transmission of HIV (Pal et al., 2011).

Morphine also undergoes enterohepatic circulation. In seven healthy volunteers given 5 mg doses of morphine intravenously, on average 57.3% of the morphine was converted to M3G, 10.4% to M6G, and 10.9% appeared unchanged in the urine, leaving 20.8% of the original morphine unaccounted for Hasselstrom and Sawe (1993). Because enterohepatic circulation is a reality, substantial quantities of morphine may be found in the gastric contents, sometimes leading to the false conclusion that morphine had been orally ingested.

When measurements of blood, urine, liver, and gastric contents were compared in 29 cases where intravenous heroin overdose was the known cause of death, the mean total and free blood morphine concentrations were 0.60 and 0.32 mg/L, respectively, while stomach contents were found to have a total morphine concentration of 1.16 mg/kg. Morphine was detectable in the stomach contents from every case, and in 24 of 29 cases (83%) the stomach contents had a higher concentration of total morphine than the blood. The mean total morphine concentration in bile was 100 times that measured in blood, and the liver total morphine concentration averaged twice that of blood levels. Clearly, enterohepatic circulation of morphine with subsequent reflux of duodenal contents back into the stomach can cause morphine to accumulate in the stomach. As a consequence the relative levels of opioids in blood and stomach contents are an unreliable indicator of the site of drug administration (Duflou et al., 2009). A handful of other studies have measured morphine in brain. The most recent (Seltenhammer et al., 2013) found morphine concentrations of 389 ng/mL in cerebrum and 275 ng/mL in cerebellum. These results are roughly in line with those reported by Spiehler and Cravey in the 1970s (Reed et al., 1975).

5.8.3 Morphine Metabolites

5.8.3.1 Morphine-3-Glucuronide

The metabolism of any drug generally involves conversion of the parent compound to more highly polar, less lipophilic, compounds. The lipophilicity of heroin is much greater than that of 6-monoacetyl morphine, and both are much more lipid soluble than morphine. Both M3G and M6G are highly polarized and minimally lipophilic (Gulaboski et al., 2007), and their ability to cross the BBB is significantly less than that of the parent compound (Wu et al., 1997a). M3G is morphine's major metabolite. It has almost no affinity for μ-receptors and no analgesic properties. For unexplained reasons, plasma concentrations of morphine itself, and of its active metabolite M6G, do not correlate with pain perception, but concentrations of M3G seem to have a strong correlation with perceived clinical effect (Sakurada et al., 2010).

The terminal half-life of M3G is 3.9 ± 1.5 h, significantly longer than that of M6G, which is only 2.6 ± 0.69 h (Osborne et al., 1990). The apparent volume of distribution for M3G in children under the age of 11 is more than twice that observed in children over that age and more than seven times the value observed in adults (0.76 vs. 0.33 vs. 14 L/kg) (Hunt et al., 1999). The clearance rate for M3G is also higher in young children than in adults (4.9 vs. 0.8 mL/min/kg) and is higher than that of M6G regardless of age. This observation suggests that bioavailability estimates derived from adults are not applicable to children and that preferential metabolism to M6G or increased clearance of M3G occurs. Although it has not been established in human trials, the results of animal studies strongly suggest

that ketamine is a noncompetitive inhibitor of M3G production (Qi et al., 2010), which means morphine would be cleared more slowly from the circulation in the presence of ketamine, an event that could have forensic significance in some polydrug abuse deaths or in deaths where both were administered for anesthesia.

5.8.3.2 Morphine-6-Glucuronide

The 3-carbon position in the morphine moiety must remain accessible in order for a molecule to have opiate activity, and it does remain open in the M6G molecule. Thus it is not surprising that this metabolite has analgesic effects in its own right (Osborne et al., 1990). In humans, M6G has approximately half the pain-relieving effect of morphine but causes fewer side effects and longer-lasting analgesia. At analgesic doses, M6G causes similar reduction of the ventilatory response to CO_2 but significantly less depression of the hypoxic ventilatory response. In postoperative surgical patients, M6G causes 50% less nausea and 75% less vomiting (van Dorp et al., 2006). When given as a pain medication, M6G has a slower onset of action than morphine because it crosses the BBB more slowly; lipid solubility is 187-fold lower than that of morphine (Wu et al., 1997a). M6G is not metabolized but it is excreted via the kidneys. Like morphine, M6G also undergoes enterohepatic cycling (Kilpatrick and Smith, 2005; Villesen et al., 2006). Chronic exposure to street heroin causes a relative increase in concentrations of M6G and may contribute to some of heroin's effects (Antonilli et al., 2003).

The volume of distribution for M6G is so low (0.42 L/kg in children under the age of 11, 0.19 L/kg in older children and 0.15 L/kg in adults) (Hunt et al., 1999) that very little M6G is found in tissues. Measurements made in fat and subcutaneous tissue, for example, disclose the presence of free morphine but none of the glucuronides (Levisky et al., 2000). The elimination of M6G is decreased in renal failure; patients who accumulate metabolite may become toxic due to its presence (Angst et al., 2000; Penson et al., 2002).

Morphine glucuronides continue to appear in the urine for days after the last episode of heroin/morphine use, even in healthy individuals who have neither liver nor kidney disease. The process will continue for as long as there is morphine in the bile to be excreted. As previously noted, concentrations of unchanged morphine in bile may reach extremely high levels. In one study of narcotic-related deaths, the average concentration of morphine in the bile was 312 mg/L (Chan et al., 1986). In Gottschalk and Cravey's series of 119 cases, the median level of morphine in the bile was 34 mg/L (Gottschalk and Cravey, 1980). These high concentrations partially explain why morphine may appear in the urine for extended periods: morphine glucuronides excreted in the bile can be deconjugated back to morphine by bacteria in the gut and then reabsorbed through the intestinal mucosa (Parker et al., 1980).

The combination of enterohepatic circulation and bacterial deconjugation makes the interpretation of postmortem morphine levels almost impossible. It is not known with any certainty just how long it takes to clear morphine from the enterohepatic circuit, but it is reasonable to suppose that patients treated with large doses of morphine, such as trauma victims maintained on respirators, and former heroin abusers who have entered detoxification programs, may continue to excrete measurable quantities of morphine glucuronides in the urine for weeks after morphine was last taken. This possibility must be taken into consideration as there now exist effective laboratory methods (liquid chromatography-tandem mass spectrometry [LC-MS/MS]), for measurement of the urinary glucuronides of nearly all opiate glucuronide conjugates (Dickerson et al., 2012). This possibility must be taken into account when interpreting drug abuse screening tests. After death, free and

conjugated morphine are stable in refrigerated blood and urine, at least for 10 days, but in the liver conjugated morphine is rapidly converted back to morphine (Moriya and Hashimoto, 1997; Alunni-Perret et al., 2003). If the postmortem interval is long, or if the tissue samples have not been frozen, inferences about the time of ingestion based on the ratio of morphine to its metabolites are almost certain to be misleading. The concentrations of nearly all abused (and many licit) drugs may increase massively once the process of putrefaction is established. Quantitative morphine measurements made in decomposing tissue, or purge, can be relied upon only to the extent that they prove the drug in question was present. Any further interpretative efforts are unwarranted (Wyman et al., 2011).

5.8.3.3 Normorphine

Like M6G, normorphine (Figure 5.19) is also psychoactive. Though not always detectable in the plasma of morphine and heroin users (Dale et al., 2007), it usually can be found in the urine of cancer patients being treated with oral morphine. Normorphine is believed to be neurotoxic. More than 90% of morphine *N*-demethylation to normorphine can be accounted for by the action of two polymorphic enzymes, CYP3A4 and cytochrome P450 2C8 (CYP2C8) (Projean et al., 2003). Based on published clinical profiles of renal failure patients treated with high-dose oral morphine, it appears that only those individuals capable of morphine demethylation are at risk for toxicity. Glare et al. (1990) described two cancer patients being treated with morphine. One, who was experiencing myoclonus, was receiving 160 mg of morphine orally every 4 h and had a plasma morphine concentration of 93 ng/mL, an M3G of 19,900 ng/mL, an M6G of 5,161 ng/mL, and a normorphine concentration of 70 ng/mL. A second patient with normal renal function received 15 mg of morphine/h but had no normorphine. The plasma morphine concentration was 81 ng/mL, with an M6G of 4600 ng/mL and an M3G of 63 ng/mL.

5.8.4 Absorption and Routes of Administration

Morphine is well absorbed, no matter the route of administration. The subcutaneous and intravenous routes of morphine administration are bioequivalent (Stuart-Harris et al., 2000). However, when heroin users "chase the dragon," bioavailability is much lower (about 50%).

5.8.4.1 Intravenous Injection

Past studies of intravenous heroin and morphine kinetics relied upon measurements made either in healthy volunteers or in cancer patients. Neither situation is especially comparable to the situation in abusers because the doses administered in clinical studies are relatively small. Studies performed in the 1970s showed that a 10 mg bolus of morphine given

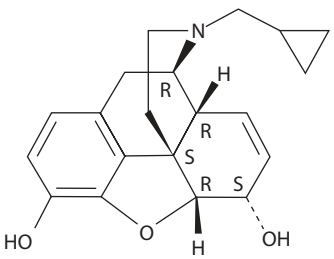


Figure 5.19 Normorphine molecule.

to healthy volunteers who were undergoing elective surgery resulted in peak blood levels of 200–400 ng/mL 5 min after injection (Berkowitz, 1976). A study performed in the early 1990s compared the pharmacokinetics of smoked and intravenous heroin in two subjects; in one subject, peak concentrations ranged from 72 ng/mL after a 10 mg intravenous dose to 401 ng/mL after a 20 mg dose. In the second subject, a 3 mg dose produced a peak level of 64 ng/mL, while a 6 mg dose gave a peak of 315 ng/mL. Levels rapidly declined thereafter and reached limits of detection within 30 min of injection (Jenkins et al., 1994).

More recent studies have been performed in addicts enrolled in heroin maintenance programs. Injecting 146 ± 48 mg of heroin into eight addicts produced maximal plasma concentrations of diacetylmorphine and 6-acetylmorphine of 3057 and 5000 ng/mL, respectively. Heroin had a clearance rate of 11.6 L/min and a volume of distribution of 0.52 L/kg, with a terminal half-life of 3.0 min for both diacetyl and 6-monoacetyl-morphine. A second, much larger and more recent study evaluated the pharmacokinetics of large doses of heroin in 74 heroin inhalers and 32 heroin injectors in a crossover trial. The terminal half-lives of heroin and 6-acetyl heroin were estimated at 7.6 and 28.1 min, respectively. The clearance rate for morphine was estimated at 73.6 L/h (CI 62.8, 85.4) and those of M3G and M6G at 6 and 10 L/h, respectively. Plasma heroin and 6-acetylmorphine concentrations were two to four times higher after injecting than inhaling (the highest concentration recorded after intravenous use was 5042 ng/mL). Interestingly, and inexplicably, the terminal half-life of 6-acetylmorphine is 13% lower in cocaine users. Another important difference highlighted by these studies was the slightly lower volume of distribution for morphine (less than 2 mL/kg) that is observed when similar studies are done with morphine rather than heroin that has been converted to morphine. This may have to do with the size of the dose or, more likely, the pharmacokinetic model used for the study (Gourlay et al., 1986; Rook et al., 2006a,b).

Trauma has an effect on morphine pharmacokinetics. Morphine clearance rates (2.5–10 mL/kg/min, with mean ± SD of 6 ± 2.6) and volume of distribution (0.28–3.30 L/kg, with mean ± SD of 1.4 ± 1.0) were found to be lower in trauma victims than in healthy volunteer test subjects or in cancer patients. These reductions are seen in trauma victims even when hepatic blood flow, as assessed by the rate of lidocaine clearance, is almost normal, suggesting that some other mechanisms besides diminished hepatic blood flow are involved (Berkenstadt et al., 1999a,b).

Diversion of sustained-release oral morphine in some countries has led to the occurrence of occasional deaths, not from morphine overdose, but because of attempts by addicts to crush the tablets and circumvent the time-release mechanism. When dissolved and injected, insoluble materials mixed with the heroin, such as talcum, cause microembolism, leading to severe tissue damage (Beer et al., 2010). Studies by researchers interested in harm reduction have shown that harmful heroin contaminants can be substantially reduced by passing the injection solution through a sterilizing (0.22 μm) filter. To prevent the filter from blocking, a preliminary coarse filter (such as a cigarette filter) should be used first. No matter how effective this field technique may be, this remains an unsafe practice due to skin and environmental contamination by particles and microorganisms, and the risks of blood-borne infections from sharing injecting equipment still remain high.

5.8.4.2 Subcutaneous Injection

Absorption via the subcutaneous route and after intramuscular injection is almost as rapid as by the intravenous route. Morphine plasma levels peak at 10–20 min, somewhat longer than after intravenous injection, but not so much longer as to have any clinical significance.

The pharmacokinetic profiles for both routes are nearly the same as after intravenous injection, and plasma levels comparable to those seen after intravenous use can be achieved after subcutaneous injection. In one recent study with healthy human volunteers, a dose of 10 mg/70 kg given subcutaneously produced a free morphine concentration of 80 ng/mL at 5 min, compared to a peak concentration of 74 ng/mL at 15 min after the same dose was given as an intravenous bolus (Stuart-Harris et al., 2000).

These similarities may explain why, in the past, subcutaneous heroin injection (known as *skin popping*, see Figure 5.35) enjoyed considerable popularity among some groups of abusers. Unfortunately, this mode of administration accounts for the high prevalence of abscesses and cellulitis among some communities of injection drug users. To some extent *skin popping* may have been replaced by smoking, but the practice still occurs, as evidenced by the outbreak of clostridial infections reported from the United Kingdom in 2000 and by continued reports of wound botulism (Brown and Ebright, 2002; Brett et al., 2004, 2005; Kimura et al., 2004; Davis and King, 2008) from Europe, the United States, and the United Kingdom (Rodolico et al., 2010). Outbreaks continue to occur in spite of intensive public health measures. An outbreak in Norway occurred at the end of 2013 (MacDonald et al., 2013).

In the State of California, during the period from 1993 to 2006, 17 intravenous drug users (IVDUs) had recurrent episodes of wound botulism, 14 with 1 recurrence and 3 with 2 recurrences. Of 25 laboratory-confirmed episodes, 22 were confirmed through serum testing and 3 through wound testing. Patients ranged in age between 32 and 61 years, and 94% were male. All patients were heroin injectors: 88% specified black tar heroin use and 76% reported subcutaneous injection. The most common presentations consisted of visible wounds, speech difficulty, double vision, respiratory difficulty, and trouble swallowing. There were no significant differences in clinical presentation between initial and second episodes (Yuan et al., 2011a).

In the United Kingdom, subcutaneous heroin delivered through a syringe injector is routinely used for hospice care (Oliver, 1985), and "pain pens" containing heroin are used for the treatment of cancer patients with breakthrough pain, providing an effective and inexpensive alternative to the use of oral transmucosal fentanyl (Enting et al., 2005). Continuous subcutaneous administration of heroin is the preferred treatment modality because it avoids the cycles of peak-level sedation and trough-level breakthrough pain, and nausea is lessened. In one study, an infusion of 165 mg/day of heroin at the rate of 3.45 mg/h resulted in a stable serum concentration of 30–40 ng/mL, even though bioavailability is considerably less after subcutaneous than intravenous dosing (Mikkelsen-Lynch et al., 2000).

5.8.4.3 Oral

Parenteral administration avoids first-pass metabolism resulting in decreased production of some morphine metabolites. After oral administration, 80% of a given dose of morphine is absorbed from the gastrointestinal tract, with nearly half that amount being metabolized during the first pass through the liver. For that reason, oral bioavailability of morphine is relatively low, at just over 33% (Lotsch et al., 1999). The amount absorbed is somewhat unpredictable because the degree to which morphine is subject to gastrointestinal extraction and metabolism is altered by age, liver function, gender, the presence of food, disease states, and genetic polymorphism (Tam, 1993). Still, there is increasing interest both in the use of oral morphine (in multiple different formulations) and even in the use of M6G. Two separate plasma concentration peaks are seen after M6G is given orally. The first is due to

hydrolysis of M6G back to morphine in the small intestine and colon. The second peak, occurring several hours later, is thought to represent the position 3-glucuronidation of morphine (Stain-Texier et al., 1998).

The oral route was popular among the "opium eaters" of the seventeenth and eighteenth centuries, when morphine distribution was unregulated and prices were low. Today, it is an impractical route for abusers because it is simply too expensive. Urine measurements have been described in only one contemporary "opium eater," an addict ingesting approximately 1 g of opium/day. Morphine, codeine, normorphine, norcodeine, and noscapine were all found in the urine, but thebaine and papaverine (normal constituents of opium) were not. The concentration of unconjugated morphine (640 ng/mL) was more than twice the concentration of codeine (Cone et al., 1982). As this practice seems to have all but disappeared, our lack of knowledge is probably of no great import.

"Brown mixture (BM)," a cough syrup used in China, contains opium powder (10.0%–10.5% morphine), opium tincture (0.9%–1.1% morphine), or camphorated opium tincture (0.045%–0.055% morphine). In a recent study, "BM" from seven different manufacturers (five tablets and two solutions) along with urine samples from alleged heroin users and volunteers with various ingestion patterns were analyzed for their morphine and codeine contents. Morphine concentrations found in urine specimens collected from volunteers ingesting BM tablets (or solutions) were always <4000 ng/mL. The following morphine-to-codeine ([*M*]/[*C*]) ratios were observed for urine specimens with morphine concentration ≥300 ng/mL: (1) <3.0 for volunteers ingesting BM solution and (2) >3.0 (mostly >5.0) for volunteers ingesting BM tablets and alleged heroin users. It appeared that (1) BM ingestion (tablet or solution) was unlikely to result in a morphine concentration > 4000 ng/mL and (2) [*M*]/[*C*] ratio might not effectively identify which individuals were "brown powder" users and which were heroin abusers (Liu et al., 2006).

Oral morphine has been a mainstay in the management of cancer pain for many years (Gourlay et al., 1986) but is gradually being supplanted by fentanyl and ondansetron (Llanes et al., 2006). Hospice patients may be treated with very high doses of morphine or methadone. In a study of 29 men and women (mean age 68 years), the average dose of morphine (controlled oral release) was 90 mg/day, but in some individuals the daily dose was nearly 1500 mg/day. The mean blood morphine in this group was 72 ng/mL, but in some individuals values of as high as 700 ng/mL were recorded (Klepstad et al., 2000). At one time concern had been expressed that such high doses of morphine might lead to M3G accumulation and toxicity but in control trials that has not proven to be the case (McCann et al., 2010).

Oral overdose, intentional or accidental, can occur (Got et al., 1994). To prevent this, and to discourage addicts from simply crushing morphine tablets, a new dosage form called Embeda has recently been introduced. It is essentially sustained-release morphine with sequestered naltrexone. This was designed to be an abuse-deterrent opioid formulation. When taken as directed, insignificant amounts of sequestered naltrexone would reach the systemic circulation because oral absorption is minimal, but upon tampering the released naltrexone counteracts the euphoria of all opioids, possibly even precipitating opioid withdrawal in opioid-dependent patients (Ruan, 2011).

In one case involving an intentional overdose with an unspecified number of sustained-release morphine capsules (MS Contin®), the patient subsequently developed rhabdomyolysis and renal failure in addition to respiratory depression. Concentrations of morphine, M6G, and M3G roughly 36 h after ingestion were 57, 154, and 798 ng/mL, respectively.

In another case report involving heroin rather than morphine, concentrations of heroin, 6-acetylmorphine, and morphine were 109, 168, and 1140 ng/mL, respectively, in blood and 17, 12, and 425 ng/g, respectively, in gastrointestinal contents. Only morphine was detected in the urine, at a concentration of 3650 ng/mL (Rop et al., 1997). A 1995 report describes a case of fatal intoxication in an 8-year-old child due to a medication error where oral morphine was dispensed instead of oral meperidine. Before going to bed, the child took one or two teaspoons of a 20 mg/mL morphine sulfate solution and was found dead in the morning. The morphine concentration was 128 ng/mL in blood, 135 mg/L in bile, and 16 mg/L in stomach contents (2.3 mg total) (Poklis et al., 1995). Case reports of infants and children with methadone overdose are much more common than reports of accidental morphine overdose. The discrepancy may be explained by the observation that, while hundreds of thousands of methadone-replacement patients are young enough to have children, oral morphine solutions are more likely to be found in the homes of older individuals with cancer.

5.8.4.4 Rectal

Plasma levels after rectal morphine administration are somewhat higher than after oral morphine but are much less than after parenteral administration (Ellison and Lewis, 1984). This route does not seem to be particularly popular among abusers, at least when compared to the rectal use of cocaine, which is a fairly common practice. One reason may be that rectal administration of morphine significantly reduces first-pass exposure in the liver, resulting in decreased hepatic transformation of morphine to its pharmacologically active metabolite, M6G (Babul and Darke, 1993). When 0.6 mg/kg of morphine was given to women undergoing cancer treatment, considerable variation between individuals was observed, but peak concentrations of 31–75 ng/mL were reached at between 45 and 120 min (Westerling et al., 1982). Fatalities have occurred at levels that were not much higher, and seizures, particularly in neonates, have been reported at levels that were much lower. Morphine-induced seizures have occurred at blood concentrations as low as 9 ng/mL (Koren and Maurice, 1989). One report described a postoperative death from cerebral hypoxia. A child was given several 4 mg morphine suppositories over the course of 4 h. Blood levels measured 1.5 h after death were 94 ng/mL (Gourlay and Boas, 1992). Different studies have shown rectal bioavailability to be extremely variable, ranging from 12% to 61% (Lindahl et al., 1981; Westerling et al., 1982). Pharmacologic manipulation of the morphine medium can improve absorption and result in levels comparable to oral administration. If the carrier medium is acidified, then the percentage of unionized drug increases, resulting in better absorption. Controlled-release morphine suppositories, containing polyglycerol ester of fatty acid, are available in some areas. Plasma levels rise more slowly than after oral administration but remain elevated for a longer period (Takatori et al., 2005).

5.8.4.5 Intranasal

Heroin and morphine can both be administered intranasally, but the transnasal absorption of morphine is poor, at least when compared to other agents such as cocaine. At the turn of the nineteenth/twentieth century, probably up to the mid-1920s, as many people took heroin by nasal insufflation as by injection. Today's abusers seem to have rediscovered this route. Government surveys report that the practice of heroin snorting has become increasingly popular on the "club" circuit, and as heroin prices continue to fall, this route can be expected to become increasingly popular.

The pharmacokinetics of intranasal and intramuscular heroin has been compared in at least one study. Peak heroin concentrations after either intranasal or intramuscular administration occur within 5 min. Resultant blood levels after 6 mg doses of heroin by either route are on the order of 30–40 ng/mL. The mean elimination half-life after intranasal administration was 5.4 ± 4.5 vs. 4.2 ± 0.12 min after intramuscular administration. Concentrations of 6-acetylmorphine reach their peak at 5–10 min after administration by either route, with peak levels of 23 ng/mL occurring after a 6 mg dose. The elimination half-life was longer for 6-acetylmorphine than for heroin: 10.8 ± 8.4 min after intranasal compared to 11.4 ± 5.4 min after intramuscular dosing with 6 mg. Once the heroin had been converted to morphine, the half-life for morphine following intranasal administration ranged from 90 ± 96 min (6 mg dose intranasally) to 168 ± 216 min (12 mg dose intranasally) (Cone et al., 1993).

Surprisingly, heroin has proven to be a well-tolerated and rapidly effective analgesic agent in the pediatric setting. The pharmacokinetic profile of intranasal heroin in adults and children has been systematically studied and compared with intramuscular heroin. In controlled studies intranasal and intramuscular heroin produce very similar physiologic responses (including pupil diameter, respiration rate, and temperature). Changes in behavioral measures (including euphoria, sedation, and dysphoria) were also similar. In other controlled trials, intranasal heroin has been compared to results with intramuscular morphine in the setting of acute orthopedic pain in children with fractures. Intranasal heroin provided the same overall degree of pain relief; pain relief was comparable but nasal administration brought a faster onset of action (Wilson et al., 1997; Kendall et al., 2001).

An abuser who dies after snorting or inhaling heroin is occasionally seen but in general such occurrences are uncommon. Autopsy in these individuals seldom discloses the typical pattern of liver and lung disease seen in chronic abusers, and the evidence suggests that nontraditional routes of ingestion lead to lower morphine blood levels. The median blood morphine concentration in 18 decedents who were non-injectors was 0.095 μg/g (range, 0.02–0.67 μg/g), significantly lower than is usually seen in heroin injectors (Thiblin et al., 2004).

5.8.4.6 Inhalation

Heroin can also be volatilized, usually by heating it on a piece of folded tinfoil, and the fumes then inhaled. In Hong Kong, in the past, heroin used for this purpose was often dyed red, and as the fumes rose from the foil, they could be imagined to have the undulating shape of a dragon's tail, explaining why the practice is called "chasing the dragon." Alternatively, the lighted end of a cigarette can be dipped in powdered heroin and then smoked. To keep the heroin from falling off the end of the cigarette, the smoker has to hold his head tilted backward. Heroin can also be mixed into the contents of a cigarette. None of these routes is particularly effective. Studies have been done in addicts comparing urinary excretion after heroin was administered by injection, volatilization, and smoking in the form of a cigarette. The mean percentage of morphine recovered after injection was 68%, after volatilization it was 26%, and after cigarette smoking it was only 14% (Kramer et al., 1991; Strang et al., 1997). The pharmacokinetics of heroin smoking and *snorting* are reviewed in Table 5.4.

Heroin treatment for otherwise resistant addicts is now being used, apparently with some success, in the United Kingdom, Switzerland, and the Netherlands. Similar programs are actively being considered in many other European countries. One consequence

of this policy shift is that controlled pharmacokinetic studies with realistic doses of heroin are possible. Inhaling, or "chasing the dragon," has always been an attractive practice among addicts, and it is also considered an attractive treatment option because it avoids disease-spreading injection practices. The pharmacokinetics of smoked heroin has been studied, both in the laboratory and clinical setting (Jenkins et al., 1994), but earlier studies were limited by the amount of heroin that could ethically be given to a volunteer. That perhaps explains why earlier studies found smoked heroin's bioavailability to be unpredictable. Jenkins et al. (1994) found that heroin could be detected in the blood within 1 min of smoking. Peak levels after smoking 10.6 mg/L were 299 ng/mL in one subject and 108 ng/mL in another and occurred within 5 min. Blood levels then rapidly declined to limits of detection (under 1 ng/mL) within 30 min of smoking. Levels of 6-acetylmorphine peaked 1–2 min after smoking. Jenkins et al. (1994) estimated that the half-lives of heroin, 6-monoacetylmorphine, and morphine were 3.3, 5.4, and 18.8 min, respectively. In general, these results are comparable to those observed after intravenous administration, but they are somewhat at variance with more recent kinetics estimates (Rook et al., 2006a,b).

The melting point of heroin is much higher than that of cocaine, and preparing free base heroin is more complicated than making "crack" cocaine, which is why this practice is relatively uncommon and mostly limited to treatment centers. However, Asians still heat heroin base on aluminum foil, usually with the flame of a cigarette lighter, and use a straw to inhale the vapors. The practice is widespread but it seems to account for relatively few deaths. A review of heroin-related deaths in Sydney, Australia, from 1992 to 1996 found that fewer than 1% of deaths were associated with smoking heroin. In the cases where smoking was responsible, the median blood total morphine concentration was 0.31 mg/L (range, 0.06–0.99 mg/L), and drugs other than morphine were commonly present (Darke and Ross, 2000). In a study of incarcerated Danish heroin users, heroin smokers accounted for nearly a quarter of the cases, with intravenous users having had a longer duration of use, earlier onset of abuse, and more serious somatic complications (Andersen et al., 1996).

Heroin treatment centers in Switzerland and the Netherlands offer addicts the option of injection or "chasing the dragon," and the pharmacokinetics of both routes has been compared. Plasma concentration data were obtained from 74 heroin inhalers and 32 injectors using pharmaceutical-grade heroin in realistic doses ranging from 66 to 450 mg. The bioavailability of smoked heroin was estimated at 53%. The terminal half-lives of heroin and 6-acetylmorphine were 7.6 and 21.8 min, respectively. The clearances of morphine and its glucuronides were estimated to be 73 L/h (Rook et al., 2006a,b).

The Drug and Alcohol Services Information System (DASIS) report for April of 2007 states that the proportion of primary heroin admissions who injected the drug declined from 69% in 1995 to 63% in 2005, while the proportion of primary heroin admissions who inhaled the drug increased from 27% in 1995 to 33% in 2005 (DASIS Report, 2007). Future trends in this area are impossible to predict. The most interesting development in recent years has been the discovery of a link between *snorting* heroin and acute leukoencephalopathy. While reports of heroin leukoencephalopathy are not rare (see Section 5.12.6.6), almost all seem to follow episodes of snorting and inhalation and not intravenous use (Brown and Bourque, 2006; Fekete and Lechan, 2006; James et al., 2010; Basak et al., 2011; Buxton et al., 2011). The most current thinking in this area is that, in some way, heroin causes mitochondrial dysfunction resulting in demyelination (Zhou and Lin, 2014), although why there seems to be a predilection for this disorder to occur in smokers rather than intravenous users is not known.

5.8.4.7 Skin

Morphine is not sufficiently fat soluble to be absorbed through intact skin, at least not in the quantities needed to produce psychological effects, unless the epidermis has been disrupted. In the mid-1990s, attempts were made at developing a morphine patch. It worked by causing a small epidermal bleb to form, allowing drug access to deeper layers of the skin. The results of initial experiments suggested that clinically relevant quantities of morphine could be delivered in this manner; however, the device has not yet come to market (Svedman et al., 1996) and at this point it appears it never will. Other opioids, particularly fentanyl and sufentanil, and also meperidine, are well absorbed through the skin. Because these other agents are also much more potent than morphine or heroin, transdermal application of fentanyl and buprenorphine has become the method of choice (Andresen et al., 2011). Both types of patches now commonly appear on the black market, and some deaths from the diverted patches have been reported (Tharp et al., 2004). Fentanyl concentrations measured in peripheral blood drawn on the day of autopsy in 10 cases (peripheral blood) were higher than those drawn the day prior to autopsy with a mean ratio (PB2/PB1) of 1.80. The ratio of heart blood concentrations to femoral blood concentrations drawn at autopsy had a mean ratio (HB/PB2) of 1.08. Some cases had blood from the same source analyzed at two different laboratories, and concentrations of fentanyl in those samples showed inter- and intralaboratory differences up to 25 ng/mL. The lesson here is that postmortem fentanyl concentrations may be affected by antemortem factors, postmortem redistribution, and laboratory variability (Krinsky et al., 2014).

5.8.4.8 Maternal/Fetal Considerations

Mothers can transfer morphine across the placenta and in their breast milk (Anon., 1861), but the degree to which morphine crosses the placenta is unpredictable. Narcotic agents do passively diffuse across the placenta, but many variables and experimental design issues could affect the outcome of any clinical studies.

There is clear evidence that morphine glucuronides can be detected in the infants born to morphine-treated mothers. However, the disposition of these metabolites is poorly understood and clinical concerns have been raised about accumulation of active metabolites in the fetus. Studies performed in the pregnant baboon model show ready transfer of the glucuronide to the fetus, with a mean ± SD fetal-to-maternal concentration ratio of 0.79 ± 0.04. The quantity of 3-glucuronide transferred amounted to very little compared to the amount of morphine that was transferred. The results of other studies clearly show that the transfer of M3G across the placenta is bidirectional. Placental transfer emerges as the major clearance pathway for the glucuronide from the fetus and suggests a component of active efflux (Garland et al., 2008).

Given the paucity of data about all drugs in breast milk, it is customary to estimate infant exposure to maternal medications by relying upon reported milk-to-maternal plasma drug concentration ratios, maternal plasma drug levels, and the volume of milk consumed over a given time by a normal child (Wilson et al., 1980).

In fact, the only really accurate way to measure breast milk drug transmission is to collect the entire volume of milk from both breasts over 24 h, then measure both the volume of milk and the amount of drug contained in it (Begg et al., 2002). This is difficult to do, and has not been done either for morphine or heroin in humans. However, when studies have been done on other drugs such as prednisolone, fentanyl, and propofol and, more recently, hydrocodone and hydromorphone, the amount of drug actually transmitted has

been such a small percentage of the amount given to the mother that most experts see little need to discontinue breast-feeding (normally, a nursing mother having elective surgery is advised to discard her milk for 24 h) (Nitsun et al., 2006). Nonetheless, it is possible for lethal amounts of morphine to be transferred via breast milk. A case report described the death of a breast-fed infant who died of a morphine overdose; his mother, who was an undiagnosed hypermetabolizer (i.e., had an overly active form of cytochrome P450 2D6 [CYP2D6]), had been prescribed codeine. Codeine relieves pain only to the extent that it is converted to morphine. Tragically, the mother converted an inordinate amount of codeine to morphine. The concentration of morphine in the breast milk was 87 ng/mL, while postmortem blood of the baby had a concentration of 70 ng/mL (Madadi et al., 2007).

After the fetus takes up morphine, it is metabolized and excreted, but neonates produce morphine glucuronides at a slower rate than older children or adults (Faura et al., 1998). Morphine and its metabolites can be detected in the amniotic fluid (Rurak et al., 1991) or in specimens of hair or meconium (Little et al., 1990; Rurak et al., 1991; ElSohly et al., 1999).

5.9 Tissue Disposition

Estimates of morphine's volume of distribution vary widely and the differences are even wider when values in the living are compared to those in the dead (Table 5.6). Jung and Reidenberg have summarized values reported from clinical and autopsy studies in a tabular format (Table 5.7) and the overlaps are, indeed, striking (Jung and Reidenberg, 2005). Values listed in Table 5.7 are from various autopsy series, some involving one drug and some involving multiple, some with 6-acetylmorphine present and some not. To date only eight case series have been published. The value of the reported results for postmortem blood is limited by the fact that the origin of the blood (cardiac versus peripheral) is not always given.

Table 5.6 Tissue Levels from Five Cases of Acutely Fatal Heroin Overdose

Tissue	Range
Blood	0.06–0.90
Urine	0.21–6.60
Bile	0.09–1.25
Stomach contents	0.01–0.03
Lung	0.09–0.18
Liver	0.07–0.29
Kidney	0.01–1.18
Heart	0.09–0.10
Spleen	0.11–0.95
Brain	0.01–0.10
Vitreous humor	0.03–0.35
Testicle	0.03–0.09
Muscle	0.01–0.04

Source: Adapted from Kintz, P. et al., *Acta Med. Leg. Soc.* (*Liege*) 39(2), 464, 1989b.

Note: Values are in mg/L or mg/kg. Urine and bile specimens were hydrolyzed to free morphine from its conjugate.

Table 5.7 Range of Reported Postmortem Blood Morphine Concentrations

Authors	*n*	Range (mg/L)
Burt et al. (2001)	82	0.90–2.1
Darke et al. (2002)	977	400–700
Gerostamoulos and Drummer (2000)	40	1.07 median
Jones et al. (2012a)	766	0.04–5.5
Logan and Smirnow (1996)	16	120–470
Minett et al. (2010)	161	103–243
Monforte (1977)	61	10–1000
Wyman and Bultman (2004)	25	2.99–3.6

Source: Adapted from Jung, B.F. and Reidenberg, M.M., *Clin. Pharmacol. Ther.*, 77(4), 324, 2005.

In smaller reported series, and case reports, some authors have reported V_{ss} values of less than 1 L/kg (Chauvin et al., 1987; Furman et al., 1990; Lotsch et al., 1996, 1999; Berkenstadt et al., 1999a,b; Rook et al., 2006a,b), while others have calculated values approaching 7 L/kg. The reason for the variation is unclear. It could be related to the health of the volunteers in general or to important genetic polymorphisms that remain undiagnosed. In patients with congestive failure and edema, fluid content would be higher, as would the volume of distribution for almost all drugs. Conversely, in patients with renal failure, where intravascular volume is often decreased, smaller fluid volume might yield a smaller value of distribution. Cancer patients are often cachectic with depleted fat stores, which would also tend to decrease the volume of distribution and confuse any attempt at interpretation, because drugs are sequestered in fat, and this extra storage space may contribute to the apparent amount of free morphine present. Finally, the extreme variation could be a function of the dose; hospital patients receive doses in the tens of milligrams; addicts may inject as much as 450 mg at one time. None of these considerations applies after death. Cadavers have neither "compartments" nor volumes of distribution.

One consequence of morphine's apparently large, but variable, volume of distribution is that less than 2% of a given dose of morphine is to be found in the circulating blood of the living. After initial intravenous administration, morphine is rapidly distributed throughout the body so tissue concentrations generally reflect the relative blood flow. After death, the time it will take morphine to redistribute and re-equilibrate, and the final tissue concentrations achieved when redistribution is complete, can be altered by all of the factors discussed earlier, most especially age. Postmortem redistribution from tissue to blood may easily double measured blood morphine concentration (Skopp et al., 1996; Bogusz, 1997), which is why the postmortem ratio of morphine to its metabolites has even less forensic value or meaning.

Another issue complicating the interpretation process has only recently become clear. The control of uridine diphosphate glucose (UDPG) production is polymorphic, as is control of P-gp. The P-gp proteins normally complex with morphine but cannot complex with either M3G or M6G because both of these molecules are too polar. Once either of the two metabolites is formed in the liver, it forms complexes with another glycoprotein called MDR1 and is immediately transported back into the circulation. UDPG is located in hepatic sinusoidal membranes. If the wrong (polymorphic) form of UDPG is present, either the expected quantity of G6P may not form or it may never make its way to the

bloodstream (Zelcer et al., 2006). In fact in animal experiments, M3G, the predominant morphine metabolite, is transported by human MRP2 (multidrug resistance-associated protein 2), a protein present primarily in the apical membrane of liver cells. When mice are genetically engineered so that they cannot secrete M3G protein, members of the MRP family transport M3G into the liver sinusoids in place of P-gp. The process is not nearly as efficient as the normal sequence and the M3G is transported across the sinusoidal membrane at a low rate. As a result M3G in plasma remains in the circulation for much longer than any of the other metabolites (van de Wetering et al., 2007). This may account for some toxicology reports showing massive amounts of M3G in the plasma even though there is little or no morphine or M6G.

A meta-analysis of 57 different studies examined the effects of age, renal impairment, route of administration, and method of sample analysis on the ratios of M3G to morphine (M3G:M) and M6G to morphine (M6G:M) and the relative concentrations of M3G and M6G in living patients. The ratios of metabolites to morphine were so wide (0.001–504 for M3G:M and 0–97 for M6G:M) as to make such calculations utterly worthless for forensic purposes in the living, let alone the dead (Faura and Collins, 1998; Jung and Reidenberg, 2005).

Muscle is an important storage site for opiates simply because of its sheer bulk. Several studies have shown that postmortem muscle morphine concentrations are similar to concentrations measured in blood (Garriott, 1991; Moriya and Hashimoto, 1999; Drummer, 2004). Other studies have shown that, while muscle is a sensitive matrix for the detection of morphine, the relationship between muscle and blood concentration is so great that muscle testing should be reserved for quantitative purposes (Hargrove and Molina, 2014). Muscle is not the appropriate testing matrix for all drugs. Indeed, animal studies have shown that, for tetrahydrocannabinol (THC), like muscle, concentrations do not necessarily bear any relationship to blood or brain concentrations (Brunet et al., 2010). The same lack of correlation is apparent with MDMA (ecstasy) measured in humans (De Letter et al., 2007).

Morphine is not as highly lipophilic as some other opioids, such as fentanyl, but it does accumulate in fat, where it can be measured after death (Levisky et al., 2000). Morphine crosses the BBB but does not possess an aromatic hydroxyl group at the C3 position so it does not enter the brain as freely as heroin. The passage of morphine across the BBB is mediated by P-gp located in brain capillary endothelium. Drugs that interfere with P-gp (such as doxorubicin) can alter brain morphine uptake and disposition. Morphine tissue disposition does not appear to be altered by the concomitant use of sympathomimetic agents such as ephedrine and phenylpropanolamine (Dambisya et al., 1992). Whether this is also true for methamphetamine and cocaine is not known, though studies of placental drug transport suggest that it may well be (Malek and Obrist, 2009). There is some evidence that cholecystokinin plays the same role for methamphetamine and cocaine that is played by P-gp for morphine (Loonam et al., 2003).

Morphine and its glucuronides are not degraded by formalin, and tissues that have been preserved in formalin can still be analyzed for morphine, with the caveat that morphine will diffuse from tissue into the fixative solution (in fact, formalin is a very efficient agent for extracting morphine). In controlled studies, when liver samples were stored in formalin for 12 weeks, poststorage concentrations were found to have decreased by approximately 25% and the missing morphine was accounted for by formalin extraction (Cingolani et al., 2001).

5.9.1 Blood

Postmortem redistribution occurs (Moriya and Hashimoto, 1999), and measured blood concentrations are site dependent. Concentrations measured in samples from the heart are almost always higher than concentrations measured in the periphery. For most drugs of abuse, concentrations measured in left ventricular blood are likely to be higher than in samples obtained from the right ventricle. Evidence suggests that the process of redistribution is most intense within 24 h of death. If the sampling interval is long, substantial changes may already have occurred between the time of death and time of autopsy. In a study of the effects of postmortem redistribution in cases of heroin overdose, the mean ratio of total morphine in the femoral artery to the femoral vein was 1.2 (range, 0–4.5), and the ratio for left heart to right heart total morphine was 1.1, but with a very wide range of 0.4–3.2 mg/L. The ratio of total morphine in the left ventricle to femoral vein was 2.0 but, again, with a very great range of interindividuality: reported values ranged from 0.6 to 6.9 mg/L. In other words, centrally obtained morphine concentrations are, on average, twice as high as in samples obtained from the periphery, and there may be a greater than threefold difference in morphine concentration between the right and left sides of the heart (Crandall et al., 2006a). However, in some cases the difference may be very small, and there simply is no way to determine the degree of change in individual cases.

Gerostamoulos and Drummer (2000) measured concentrations of morphine and its metabolites in a group of 40 patients. The average postmortem interval was 59 h. The results of their study are shown in Table 5.8. The median total morphine concentration was 1.07 mg/L, with corresponding values of 0.32 ± 0.23, 0.03 ± 0.07, 0.16 ± 0.13, and 0.66 ± 0.56 for free morphine, normorphine, M6G, and M3G, respectively. These values are higher than those reported by Logan and Smirnow in another moderate-sized series of 48 decedents where the median morphine concentration in femoral blood was 0.082 (range, 0.006–1.20), with a mean of 0.143 mg/L. In "ventricular blood" (side not stated), the median was 0.141 mg/L (range, 0.008–836) with a mean of 0.230 mg/L (Logan and Smirnow, 1996). As earlier workers demonstrated, blood taken from the heart has consistently higher morphine concentrations than blood taken from the periphery.

In order to minimize the effects of postmortem redistribution, blood samples should always be taken from femoral vessels and never the heart (unless the intent is to do primary screening directly, using cardiac blood). It has been accepted for years that femoral blood samples should only be collected after the vessels themselves have been ligated, but the results of one study cast doubt on that position. Hargrove and McCutcheon studied blood concentrations obtained by "blind" femoral stick with concentrations in clamped vessels

Table 5.8 Postmortem Blood, Morphine Concentrations (n = 40)

	Median Total Morphine (mg/L)	Median Free Morphine (mg/L)
Subclavian	0.58	0.16
Heart[a]	0.76	0.19
Femoral	0.64	0.25

Source: Adapted from Gerostamoulos, J. and Drummer, O.H., *J. Forensic Sci.*, 45(4), 843, 2000.

[a] The original paper does not specify from which side of the heart the samples were obtained (samples from the left would be expected to contain higher concentrations than those from the right).

and found no significant differences (Hargrove and McCutcheon, 2008). However, until this observation has been confirmed by others, it would seem prudent to continue clamping the femoral vessels before obtaining blood.

Body packers dying from ruptured drug packets may have heroin and morphine levels that exceed 100,000 ng/mL (Joynt and Mikhael, 1985). Blood samples in 21 heroin-related deaths did not have detectable blood heroin levels in every case. Mean 6-acetylmorphine levels were 9.9 ng/mL (range, 0–82.9), while mean free morphine levels were 222 ng/mL (range, 11.2–1277 ng/mL). Smugglers may have extraordinarily high blood morphine concentrations. In one study of 10 smugglers from Colombia, blood concentrations of morphine were <1.0 mg/L in 4 victims; no morphine was detected in 1 (who had died of peritonitis). Two victims had blood morphine concentrations of 4.4 and 6.7 mg/L, respectively, and three had morphine concentrations of 35.8, 39.4, and 52.6 mg/L, respectively (Wetli et al., 1997).

Very high blood morphine concentrations may also be seen in individuals with patient-controlled analgesia (PCA) devices, as the machine may continue to infuse after death. One published report describes a 44-year-old male with end-stage pancreatic cancer. He was receiving morphine for pain control via a single subclavian intravenous catheter that continued to infuse for 45 min after his death. Even though death was ruled to be the result of the adenocarcinoma, free morphine concentrations in heart blood, vitreous fluid, brain, liver, stomach contents, and urine were 96 mg/L, 52 mg/L, 26 mg/kg, 88 mg/kg, 82 mg/L, and 976 mg/L, respectively. Total morphine concentrations in those same organs were 421 mg/L, 238 mg/L, 65 mg/kg, 256 mg/kg, and 325 mg/L, respectively (Kerrigan et al., 2004).

Another hazard of PCA morphine administration is medication error, and there are several reports in the literature. One report describes a 19-year-old woman who underwent Cesarean section and delivered a healthy infant; postoperatively, morphine sulfate (2 mg bolus, lockout interval of 6 min, 4 h limit of 30 mg) was ordered. A drug cassette containing 1 mg/mL solution of morphine was unavailable, so the nurse used a cassette that contained a more concentrated solution (5 mg/mL). The patient was pronounced dead 7.5 h later. Postmortem blood had a free morphine concentration of 170 ng/mL, with a total morphine concentration of 761 ng/mL. Analysis of the contents of the morphine cassette itself disclosed that the morphine concentration was 3.8 mg/mL (Vicente et al., 2003). For purposes of comparison, plasma morphine concentrations after 72 h of PCA treatment averaged 43 ng/mL, while concentrations of M6G and M3G were 60 and 510 ng/mL, respectively (Sam et al., 2011).

In a systematic study of PCA-related events, Schien et al. found that 6.5% of PCA events were a consequence of operator error. Most (81%) of these errors were due to pump misprogramming, of which almost half were associated with patient harm; 76.4% of adverse events were attributed to device malfunction (e.g., due to frayed wires or a crack in the drug cartridge), although only 0.5% of these were associated with harm to patients. In a report based on data from MEDMARX, a voluntary database that captures reports on medication errors, 7.9% of the PCA-related errors captured over a 5-year period were described as causing harm to patients (Schien et al., 2009).

In general, decedents of heroin overdose usually have higher median morphine concentrations than living patients who are being maintained on heroin (0.35 vs. 0.09 mg/L); however, the blood morphine concentrations of the two groups overlap substantially, ranging from 0.08 to 1.45 mg/L. Living heroin users often have morphine concentrations higher than the median concentration recorded for fatal cases (Darke et al., 1997), and there is no

logical or compelling reason to assume that the chemistry of the living should in anyway resemble the chemistry of morphine in a decaying corpse. As Jung and Reidenberg succinctly expressed it, "Opioid levels obtained at autopsy can be misinterpreted if levels presumed to be fatal in nontolerant patients are applied to opioid-tolerant patients receiving adequate opioid therapy for chronic pain" (Jung and Reidenberg, 2005).

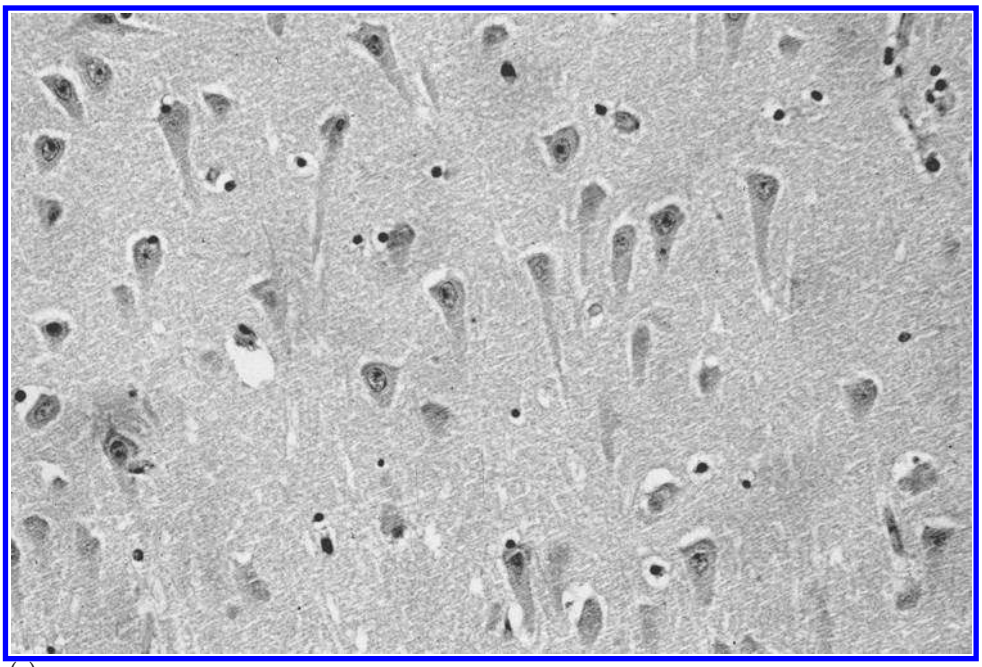
5.9.2 Brain

Immunohistochemical studies of heroin overdose show morphine localizing in the neuronal cytoplasm of the cerebral cortex, hippocampus, basal ganglia, thalamus, brain stem, and cerebellum. Binding also occurs, but to a lesser degree, in the endothelium of some brain capillaries (Liu et al., 1996). In animal studies, morphine is also taken up by astrocytes and GABAergic cells (Laux et al., 2011). The human hippocampus is particularly rich in μ-receptors (Figure 5.20a and b), and the ganglion cells located in the hippocampus, as well as their axons and dendrites, concentrate morphine to a very significant degree, particularly in cases of heroin/morphine overdose (Wehner et al., 2000). The morphine concentrations observed in different regions of postmortem brain are at least partially dependent on the number of μ-opioid receptors in that region. The number of receptors has been studied in the brains (specifically in Brodmann areas 11, 24, and 25) of dead heroin users and compared to controls (Schmidt et al., 2003). It appears there are no differences in areas 24 and 25, but the number of receptors is increased in area 11. This suggests that the frontal lobe would be the optimal sampling site for measuring morphine concentrations.

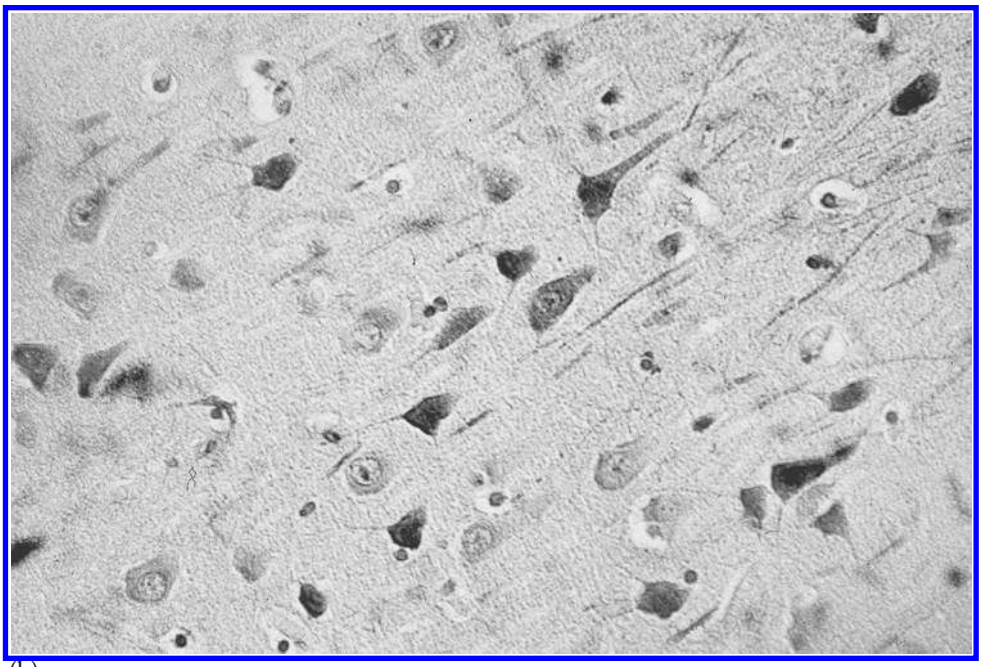
Morphine in the hippocampus exhibits a rather narrow range of concentrations. In one human postmortem study, values ranged from 134 to 298 ng/g, with good correlation between morphine concentrations in peripheral blood and hippocampus, although the concentration ranges observed in blood were much wider. For example, one victim of a lethal overdose had a total blood morphine concentration of 1.5 mg/L but only 298 ng/mL in the hippocampus (Wehner et al., 2000). The limited concentration ranges observed in the hippocampus are presumably explained by receptor saturation. Once all of the μ-receptors have bound to morphine, drug still remaining in the blood will be deposited in other tissues.

After heroin ingestion there is a significant change in the activities of glutathione *S*-transferase (GST) (detoxifying electrophilic glutathione transferases) and glutathione peroxidase (GSHPx) (nonelectrophilic glutathione transferases). When the brains of nine heroin abusers were compared with those of eight controls, GSHPx and GST activities, as well as levels of glutathione itself, were decreased in the frontal, temporal, parietal, and occipital cortices, brain stem, hippocampus, and white matter of heroin abusers. There is clear evidence that heroin (actually morphine) affects all regions of human brain that contain glutathione *S*-transferase detoxifying electrophilics and especially the brain stem. Glutathione *S*-transferase plays an important role during heroin intoxication; however, its protective effect is lower in the brain stem than in the brain cortex or hippocampus (Gutowicz et al., 2011).

Overflow from the hippocampus is very likely the explanation for the wide range of blood/brain concentration ratios that have been reported in the literature. In the three heroin users described by Kintz et al. (2005b), the blood/brain ratios were 13, 0.24, and 1.5, with tissue concentrations ranging from 0.005 to 0.089 mg/kg of wet brain. In a second small study, cerebrospinal fluid (CSF) and brain levels of 6-acetylmorphine were found to



(a)



(b)

Figure 5.20 (a, b) Histochemical demonstration of opiate receptors. The human hippocampus is particularly rich in μ-receptors, and immunohistochemical studies show that the ganglion cells located in the hippocampus, as well as their axons and dendrites, concentrate morphine to a very significant degree. The micrograph on the top is from a drug-free control brain and has been stained with anti-morphine antibodies. The bottom micrograph shows intense uptake of anti-morphine antibodies in brain tissue from a heroin overdose. (Courtesy of Professor Frank Wehner, University of Tubingen, Tubingen, Germany.)

be much higher than levels in blood, liver, lung, and kidney. One individual had a blood 6-acetylmorphine level of 11.3 ng/mL, compared to levels of 58 ng/mL in the CSF and 158 ng/mL in the brain. In a second case, blood levels were 16.2 ng/mL, while levels in the CSF and brain were 38.5 and 53.6, respectively (Goldberger et al., 1994). With the exception of CSF, the concentration ratio of blood morphine to the morphine concentrations in other tissues varies so widely as to make such determinations useless.

Blood/CSF ratios, however, appear to have more predictive value (2.74 ± 1.69). In 89% of reported cases, morphine levels were lower in the CSF than in the blood (Wahba et al., 1993; Moriya and Hashimoto, 1997). Some information about drug distribution has been derived from studies of pain management. Plasma and CSF steady-state concentrations of morphine, as well as M3G and M6G, were studied in 21 cancer patients being treated with chronic subcutaneous morphine infusions. A moderate but still statistically significant correlation was found between the daily dose of morphine administered and the concentrations of morphine and its metabolites in the CSF. The mean CSF/plasma morphine concentration ratio was 0.36 ± 0.07. As is true for brain/plasma ratios, the correlation between CSF and plasma values was poor (Wolff et al., 1996). The mean M3G and M6G concentrations in CSF were less than 10% of the concentrations found in the plasma.

Comparison of the morphine concentrations in the medulla oblongata and the cerebellum provides information on the interval between morphine administration and death. If the postmortem interval is relatively short, the ratio of morphine in the brain stem to the concentration in the cerebellum will be less than one. Higher ratios suggest that a much longer time has elapsed (Vycudilik, 1988). The ratio rises above one if at least several hours have passed.

5.9.3 Liver

Hepatic morphine concentrations have been measured in several series. The first series, consisting of 10 cases, was reported by Felby et al. (1974); the mean morphine concentration was 3.0 mg/kg, and the range was from 0.4 to 18 mg/kg. Two cases reported by Chan et al. (1986) had liver concentrations of 7.0 and 2.9 mg/kg, respectively. In a series of 20 narcotic-related deaths reported by Goldberger et al. (1994), liver concentrations of free morphine ranged from 0.039 to 0.55 mg/kg, with an average value of 0.21 mg/kg. In those same individuals, the average blood concentration was 0.099 mg/L (Goldberger et al., 1994). Chan et al. (2006) reported biliary concentrations of 312 and 248 mg/L in two cases. These values were nearly 30 times higher than the blood levels in the same individuals. Others have reported less striking differences between liver and bile concentrations. Kintz et al. (1989a,b) found bile levels of 0.087–0.363 mg/L and liver morphine concentrations of 0.067–1.424 mg/kg. In the most recently reported series of 25 heroin-related deaths, the mean liver concentration was 0.33 mg/g (range, 0.04–1.56), while the concentration of free morphine in femoral blood was 0.135 mg/L, a 10-fold difference (Wyman and Bultman, 2004). The differences have partly to do with the amount of drug taken before death and partly with the chronicity of use. It also appears that the rate of hepatic morphine metabolism may depend on the size of the liver itself. In controlled studies, plasma morphine concentrations are higher in patients with liver resection than they are in controls or in patients with colon resections who had been anesthetized with equivalent amounts of morphine (Rudin et al., 2007). The phenomenon is probably explained by the fact that less of the enzyme required to convert morphine to its glucuronides (UTG1A1 and UGT1A8) is available to perform the conversion (Ohno et al., 2008).

Concentrations of free morphine in the liver may be very high, but that is not the case for heroin. Even when heroin and its metabolites are measured, no heroin or 6-acetylmorphine is detected in samples from the liver, though high concentrations may be found in the brain: 158 ng/mL in one case report and 54 ng/mL in another (Goldberger et al., 1994). The same finding has been repeated in several studies (Wyman and Bultman, 2004). Quantitation of hepatic morphine levels is a particularly useful approach in the case of exhumations. Blood and urine are unlikely to be available; however, at exhumation soft tissue will be available, and formalin embalming does not interfere with the extraction and measurement of free morphine (Levine et al., 1994). Concentrations of morphine glucuronides remain stable in liver for several days after death, but if the cadaver is not refrigerated, or if the postmortem interval is long (>3 days), free morphine will be liberated, and the ratio of free to conjugated morphine will not be a valid indicator of concentrations prior to death (Skopp et al., 1996; Bogusz, 1997; Moriya and Hashimoto, 1997). Some believe that limb skeletal muscle can be used for the same purpose (Pounder et al., 1996).

5.9.4 Lymph Nodes

Enlargement of abdominal lymph nodes occurs more often in active drug users than in normal controls. In one series, birefringent material was detected in portal lymph nodes in 42% of addicts studied, and signs of antigen stimulation, as evidenced by the number of germinal centers and plasma cells, were twice as common in heroin addicts as in controls (60% for heroin addicts vs. 30%–40% in normals) (Kringsholm and Christoffersen, 1987). Enlarged hepatic lymph nodes are a very common finding in heroin addicts. Whether or not the enlargement is the result of some toxic effect exerted by morphine itself or the contaminants injected with the heroin is not known. Human lymph nodes concentrate morphine, and in some cases nodes taken at autopsy may have higher concentrations of morphine than blood and bile. Reported levels have ranged from 0.03 to 0.87 mg/100 g of tissue (Nakamura and Choi, 1983).

Narcotic abuse has long been associated with depressed immunity, but a mechanism to explain this defect has not been forth coming, although decreases in the numbers of lymphocytes have always been suspected. Now it appears that an anatomic explanation may have been identified, at least in an animal model. In a recent animal study, mice were implanted with morphine pellets following which B- and T-cell subsets in the bone marrow, thymus, spleen, and lymph nodes were analyzed at various time points afterward. The lymphocyte populations most susceptible to morphine-induced depletion were the precursor cells undergoing selection. But, surprisingly, the recovery of lymphocytes following morphine-induced depletion occurred in the presence of morphine and via increased proliferation of lymphoid precursors and homeostatic proliferation of T cells (Zhang et al., 2011).

In still another animal study, proteomic analysis revealed a morphine-induced suppressive effect in lymph nodes, with decreased abundance of protein mediators involved in the functional categories of energy metabolism, signaling, and maintenance of cell structure (Brown et al., 2012). However, when the histopathology of 34 human addicts was reviewed, moderately active chronic hepatitis was evident (Lun'kova et al., 2002). Given the extremely high rate of hepatitis C infection among intravenous drug abusers, chronic infection seems to be the most likely explanation for lymphoid hyperplasia.

5.9.5 Other Biofluids

Most of the opiates can be detected in saliva, but results must be interpreted with some caution. The oral or intranasal use of these drugs may result in very high saliva levels due to high concentrations of drug in the oral cavity and the high degree of partitioning into fluids secreted in the oral cavity (Wang et al., 1994). The ingestion of poppy seeds may cause unexpectedly high morphine concentrations (Rohrig and Moore, 2003). As might be expected, for the first hour after intranasal heroin administration, saliva morphine concentrations far exceed those in the plasma (Cone et al., 1993), although there is still a higher concentration in saliva many hours later due to partitioning.

Simultaneous measurements of morphine in saliva, plasma, and urine have shown that urine concentrations of morphine may be as much as 100 times greater than concentrations measured in saliva and 16 times higher than levels in the plasma (Cone et al., 1991). Because it is much more soluble in water than morphine, heroin appears in saliva much more quickly (Wang et al., 1994), but neither compound is likely to be detectable in saliva for much more than 12 h. Low doses of heroin (less than 5 mg) are unlikely to be detected in saliva at all (Gorodetzky and Kullberg, 1974).

Heroin is metabolized too quickly ever to be detected in CSF levels, but 6-acetylmorphine may be detectable, and sometimes at very high concentrations (>0.20 mg/L), even when no heroin or 6-acetylmorphine is detected in the blood. However, 6-AM is more likely to be found in the vitreous than in CSF; Wyman and Bultman (2004) found it to be present in all 25 cases analyzed in their study. The codeine/morphine concentration ratio in vitreous humor is generally similar to that reported for blood and urine, suggesting that vitreous measurement can be used as the basis for differentiating among fatalities induced by codeine or morphine (heroin) (Lin et al., 1997). In one study of 29 heroin-related fatalities, the mean concentration of 6-AM was 10 ng/mL in the CSF and 17 ng/mL in the vitreous (Pragst et al., 1999).

Morphine concentrations peak in the CSF 3 h after intramuscular administration, and at equilibrium the ratio of CSF to plasma morphine is very nearly 1:1. The elimination half-life of morphine from CSF is the same as that from the blood (Nordberg, 1984). Measurements made in patients undergoing lumbar myelography 1.5 h after they had been given 10 mg intramuscular doses of morphine revealed CSF levels of morphine, M6G, and M3G of 8.8, 35, and 55 ng/mL, respectively (Laizure et al., 1993). CSF morphine levels higher than 20 ng/mL are thought to be consistent with narcotic-induced fatal respiratory depression (Logan and Luthi, 1994).

Recent attempts at using saliva or oral fluid (toxicologists prefer this nomenclature because, in addition to saliva, secretions from other glands are also present) continue to suggest limited forensic use, and the distributions of drug concentration ratios are sufficiently wide for most drugs to prohibit the estimation of plasma drug concentrations from measurements made in oral fluid. In one study the median oral fluid/blood drug concentration ratios for the most prevalent drugs were 0.036 diazepam, 0.027 nordiazepam, 7.1 amphetamine, 2.9 methamphetamine, 5.4 codeine, 1.9 morphine, and 4.7 THC (Gjerde et al., 2010). The correlation coefficients between drug concentrations in oral fluid and blood ranged from 0.15 to 0.96 for the six most prevalent drugs. While these findings may be of interest to clinical pharmacologists, even swabbing the mouth of a cadaver would not provide any useful evidence except to confirm the presence or absence of a drug.

5.9.6 Urine

After morphine has been converted to glucuronide, it is excreted in the urine. In autopsy studies, urine concentrations of conjugated morphine have ranged from 100 to 120,000 ng/mL (Sawe, 1986). The concentration ultimately measured depends largely on the volume of urine that is allowed to collect between measurements (Cone, 1990). In a study of 29 victims of heroin overdose, the blood/urine ratio for morphine was 2.53 ± 5.45, but the range was so wide (0.006–25.2) that drawing any sort of inference is impossible (Wahba et al., 1993). In a study of 168 heroin-related deaths investigated by the San Francisco Medical Examiner's office in 1999, total morphine concentrations in the urine ranged from less than 10 to 85,000 ng/mL, a result strikingly similar to the results in Sawe's original study performed more than 15 years earlier (Karch et al., 1999, unpublished data).

Racial and interethnic differences must also be considered. Chinese subjects have a higher clearance rate for morphine than Caucasians, primarily because they form more glucuronide than Caucasians do, and they do so more quickly. Whether or not these differences will have a bearing on drug detection is not clear, but they certainly can have clinical significance; in nontolerant subjects equal doses of morphine produce more respiratory depression and a greater drop in blood pressure in Caucasians than in Chinese (Zhou et al., 1993). The differences are, no doubt, a consequence of UDPG2 polymorphisms (see Sections 5.8.9 and 5.9.6.3). Except for demonstrating the presence of morphine or 6-AM, urine measurements provide very little information relevant to the forensic pathologist.

Nonetheless, the method is still widely used by pain physicians who are trying to monitor compliance. Laboratories testing for pain medications use cutoffs established by the manufacturers of immunoassay reagents themselves, not by any peer-reviewed scientific organization. Many feel that these cutoffs may be inappropriate for monitoring patients being treated for chronic pain with opioid therapy because the cutoffs are set too high (Pesce et al., 2011). The utility of urine testing would seem to be confined to (1) obscure poisonings and (2) the monitoring of patients in methadone and alcohol withdrawal programs, where abuse of other drugs is suspected.

5.9.7 Hair

The use of hair is increasingly accepted by forensic practitioners and for a number of important reasons. In the living, collection is noninvasive. At autopsy, collection of an ample sample takes only a few seconds. Though hair samples are susceptible to external contamination, particularly in the autopsy suite, drug stability is not an issue, adulteration is not a problem, and the window of detection is enormous (from a few hours after death until centuries later). The technology of the process is constantly improving, not just in sensitivity and specificity but also in price. Morphine and 6-AM are both detectable in hair (Puschel et al., 1983; Kintz and Mangin, 1995).

Although the appropriateness of hair testing for some purposes has been questioned, its value in forensic pathology cannot be overestimated. Drugs remain stable in hair for as long as the hair is present. The position of drug along the hair shaft can give a good indication of when, in relation to time of death, the drug was ingested. The failure to detect drug in hair, even when massive amounts are present elsewhere in the body, suggests overdose. Conversely, when morphine and, perhaps, 6-AM can be detected all along the hair shaft, chronicity of use is established. However, the greatest advantage of hair testing is that it need not be performed

immediately. Should the autopsy disclose an unexpected natural cause of death, no testing is required. Should a question about possible drug involvement arise at some later date, the specimen can be retrieved and tested with full confidence that any drugs that were present at the time of death will still be present in the same concentrations. The most frequently detected drugs of abuse in hair are cocaine and its metabolites, amphetamine-type stimulants, and opiates. The most critical and time-consuming step in hair analysis for opiates is their quantitative extraction from the hair matrix. Extraction can be performed in several ways, and many different procedures have been published. Most of the extraction techniques include mild acidic hydrolysis or methanol extraction. One important issue regarding hair analysis for opiates is the real possibility of heroin or 6-AM being accidentally converted to morphine, which is likely to occur under uncontrolled acidic or alkaline conditions.

Spontaneous conversion (which would not lead to the production of any 6-AM) would make it impossible to prove that heroin had actually been used. Since heroin is hardly the only opiate ever abused, a technique that can detect other related opioids, but not destroy 6-AM that may be present, is desirable. Several methods utilizing mixed-mode solid-phase extraction and GC-MS are known (Barroso et al., 2010).

5.9.8 Excretion and Detectability

The conversion of heroin to morphine is so rapid that the probability of detecting heroin in either blood or urine is small and the possibilities of detecting 6-AM are not that much greater. However, once the conversion to morphine is complete, the limits of detection for the metabolites are the same as for morphine itself. Testing for opiates in urine is a problem. Poppy seeds are widely eaten and they contain both morphine and codeine, but not heroin. Poppy seeds from different origins contain widely variable amounts of morphine (2–251 mg/g) and codeine (0.4–57.1 mg/g) (Pettitt et al., 1987; Pelders and Ros, 1996), but under no circumstances will innocent poppy seed eaters have detectable levels of 6-AM. Small amounts of 6-AM may also be ingested directly as contaminants produced along with heroin in the manufacturing process (O'Neil and Pitts, 1992).

German researchers compared concentrations of urine morphine and its metabolites in two groups of volunteers. On two separate occasions the volunteers consumed cake that had been baked with one of two different kinds of poppy seeds (10–60 g of poppy seeds): one contained high concentrations of papaverine and noscapine, two compounds considered as surrogate markers for heroin, while the other type of poppy seeds was also very high in morphine content, but contained relatively little of the marker compounds. The researchers then collected serial urine samples and tested them using LC-MS/MS. Peak concentrations of morphine, codeine, and their glucuronides appeared 4–8 h after ingestion of poppy seeds, and morphine concentrations were often in excess of 10 μg/mL. Morphine glucuronides were present in serum samples taken up to 6 h after consumption. Free morphine was only detected in traces (1–3 ng/mL) within 2 h of consumption. Neither noscapine nor papaverine was detectable in urine or blood samples after the consumption of poppy seeds containing up to 94 μg noscapine and up to 3.3 μg papaverine. Both of the latter were rapidly metabolized, whereas desmethylpapaverine and, especially, its glucuronide were found in urine samples even 48 h after consumption. Thus the presence of papaverine and its metabolites should not be regarded as markers for the consumption of heroin, which can only be inferred if a specific marker such as acetylcodeine is detected. Unfortunately, the chances of finding 6-AM are small (Trafkowski et al., 2006).

To date only one study has been published where the actual amount of morphine and codeine given to volunteers was measured beforehand (Smith et al., 2014). Twenty-two volunteers submitted 391 urine specimens after ingesting two doses of seeds containing 15.7 mg morphine and 3 mg codeine. All urine samples were collected over 32 h: 26.6% and 83.4% were positive for morphine at 2000 and 300 μg/L gas chromatography–mass spectrometry (GC/MS) cutoffs, respectively. For the 19 subjects who completed the study, morphine concentrations ranged from <300 to 7522 μg/L with a median peak concentration of 5239 μg/L. The median first morphine-positive urine sample at 2000 μg/L cutoff concentration occurred at 6.6 h (1.2–12.1 μg/L), with the last positive from 2.6 to 18 h after the second dose. No specimens were positive for codeine at a cutoff concentration of 2000 μg/L, but 20.2% exceeded 300 μg/L, with peak concentrations of 658 μg/L (284–1540 μg/L).

If 6-AM is not detected, distinguishing innocent poppy seed ingestion from heroin abuse can be problematic. In the past, the distinction was made by relying on the presence of confirmatory evidence such as track marks. That approach was never very effective, and now that many heroin users insufflate the drug, the presence or absence of track marks cannot be relied upon to make that distinction. Workplace testing regulations in the United States now recognize that reality and the urinary opiate cutoff has been raised to 2000 ng/mL of urine. If concentrations exceed the 2000 ng/mL cutoff, 6-AM must also be detected to prove heroin use.

Thebaine, a naturally occurring compound found in poppy seeds, also offers potential as a surrogate for the diagnosis of heroin consumption. It is not present in refined heroin but does appear in the urine of poppy seed consumers. Volunteers given an 11 g dose of poppy seeds were found to have urine thebaine concentrations ranging from 2 to 81 ng/mL (Cassella et al., 1997). Another alternative is to test the hair. Both 6-AM and M6G are deposited within the hair matrix and remain stable there for many months (Rothe and Pragst, 1995). The only problem with this approach, and it is largely theoretical, is that heroin samples can also contain 6-AM. That means that the presence of 6-AM, like the presence of heroin itself, might be the result of external contamination (as in the case of a customs officer who confiscates a large quantity of heroin).

5.9.9 Postmortem Tissue Measurements

In the past, the half-life of 6-AM was believed to be too short to routinely quantitate. In six volunteers given single doses of heroin (3.0 and 6.0 mg of heroin), the urine half-life averaged 0.6 h, with a total detection time of 2–8 h. In contrast, free morphine and total morphine were detectable in the urine for up to 24 h after heroin administration (Cone and Welch, 1991). With advances in technology, it is now possible to monitor heroin and all of its metabolites simultaneously. The measurement process is straightforward when liquid chromatography–mass spectrometry (LC-MS) is used, and this approach also has much higher sensitivity than previous mass spectrometry (MS) methods.

Postmortem measurements of morphine and its metabolites in cases of heroin overdose have been reported. In a German study, morphine, M3G, M6G, and 6-AM were quantitated simultaneously in 21 heroin overdose victims. Blood concentrations of morphine ranged from 8 to 1539 ng/mL, M3G from 111 to 941 ng/mL, M6G from 32 to 332 ng/mL, and 6-AM from 0 to 73 ng/mL. The levels of morphine were correlated with glucuronide values and with 6-AM (Bogusz, 1997). Very similar results, at least for the glucuronides, have been reported from Australia (Gerostamoulos and Drummer, 2000) and from the United States (Logan and Smirnow, 1996).

Concentrations of morphine, M3G, and M6G are generally lower in blood and in vitreous humor than in CSF, but the concentrations of morphine and molar ratios of M6G to morphine in blood and CSF correlate very well. Thus, the presence of much more morphine than glucuronide is thought to be consistent with ingestion immediately prior to death. However, the ratio of morphine to its metabolites is highly dependent on (1) the time elapsed from death until tissue sampling (postmortem interval), (2) the temperature, (3) existing but unsuspected UDPG polymorphisms, (4) the tissue being sampled, and finally, (5) the volume of distribution of the three molecules.

Gerostamoulos and Drummer (2000) did not find significant differences between morphine and morphine metabolite concentrations in samples taken from subclavian, heart, and femoral vessels, nor did they observe any significant differences between concentrations measured on arrival at the morgue and concentrations measured, on average, 59 h later. There are two possible explanations for this finding: either significant postmortem redistribution of morphine and its metabolites simply does not occur, which seems unlikely, or redistribution and equilibration had already occurred before the first blood samples were drawn. The latter seems more likely, given that, when others have obtained samples from multiple sites, great concentration differences have been observed (Skopp et al., 1996).

The results of animal studies also support the second possibility. In a study of experimental morphine overdose, 20 New Hampshire swine, each weighing between 50 and 70 kg, were injected with a morphine dose of 2 mg/kg. Blood concentrations were then measured and compared with concentrations in the vitreous humor, as well as in the femoral artery and vein and left and right ventricles (Table 5.9). Samples were obtained at regular intervals starting 30 min before the injection and continuing for 95 h after the time of death.

Comparisons were then made between antemortem and postmortem values. The researchers found that concentrations of both free and total morphine varied significantly between animals, between sampling sites, and over time. Free morphine values were generally higher after death than before, but total postmortem morphine levels were similar to antemortem levels. Time had an effect, but a small one (Crandall et al., 2006b). Concentrations of free morphine had doubled within 1 h of death, and then dropped by nearly three quarters in 1 h.

Overreliance on morphine-to-metabolite ratios can lead to erroneous conclusions (Skopp et al., 1996). As previously discussed, morphine has a very large steady state volume of distribution (V_d of 2–5 L), but the volume of distribution of the metabolites is small ($V_d < 1$). At equilibrium, virtually all of the free morphine is found in tissue, while all the glucuronides are found in the blood. The movement of less than 1% of free morphine from tissue back into postmortem blood would double the observed concentration and lead to the erroneous conclusion that morphine concentrations at the time of death were much higher than they actually were. Just what can be done to correct for this reality is not

Table 5.9 Postmortem Morphine Values in Swine (Concentrations Are in ng/mL)

Time	Left Ventricle	Femoral Artery	Right Ventricle	Femoral Vein	Vitreous Humor
10 min	424	407	394	576	880
60 min	1061	1346	1071	1039	439
8 h	706	461	565	778	198

Source: Adapted from Crandall, C.S. et al., *J. Anal. Toxicol.*, 30(9), 651, 2006b.

known, as there is no reproducible scientific method by which one could determine how much free morphine had migrated back into the tissue.

Tissue levels in heroin body packers carrying balloons full of heroin in their intestines may reach astronomic levels. A woman who had swallowed a number of packets containing 25% heroin was found to have a 6-AM level of 184,000 ng/mL. Morphine and codeine levels were equally impressive (120,000 and 1700 ng/mL, respectively) (Joynt and Mikhael, 1985; Wetli et al., 1997). A report from Thailand indicates that the average deceased body packer carries 30–50 g of heroin that is 50%–90% pure (Sribanditmongkol et al., 2006), though this amount seems quite small compared to other reports that have described cases where more than half a kilogram of heroin was contained in the intestines (Wetli et al., 1997), the amount was still more than adequate to produce a lethal outcome.

5.10 Interpretation of Opiate Blood and Tissue Concentrations

5.10.1 Introduction

No matter which tissue is analyzed, the cause of death cannot be determined from an isolated toxicologic measurement, although with some drugs very firm inferences can be drawn. It is accepted dogma that specific postmortem blood concentrations cannot be said to have caused death, morbidity, or even significant impairment without also knowing the clinical history and autopsy findings. The problem with this concept is that nearly 10% of all autopsies are found to be *negative*, a result mainly of the fact that some natural causes cannot be determined easily or at all. Recently, an editorial published in the journal *Circulation* (June 3, 2014) observed that if coronary artery disease is not responsible, most cases of sudden infant death syndrome (SIDS) and sudden unexpected death syndrome (SUDS) (200,000–400,000/year) were due to cardiac arrhythmia where syncope and sudden death are often the first manifestations of heart disease. Today, we now know that ~35% of SUDS and ~20% of SIDS cases may be explained by mutations in cardiac ion channels (*cardiac channelopathies*) (Deo and Albert, 2012; Giudicessi and Ackerman, 2012 ; Krexi et al., 2015). The implications of these observations are clear and daunting. Neither the presence of viruses nor the presence of polymorphisms can be reliably detected without DNA testing.

Even if gross autopsy changes are visible, the investigator still needs to observe the scene, obtain the individual's past medical and drug use history, and factor all these components together before an accurate cause of death can be determined. There is a tendency among death investigators to forget that historical information may be available from many sources, including the emergency room records and interviews, the records of physicians who attended the patient in the past, and the pharmacies that provided the drugs (Harding-Pink and Fryc, 1988). In the following sections, the usefulness of each testing modality is reviewed. The need for testing antemortem blood samples, if available, is so obvious as not to need stating.

5.10.2 Urine Testing

Poppy seed ingestion ensures the presence of both codeine and morphine in the urine. A prescription for codeine could explain the presence of some, but not massive, amounts of morphine detected in the urine. After oral dosing with codeine, 5%–15% may be detected

in the urine as free or conjugated morphine (Fell et al., 1983; Gjerde and Morland, 1991). The conversion of codeine to morphine is believed to be the method by which codeine produces analgesia. Under current federal workplace testing rules, urine specimens are considered to be presumptively positive if an opiate concentration in excess of 2000 ng/mL is detected. For confirmation, the sample must be tested again with a different method, preferably MS. Morphine or codeine must be present in a concentration of at least 2000 ng/mL, and to specifically prove heroin ingestion, at least 10 ng/mL of 6-AM must also be detected. However, the half-life of this latter compound is so short that these criteria often cannot be met. In the setting of a drug death investigation, the vitreous humor is the matrix most likely to yield a positive test for 6-MAM, as that molecule persists in the vitreous long after it has been cleared from the bloodstream (Wyman and Bultman, 2004).

Heroin use can also explain the presence of both morphine and codeine in the urine, simply because it is rapidly converted to morphine and also because heroin is often contaminated with small amounts of codeine (Bastos et al., 1970). Humans do not metabolize morphine to codeine (Mitchell et al., 1991). Codeine-containing cough syrups (formerly, one syrup sold in Japan and Southeast Asia was responsible for a large percentage of positive tests at the U.S. Army testing lab in Hawaii) are an important cause for false-positive workplace drug tests, as are poppy seed-containing pastries. Poppy seeds, as discussed in Section 5.9.8, naturally contain morphine and codeine, and very high levels of both drugs can sometimes be found in some individuals (ElSohly et al., 1988). One must accept that if the individual being tested has a prescription for codeine, and also claims to have eaten poppy seeds, it is possible that their urine might contain more morphine than codeine, even if the person was not abusing drugs!

The commercial opiate assays currently in general use are unlikely to cross-react with synthetic and semisynthetic opiates, partly because the original federal regulations regulating workplace programs are specific for morphine. However, newer assay systems are being introduced and federal rules modified so that the detection of drugs such as oxymorphone and oxycodone is not only possible but also allowable. Recent events suggest that the screening issue may not be as straightforward as once thought. Black market fentanyl (usually in the form of alpha methyl-fentanyl) seems to be emerging, as are a number of newer synthetic drugs such as the bk-amphetamines. The new drugs are appearing at an alarmingly fast rate and manufacturers of immunoassays are unlikely to test them for cross-reactions with their opiate assays.

5.10.3 Blood Testing

Regular opiate users rapidly become tolerant to opiate-induced respiratory depression, making the interpretation of blood or plasma concentrations (from the living or dead) extremely difficult. In acute overdose, where death is obviously due to respiratory depression and frothy pulmonary edema is present, blood concentrations have ranged anywhere from 100 to 2800 ng/mL (Felby et al., 1974; Richards et al., 1976; Reed et al., 1977; Logan et al., 1987; Sawyer and Forney, 1988; Steentoft et al., 1988; Kintz et al., 1989a; Logan and Smirnow, 1996; Bogusz, 1997; Gerostamoulos and Drummer, 2000; Jung and Reidenberg, 2005; Crandall et al., 2006a,b; Jones et al., 2012a).

Much of the early literature on morphine toxicity was published before it was understood that M6G had roughly the same psychoactive effect as morphine and even greater binding affinity for the μ-receptor than morphine itself. The problem was that the very

early studies of drug excretion and metabolism were performed with the first generation of radioimmunoassay; the value of these tests is largely diminished by the fact that they had such wide cross-reactivity. The final results did not give an accurate picture of the free morphine in circulation. Thus the ranges reported using early immunoassay techniques were neither very accurate nor very meaningful. Even if these numbers did have some meaning, the same absolute morphine concentration may be associated with death in a naïve individual, while it may produce minimal, if any, symptoms in an experienced user. Morphine blood concentrations in living addicts receiving maintenance heroin may, in fact, be substantially higher than in individuals dying of heroin overdose (Darke et al., 1997). Postmortem blood morphine concentrations cannot be interpreted in isolation.

In 2012 a study from Sweden measured the concentration of free morphine in femoral blood from heroin-related deaths and compared the results with the concentration measured in venous blood taken from impaired drivers. The presence of 6-MAM in blood or urine was considered a biomarker for recent heroin use. The researchers found that concentrations of free morphine in blood were not associated with age of heroin users and that the median concentration of free morphine was higher in autopsy cases (0.24 mg/L, $n = 766$) compared with apprehended drivers with 6-MAM in blood (0.15 mg/L, $n = 124$, $p < 0.05$) and appreciably higher than in drivers with 6-MAM in urine but not in blood (0.03 mg/L, $n = 1823$, $p < 0.001$). The free morphine concentration was above 0.20 mg/L in 65% of autopsy cases. Interestingly, free blood morphine concentrations in individuals dying from polydrug abuse were not significantly different from those in heroin-only deaths (0.25 mg/L, $n = 63$). The concentration of morphine in drug overdose deaths (median 0.25 mg/L, $n = 669$) was about the same as in traumatic deaths among heroin users (0.23 mg/L, $n = 97$). However, the concentration of morphine was lower when the deceased had consumed alcohol (0.18 mg/L, $n = 104$) compared with taking a benzodiazepine (0.32 mg/L, $n = 94$) (Jones et al., 2012a).

Some special considerations apply to postmortem testing that are not encountered in the clinical laboratory. Morphine and its glucuronides are extremely stable in refrigerated blood and in the plasma, as is morphine in unrefrigerated blood. But the glucuronides are not stable in unrefrigerated postmortem blood (Skopp et al., 2001). Temperature, exposure to light, length of sample storage, and bacterial overgrowth all affect the final measured morphine concentration. The longer the sample is stored and the higher the temperature, the more likely decomposition is to occur. Most of the change is a result of bacterial hydrolysis of morphine glucuronides; bacteria can be cultured from postmortem blood within 5 h of death (Melvin et al., 1984).

Other than the fact that the respiratory drive ceases and the lungs fill with protein-rich edema, the actual cause of these deaths is not completely understood, partly because nothing can be determined about the state of tolerance after death. At the trial of Harold Shipman, a UK physician who murdered hundreds of his patients with heroin injections, it was argued that the absence of heroin in the hair, in the face of massive concentrations of heroin in muscle and liver, proved that the decedents could not have been tolerant. Had they been taking heroin for any length of time, heroin or its metabolites would have been detected in the hair and it was not. Tagliaro et al. (1998) observed that morphine concentrations in the hair of heroin overdose victims are comparable to those in former heroin users enrolled in rehabilitation programs, and that hair from individuals in both groups contained substantially less morphine than hair from living, active heroin users.

While it is reasonable to presume that high postmortem blood morphine concentrations in the face of low (or undetectable) hair morphine concentrations indicate a lack of opiate tolerance, there are some who dispute this contention. In a study of 60 cases, death was considered related to heroin intake in 28. In 18 of the 28 cases where heroin was clearly the cause of death, opioids were absent in the most recent hair segments, suggesting a reduced tolerance to opioids. But in the remaining cases, where morphine was found throughout the hair, the blood morphine levels were similar to levels in the decedents without morphine at the hair root (Druid et al., 2007). The results seem to fly in the face of common wisdom, yet may be true. Alternatively, another undetected adulterant may have been present. Clearly, more research is needed.

5.10.4 Determining the Cause of Death

5.10.4.1 Value of Scene Investigation

Examination of the death scene may reveal details that can confirm or discredit the autopsy and toxicology results. Halpern was one of the first to point out that there is certain sameness about heroin-related deaths (Halpern, 1972). More often than not, heroin users are found on the street or in an alley, injecting by themselves and dying in isolation. Decedents are much more likely to be male (>70%) and mostly in their mid-20s (Louria et al., 1967; Cherubin et al., 1972; Wetli et al., 1972). Since Halpern published his original observations, the percentage of male decedents has increased and in most areas men now account for more than 90% of opiate-related deaths.

Drug paraphernalia is likely to be found at the victim's side. Even in the 1970s, before anyone had ever thought to add fentanyl to heroin, addicts were occasionally found dead with needles still in their arms. Some of those cases may have been misdiagnosed homicides; at this point there is simply no way to tell. Today this finding suggests use of heroin laced with fentanyl. In the past, use of fentanyl was mainly the prerogative of medical workers who had ready access. Medical workers who die of fentanyl are usually found at home or at work (often a hospital) (Henderson, 1991). Now that illicit fentanyl is being added to street heroin, the profile of decedents has changed, and the possibility of fentanyl overdose must always be considered, even in what appears to be a straightforward heroin-related death.

In spite of recent studies suggesting abstinence is not an issue, experience suggests that deaths in opiate abusers are much more likely to occur when the user has been abstinent, and it is important to establish whether the decedent had just been released from jail or a detoxification program (Harding-Pink and Fryc, 1988). It is also important to establish whether or not ethanol or other drugs have been consumed. The combination of ethanol and opiates is said to be more lethal than the consumption of either drug alone; however, this assumption remains somewhat controversial. The case seems to be stronger for benzodiazepines—their simultaneous use with opiates clearly increases the risk for sudden death (Gerostamoulos et al., 2001).

Ruttenber et al. (1990) studied 505 heroin-related deaths and found that those who had not been drinking had higher morphine levels in their blood and bile (500 and 7500 ng/mL, respectively) than those individuals who had been drinking (300 and 3000 ng/mL, respectively). Similar findings were reported in an Australian study (Darke et al., 1997). When blood toxicology results for deaths attributed to heroin overdose were compared with those

of a sample of 100 living heroin-replacement patients who had injected within the preceding 24 h, the fatalities had higher median concentrations of morphine than the living heroin users (0.35 vs. 0.09 mg/L), but there was massive overlap between the two groups (0.08–1.45 mg/L). Ethanol was detected in 51% of the fatalities (median = 0.10 g/100 mL) but in only 1% of the heroin-replacement patients, and there was a significant negative correlation among fatal cases between blood morphine and blood ethanol concentrations. These results led the authors to suggest a possible role for ethanol, and possibly benzodiazepines, in some of the deaths. The suggestion is often heard in the investigation of buprenorphine-related deaths (Drummer, 2005).

The historical record is especially important when investigating deaths related to other opioids, particularly methadone, where interindividual responses are known to vary considerably because of genetic polymorphisms (which are never measured by medical examiners), sex, weight, use of concomitant medications, duration of methadone treatment, previous exposure to other opioids, and plasma concentrations of alpha-1-acid glycoprotein (Garrido and Troconiz, 1999). Where the death occurred also matters, particularly in the case of methadone, where it is now understood that the *l*-form binds to human Ether-à-go-go Related Gene (hERG) channels and that methadone and can, potentially, cause torsades de pointes (Ehret et al., 2007).

Some countries sell a racemic mixture of methadone and others do not, and so the specific type of methadone must be considered (Karch, 2011; Karch et al., 2014). Methadone induces the enzymes required for its own metabolism, which means that death is more likely to occur early, rather than late in therapy. Naïve users are at much greater risk for overdose than individuals who have been taking methadone for some time (Wu and Henry, 1990; Caplehorn and Drummer, 2002). A history of liver disease (from alcoholism or hepatitis) may imply decreased production of alpha-1-acid glycoprotein and a sudden rise in plasma methadone levels.

As this book comes to press, there is an ongoing debate about who should be the individual to classify the manner of death and whether forensic toxicologists should have this responsibility at all. The debate will continue long after publication of this edition, but from all indications it appears that The National Institute of Standards and Testing (NIST—a federal U.S. agency) will produce a set of standards for forensic toxicologists and forensic pathologists, even though there is only token representation of both disciplines writing the standard! The standards, when they are finally promulgated, will resemble existing textbooks on both forensic toxicology and forensic pathology, at least to the extent they are certain to contain some sort of statement to the effect that the true cause of death can only be determined by integrating the findings of the scene investigation, autopsy, and toxicologic analysis. Who is considered to be the most suited to the job depends on the discipline of the author, and the decision will be sure to displease multiple disciplines.

One of the many problems bound to result from the implementation of a universal protocol is that toxicologic analysis is not an independent element of death investigation. In fact, the results of toxicologic testing are a direct function of the method used to perform the autopsy and collect the specimens that are eventually tested. It also depends upon the tests that have actually been ordered by the autopsy pathologist—toxicologists are not free agents (for that matter, neither are the pathologists since, in many jurisdictions, such as the United Kingdom, they must first receive permission from a coroner before they can even order a test).

Several authors have dealt with this issue, referring to the problems collectively as *preanalytic issues* (Drummer and Gerostamoulos, 2002; Skopp, 2004; Flanagan and Connally, 2005; Flanagan et al., 2005). As Skopp (2004) observes, even though these issues do not involve the actual performance of an analysis, answers to these questions are nonetheless vital, because they can alter the final analytical result. A sample from the left side of the heart or subclavian vessels may contain a much higher morphine concentration than existed at the time of death. The longer the postmortem interval, the greater the chance that any concentration measured will not accurately reflect the concentration at the time of death, and the greater that drug's steady state volume of distribution (V_{ss}), the greater the probability that the concentration will have changed after death. The list of *preanalytic issues* is very long and it is hard to see how one government standard can anticipate, let alone solve, them. Only several of the most important will be dealt with here.

5.10.4.2 Sample Provenance

Without knowing the provenance of a sample, there is no guarantee of accuracy. Every report should state how many tubes of blood were collected and from where in the body they were obtained. The report should also note who collected the specimens, as they are likely to sample the same site every time. What preservative did the tubes contain? How were the tubes stored until the time of analysis? How many tubes were actually collected? What size syringe was used to draw the blood? If more than 40 mL of blood were taken from the inferior vena cava, some blood (and therefore some drug) drawn directly from the liver would be included in the sample. Since all of the abused drugs are metabolized in the liver, inclusion of hepatic blood in a peripheral specimen will yield a falsely elevated value. How long did the samples sit at room temperature until they were refrigerated? (Prouty and Anderson, 1990; Drummer et al., 2004) Not all metabolites are stable. Were the containers made of glass or plastic? Some drugs adhere to the side of plastic, but not glass tubes. If blood was drawn from the femoral vessels, were they ligated first? (Pounder, 1993) If not, how many milliliters of blood were drawn? Were other fluids and tissues were collected and how were they collected and stored? Was blood drawn in the hospital prior to death collected? If it was, was it analyzed, and how did the result at autopsy compare with the results obtained antemortem? What color Vacutainer was used, and what preservatives were added? Was the sample processed within the office performing the autopsy, or were samples shipped to a reference laboratory? If the latter, was the sample refrigerated or frozen? What was the condition of the specimen when it arrived at the laboratory? Ideally, all of this information would be provided in a standardized specimen collection form. The form should include comments on any potential problems with the specimen and what impact these problems may exert on the final analytic results. If the blood sample is old, has the stability of the drug during storage ever been studied? If so, what were the results? Was the chain of custody followed? Were the samples stored in a secure area? Was a log maintained to record those with access to the specimen? These are all analytical issues, even if they do not involve analysis per se hence the term *preanalytic*. The pathologist needs to have this information as it is almost a certainty that he will be asked each and every question in court. No matter the precision and accuracy of the test itself, the results can be invalidated by any of the previously mentioned issues.

Perhaps the most important issue of all is provenance. Under the best of circumstances, drug concentrations measured in postmortem blood are unreliable indicators of what the concentrations actually were during life. If the testing medium is not blood but, rather, *cavity fluid*, the toxicology laboratory should reject the sample as being inadequate

and inappropriate for anything but qualitative assessment. Quantitative measurements of cavity fluid are not worth considering. The same considerations apply to decomposing tissue and purge.

5.10.5 Postmortem Chemistry

5.10.5.1 Redistribution and Diffusion

Blood concentrations measured in postmortem material are not equivalent to measurements made in the living. "Heart blood" collected at autopsy is blood in name only. Blood is a living tissue; except to the degree that bacteria can be recovered from this material, postmortem "blood" is not a living thing, it is water and tissue debris (Jones, 1998). Not one single control study, even in animals, has ever shown that postmortem drug concentrations accurately reflect drug concentrations at the time of death, but a goodly number have shown quite the opposite to be true, chiefly because of the problem of postmortem redistribution (Pounder et al., 1996; Hilberg et al., 1999; Moriya and Hashimoto, 1999; Drummer and Gerostamoulos, 2002; Flanagan et al., 2003; Ferner, 2008). *Postmortem redistribution* is defined as the movement of a drug down a concentration gradient after death. The process begins immediately and continues indefinitely, but it appears that the greatest changes occur within the first 24 h. The greater the apparent steady-state volume of distribution of a compound, the more likely the process will occur. Redistribution is one of the chief reasons why the results of quantitative postmortem blood testing can be so misleading.

Perimortem aspiration of stomach contents is just one reason why an accurately measured blood concentration may still lead to a completely inaccurate and misleading result (Knight, 1975; Pounder and Yonemitsu, 1991). If there is drug in the stomach, aspiration will produce spuriously high drug concentrations within the bronchial tree. Simple diffusion out of the bronchi then allows drugs to traverse thin-walled pulmonary vessels, and if aspiration occurs into the left lung, the result may be misleadingly high drug concentrations, especially if cardiac blood is the analyte. Because of these two problems—aspiration and drug redistribution—blood samples obtained from different parts of the body are likely to contain different concentrations of the same drug.

The physical properties of the drug determine, to a large extent, the degree of postmortem redistribution. Drugs with a low steady-state volume of distribution, such as ketoconazole, will penetrate tissues poorly. In the living, at equilibrium, most ketoconazole will be in the plasma compartment. After death, it would still be in the plasma compartment, and not really subject to redistribution. The exact opposite is true for most drugs of abuse, which have larger apparent volumes of distribution. Their metabolites (and most glucuronides of most drugs for that matter) have a very low volume of distribution. In the living, at equilibrium, most morphine is in the tissue, but most morphine metabolite is in the plasma, providing an opportunity for redistribution of free morphine to occur after death (Skopp et al., 1996, 1998; Klingmann et al., 2000). The postmortem movement of free morphine out of the tissue, back into the plasma, makes it impossible to estimate the time of ingestion by calculating the ratio of free to conjugated morphine.

5.10.5.2 Apparent Steady-State Volume of Distribution (V_{ss})

The rate of postmortem redistribution is also a function of the V_{ss} constant of each individual drug. V_{ss} constants are not true physiologic measures; they are "fudge" factors, relating the amount of drug in the body and the concentration of drug measured in any particular

body compartment, usually the plasma (Shargel and Yu, 1992). Published values for V_{ss} are based upon measurements made in healthy volunteers (unless they are simulated from computer models), and their relevance to drug abusers, who are taking massive doses of drug, remains open to question. V_{ss} is a physiologic measure and it may be altered by many factors (Noble, 2003). V_{ss} increases with age as muscle mass decreases and fat content rises. For the same reason, muscle-wasting diseases cause V_{ss} to increase. V_{ss} is altered by blood flow, and in shock states both muscle flow and renal clearance are decreased. Drug–protein binding, glycolization, liver disease, and drug interactions all can alter V_{ss}. For the reasons discussed, there is enormous interindividual variation of apparent V_{ss}, even in healthy volunteers, under controlled conditions. For example, the V_{ss} for methamphetamine, in healthy volunteers given fixed doses of drug and then living in a locked ward, was found to range from 2 to 11 L/kg (Schepers et al., 2003). This enormous and unpredictable degree of variability, even in the living, makes it impossible to establish any sort of relationship between postmortem drug concentrations and reported dosage, though it would seem reasonable to conclude that if low levels of drug are detected, the drug was consumed. Some of the less appreciated confounders will be discussed in this section. One is alpha-1-acid glycoprotein (AGP), which binds to methadone and other opiates. AGP is classified as an orosomucoid (ORM) or alpha-1-acid glycoprotein (AGP or AAG). It is a plasma alpha glycoprotein modulated by two polymorphic genes. It is synthesized primarily in hepatocytes and has a normal plasma concentration between 0.6 and 1.2 mg/mL (1%–3% plasma protein) (Colombo et al., 2006).

Plasma levels of AGP are affected by pregnancy, certain drugs, and some diseases, especially HIV. The most notorious of these confounders is liver disease. Methadone patients with apparently stable disease may become unstable due to alcoholism and die of methadone overdose even though they are taking the same dosage of methadone they had in the past. ORM acts as a carrier of basic and neutrally charged lipophilic compounds. In medicine, it is known as the primary carrier of basic drugs (whereas albumin carries acidic drugs), steroids, and protease inhibitors (Urien et al., 1991). ORM concentrations increase in obstructive jaundice but diminish in hepatocellular jaundice and in intestinal infections.

If less AGP is produced, concentrations of free methadone are forced to increase and so, presumably, is the narcotic effect exerted (Israili and Dayton, 2001). Free methadone levels may also be increased in the presence of other drugs that competitively bind AGP. Conversely, AGP is increased in some inflammatory conditions, which implies that as the disease evolves, the effects of a given dose of methadone would diminish (Herve et al., 1996).

P-gp is a drug efflux transporter found in the liver, kidney, and gut. Many drugs (digoxin, fexofenadine, CyA, and some protease inhibitors) cannot be excreted in its absence. As a practical matter, most drugs that affect CYP3A4 usually also exert an effect on P-gp (Balayssac et al., 2005). Widely used drugs such as carvedilol, CyA, ketoconazole, and verapamil all inhibit P-gp production. Rifampin and St. John's wort, among others, induce P-gp production (Zhou et al., 2004). Could the death of a cocaine and heroin abuser, with cardiomyopathy, be related to the fact that his cardiologist had just begun treatment with carvedilol, or did the doctor at the methadone maintenance clinic administer the wrong dose of methadone? It may be impossible to say, but these complex issues exist and cannot be ignored.

Chiral variation is one reason the investigation of methadone-related deaths is so complex. In most countries, methadone is sold as a racemic mixture; even though the *l*-form

does not bind opiate receptors, it does bind hERG potassium channels (responsible for the delayed rectifier current), causing QT prolongation and fatal episodes of torsades de pointes. Luckily, this occurs only in polymorphic individuals with an abnormal form of CYP3A4 (Eap et al., 2007). This is an irrelevant consideration for most methadone users, but not for those who are polymorphic for the needed enzyme. The apparent V_{ss} constants for the *d*- and *l*-forms are different, which almost certainly guarantees that the rate of postmortem redistribution is also different, and the *d*-form also has a longer half-life than the *l*-form, which further complicates the situation (Baumann et al., 2002; Rentsch, 2002; Gerber et al., 2004). Neither chiral separation nor CYP3A4 activity measurements are routinely performed in the postmortem setting, so a pathologist trying to make sense of a report stating that a methadone concentration of 700 ng/mL was found in "heart blood" has no way of knowing what proportion of that 700 ng/mL is an active drug, let alone what the total concentration was at the time of death, or whether the decedent was CYP3A4 deficient. In short, he is guessing.

To date, only one study has sought to determine whether chiral separation of the methadone isomers might improve the diagnostic accuracy of the autopsy, and the results have not been encouraging. When *d*- and *l*-methadone were measured in femoral blood from 10 postmortem cases, the *d*-methadone concentrations ranged from 0.006 to 1.235 mg/kg with a median of 0.41 mg/kg, and the *l*-methadone concentrations ranged from 0 to 0.794 mg/kg (median 0.33 mg/kg). The median *d*:*l* ratio was 1.46 (total range from 1.00 to 2.62), which tends to be higher than that reported in the plasma of living subjects but is still hardly sufficient for diagnostic purposes (Johansen and Linnet, 2008) and some new method of discrimination will be required.

5.10.5.3 Other Confounding Variables

It is not uncommon to hear an "expert" offer an opinion on how much time had elapsed between the last dose of heroin/morphine and death. Such speculation is unscientific, not just because of the differences in V_{ss} between parent drug and metabolite, because bacteria translocate. That is, they migrate through the walls of the bowel, invading the rest of the body within a few hours of death (Kellerman et al., 1976). *Escherichia coli* bacteria, which constitute the bulk of the bacteria in the gut, contain multiple glucuronidases. In fact, they are the main source of commercially prepared glucuronidase sold by reagent makers. Some of these enzymes are capable of both conjugating and deconjugating morphine (Moriya and Hashimoto, 1997), and the direction of the reaction is generally unpredictable. Thus, even if redistribution did not occur, the ratio of free to conjugated morphine would still not provide an accurate measure of the time of last dose, since there is no way to tell which free morphine came from ingestion and which was generated by bacteria. There is also evidence that *E. coli*, clostridia species, and other bacteria may be responsible for producing some of the γ-hydroxybutyric acid found in postmortem blood (Elliott et al., 2004).

Morphine is converted to glucuronide by the action of a liver enzyme called UDPG. Multiple polymorphic forms of this enzyme exist. If an individual is a poor metabolizer (PM) and has an ineffective form of UDPG, then very little conjugated M6G will make its way back from the liver to the systemic circulation. Thus, even in the living, without knowing an individual's ability to conjugate morphine (which would require DNA resequencing), comparing the ratio of bound morphine to free morphine is a meaningless exercise.

The state of hydration is rarely considered and, except for the occasional patient dying in hospital, rarely addressed. The body begins to dehydrate after death; blood clots liquefy

and without actually measuring the hematocrit of the sample being analyzed, there is no way to tell whether the drug present has been diluted or concentrated (the additional problems posed by the increased blood volume associated with heart failure and the decreased blood volume associated with renal failure are discussed in Section 5.9). Similar considerations apply to urine testing. Ingestion of very small quantities of drug can result in very high urine concentrations, depending on water intake and underlying renal function. The pathologist is unlikely to know about either.

Genetic polymorphism is, no doubt, responsible for more than a few cases of drug toxicity, but the true incidence of this problem is not really known. The metabolism of codeine provides a good example. Codeine is metabolized by two different enzymes within the P450 system. These enzymes are located in the liver. This family of enzymes is involved in the metabolism of many different compounds. In general the P450 enzymes are the first enzymes to come into contact with a molecule that is to be inactivated (called type I reactions). Then type II enzymes conjugate the drug, either via glucuronidation or sulfation, so that the drug complex can be excreted by the kidney. Codeine is metabolized by two members of this family, cytochrome P450 2DA (CYP2DA) and CYP3A4 (Leppert, 2011).

Codeine undergoes *O*-dealkylation to form morphine. The conversion is catalyzed by a polymorphic form of CYP2D6 (Lotsch et al., 2004). Without testing to see which polymorphic form is present, there is no way to predict how much morphine an individual will form from codeine. Individuals entirely lacking CYP2D6 activity (7%–10% of Caucasians) are called PMs and are not likely to get much pain relief from codeine because they cannot convert enough of it into morphine. But it is also possible to have multiple duplications of CYP2D6, making the individual into an ultrametabolizer who can transform abnormally large amounts of codeine into morphine. Thus it is quite conceivable that a person with a gene duplication might experience an overdose even though only a modest dose of codeine had been taken. Duplication might also explain the finding of very high levels of codeine in postmortem blood samples, even though the clinical history indicated that very little drug had been taken. The latter scenario has already been reported. Koren et al. (2006) described the case of an 11-day-old who died of apparent morphine overdose from breast-feeding. Genotype analysis for *CYP2D6* showed that the mother was heterozygous for a *CYP2D6*2A* allele with *CYP2D6*2×2* gene duplication, which qualified her for the status ultrarapid metabolizer (UM). Free morphine in the infant was 70 ng/mL, although the established range for breast-fed infants of mothers taking codeine is only 0–2.2 ng/mL (Meny et al., 1993).

5.10.5.4 What Is a Complete Autopsy?

If toxicology results cannot be considered in a vacuum, neither can autopsy findings. When a doctor "certifies" a cause of death, his certification is based upon his evaluation of the evidence available to him, but it is still just his opinion and does not set a precedent for similar cases. If the decedent was a known cocaine user, the body was noted to have frothy pulmonary edema, blood cocaine concentration is 1 mg/L, and a crack pipe was found at his side, the decision is not difficult. But what if the blood cocaine concentration had been 0.050 mg/L, there was minimal pulmonary edema, and no other apparent stigmata of drug abuse or anatomic abnormalities were observed, but concentric cardiac hypertrophy was evident? How would the pathologist determine which abnormality was causal—the one he could see and weigh or the hERG channel–cocaine interaction he knows might exist, but cannot measure?

In the same fashion, suppose the pathologist was confronted with a decedent who had a remote history of heroin abuse and only mild pulmonary edema with a blood morphine concentration of 20 ng/mL evident at autopsy. In the absence of other identifiable anatomic changes, the pathologist would, no doubt, determine the manner of death as heroin overdose and an accidental death. But that would be incorrect for at least two reasons. First, unless hair testing had been performed, the pathologist would have no idea whether the decedent was a regular user and therefore tolerant or whether the decedent was naïve and intolerant. Second, the cocaine that was present could have unmasked one of many genetic defects that can interfere with the metabolism of morphine or exaggerate its effects (Oertel et al., 2009). Whether such a death would be classified as natural or accidental could be a matter for debate. What is not debatable is that the autopsy pathologist has no way to look at the body and determine whether or not the decedent suffered from a P-gp variant or an *SCN9A* mutation, at least not without performing genetic resequencing first.

If no slides were ever taken of the myocardium, then the pathologist might be very impressed by the history, modest morphine concentration, and cardiomegaly, but without genetic resequencing, the pathologist would never know whether the decedent was an addict suffering from an undiagnosed case of myocarditis (obscure cardiac infections are not rare in intravenous heroin users) or an addict who died from a heroin overdose. Or what if the decedent, with his known history of occasional heroin use, were to catch the flu, run a mild fever, and die an arrhythmic sudden death because the fever had unmasked a previously undiagnosed case of Brugada syndrome (Karch, 2007; Sanchez and Kates, 2004)? Yes, some gross abnormalities would have been identified at autopsy but the underlying cause of death, which cannot be seen with the naked eye, would have been missed. The sorry fact is that most "unremarkable" autopsies are actually just incomplete autopsies. This is not to condemn any shortcomings of forensic pathology. It is simply to point out that the tools of molecular biology have become vastly more important than could ever have been anticipated. To argue otherwise is simply to mislead ourselves and the trier of fact. The good news is that genetic testing has also evolved more rapidly than could ever have been imagined, bringing hope that some of these problems will be resolved in the near future.

5.11 Synthetic Opioids: General Considerations

The prevalence of opioid use has been increasing globally since 2006. Surprisingly, this is thought to be a consequence of the increased misuse of prescription opioids. UNODC attributed 43,000 deaths to opioid in 2010. On the other hand, evidence suggests that the use of heroin in the United States is increasing. This increase is thought by many to be a consequence of drug users having discovered that heroin is cheaper to come by than diverted opiates. The increase in heroin abuse is reflected by the increasing nonmedical use of pain relievers. Taken together, admissions for opiates and prescription opioids are rising, though the increase is due more to increasing use of opiates than heroin, and now more patients are now admitted for treatment of prescription opiate abuse than heroin (UNODC, 2014).

According to figures released by UNODC, admissions for opioid abuse now account for more hospital admissions than either cocaine or methamphetamine (SAMHSA, 2011). Worse still is the fact that the number of deaths resulting from prescription painkiller overdose also continues to rise, especially among women (CDC, 2014a). Medical emergencies related to the nonmedical use of pharmaceuticals increased 132% over the period

2004–2011, with the number of medical emergencies involving opiates and/or opioids rising 183%. The increasing popularity of these drugs is confirmed by the associated death rate. According to the Centers for Disease Control and Prevention (CDC), deaths from prescription drug abuse have tripled since 1990. In 2008 synthetic opioids were involved in 14,800 deaths—more than cocaine and heroin combined (CDC, 2011). In 2010, 2 million people reported using prescription painkillers nonmedically for the first time within the last year—nearly 5500 a day (SAMHSA, 2011). From 1999 to 2006 the number of fatal poisonings involving opioid analgesics totaled more than 5000 (Warner et al., 2011) and now far exceeds the number of deaths attributable to either heroin or cocaine (Figure 5.21). In fact, the numbers may be a good deal higher because only 36 states operate Prescription Drug Monitoring Programs (Alliance of States with Prescription Monitoring Programs, 2011). To put the issue in more concrete terms, the quantity of prescription painkillers sold to pharmacies, hospitals, and doctors' offices was four times larger in 2010 than in 1999. Enough prescription painkillers were prescribed in 2010 to medicate every American adult around the clock for 1 month (CDC, 2010).

U.S. government agencies charged with tracking illicit drug use state that the most commonly diverted prescription drugs are the OPRs. The government includes, within this category, codeine, fentanyl (Duragesic®, Actiq®), hydromorphone (Dilaudid), meperidine, morphine (MS Contin), oxycodone (OxyContin), pentazocine (Talwin), dextropropoxyphene (Darvon), methadone (Dolophine), and hydrocodone combinations (Vicodin, Lortab, and Lorcet), and several new therapeutic agents, such as hydromorphone, are added to the list each year. Combined mortality data from the 28 states followed by the government (encompassing 56% of the U.S. population) from 2010 to 2012 indicate that the death rate from heroin overdose doubled in the 28 states, increasing from 1.0 to 2.1/100,000

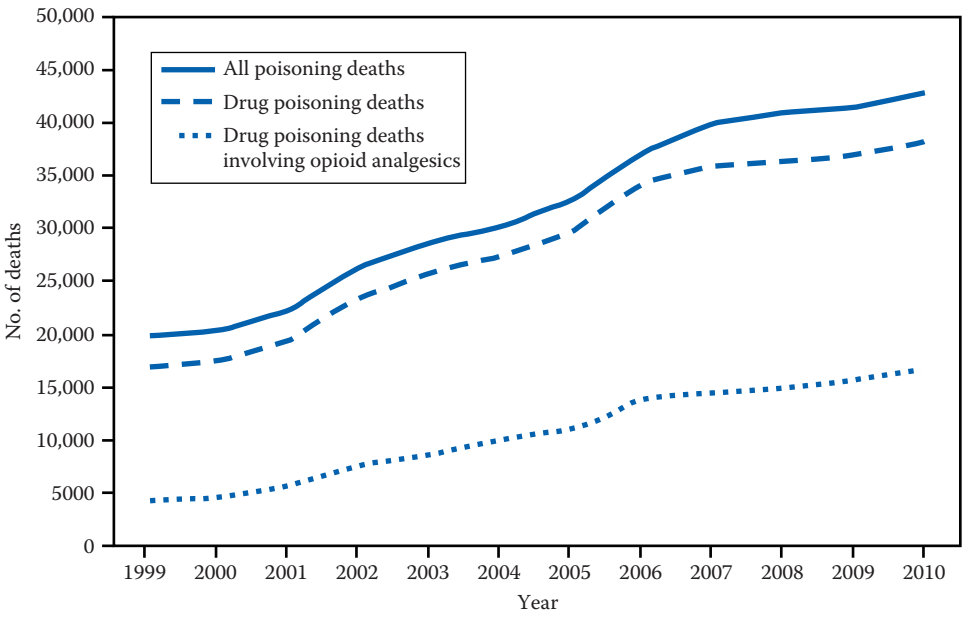


Figure 5.21 Proportion of all poisoning deaths involving drug poisoning and opioid analgesic poisoning in the United States, 1999–2010. The reported death rates from prescription opioid pain reliever (OPR) overdoses quadrupled during 1999–2010, whereas rates of deaths from heroin overdoses increased by <50%. (From *MMWR*, 62(12), 234, March 29, 2013.)

population, reflecting an increase in the number of deaths from 1779 to 3630. Comparing the same years, the death rate from opiate-related overdose declined 6.6%, from 6.0 to 5.6/100,000, a decline from 10,427 to 9,869 deaths. Nonetheless the overall drug overdose death rate increased 4.3%, from 13.0 to 13.6. Heroin death rates increased after 2010 in every subgroup examined. Heroin death rates doubled for males and females, whereas opiate-related death rates declined 12.4% in males and were unchanged in females. Heroin death rates increased for all age groups, whereas opiate-related death rates declined for age groups <45 years. Opiate-related death rates increased for persons aged 55–64 years. Heroin death rates doubled in non-Hispanic whites and Hispanic whites and nearly doubled in blacks. Opiate-related death rates decreased 8% in non-Hispanic whites and remained level in all other races/ethnicities. The Northeast and South had much larger heroin overdose death increases (211.2% and 180.9%, respectively) than the Midwest and West (62.1% and 90.7%, respectively). Opiate-related death rates declined only in the South.

Combined mortality data from the 28 states indicate an increasing problem with fatal overdoses from heroin from 2010 to 2012. Death rates from opiate-related overdose declined overall but still remained more than twice as high as heroin overdose death rates. Changes in heroin death rates were positively correlated with changes in opiate-related death rates. Mortality from overdoses of any type of drug rose slightly (Degenhardt et al., 2011; Rudd et al., 2014).

Interestingly, there is an unexplained disconnect between the rapidly increasing rates of death reported by the federal government and the experience of local police authorities, who report that illicit diversion of legal opiates accounts for less than 10% of the drugs in their jurisdiction. What follows is a detailed account of the toxicology and pathology of the most popular licit drugs that, ultimately, become abused drugs. Several agents such as buprenorphine and kratom are included for completeness, even though the degree to which they really are abused is open to question.

5.11.1 Buprenorphine (Table 5.10)

5.11.1.1 General Comments

Buprenorphine is a semisynthetic opioid. Like oxycodone, it is derived from thebaine. It is 25–50 times more potent than morphine and is classified as a partial opioid agonist (Wallenstein et al., 1986). Buprenorphine has a slower onset of pain relief, has longer duration of action, and is thought to cause less respiratory depression than morphine. The Drug Addiction Treatment Act of 2000 (DATA) allows physicians who receive specialized training to treat opiate addicts, in their offices, with Schedule III, IV, and V medications that have been approved by the Food and Drug Administration (FDA) specifically for addiction treatment. Buprenorphine is one of these drugs.

Two formulations, Subutex® and Suboxone®, are now available. Subutex (buprenorphine hydrochloride) is intended for use in the initial stages of withdrawal therapy, while Suboxone (buprenorphine hydrochloride and naloxone hydrochloride) is intended for use in the maintenance stage. As of this writing, no other medication has met the requirements of the act passed by Congress in 2000 (Buprenex is intended for pain relief, not replacement therapy, and cannot be used in these programs). Similarly, methadone remains the only drug that can legitimately be prescribed to treat opiate dependency during pregnancy. The results of large clinical trials suggest that buprenorphine may be at least as safe and effective as methadone. Subutex/Suboxone can be used to treat addiction to any opiate.

Table 5.10 Physiochemical Properties and Pharmacokinetics of Buprenorphine

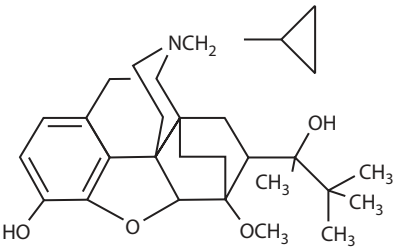
Chemical names	[(5α,7α,*S*)]-17-(cyclopropylmethyl)-α-(1,1-dimethylethyl)-4,5-epoxy-18,19-dihydro-3-hydroxy-6-methoxy-α-methyl-6,14-ethenomorphine, 1-methyl-4-phenyl-4-piperidine-carboxylic acid ethyl ester
Physiochemical properties, structure, and form	Available as hydrochloride salt, soluble in water CAS: 52485-79-7 MW: 247.34 p*K*a: 8.4, 9.9 Protein binding: 96% V_d: 2.6–5.0 L/kg Clearance: 18 mL/min/kg 
Synonyms	Subutex, Suboxone, Temgesic, Buprenex, Buprex, Finibron, Prefin tablets for sublingual use (Figure 5.22), solution for injections, skin patches
Pharmacokinetic parameters	Bioavailability: Sublingual solution: 28%–51% Sublingual tablet: 49%–64% Intramuscular: Not known Patch: First approved by FDA in 2011, designed to deliver 5 μg/h for 7 days. According to the package insert, doses higher than 20 μg/day may be associated with QT prolongation. C_{max}: Buccal: 1.98 ± 0.17 Solution: 3.3 ± 0.81 IV: 0.001 mg/kg × 12 volunteers, 207 ng/mL Skin patch: <0.2 ng C_{max} at 1 h
Common blood concentrations in drug users	Up to about 10 ng/mL
Blood terminal elimination half-life	2–6 h
Metabolism	CYP3A4 and CYP2C8 account for 95% of the metabolism of buprenorphine to norbuprenorphine, although CYP3A5, CYP3A7, CYP2C18, and CYP2C19 also contribute to the process, plus conjugation.
Excretion	Fecal: 10%–30% (almost all free) Urine: Excreted largely unchanged (27%) and feces (68%); high levels in bile
Postmortem artifacts	Increases of up to 100% possible in femoral blood, higher in central blood
Interactions	CYP3A4 inhibitors—antiretrovirals, antifungals, benzodiazepines. Rifampin induces its own metabolism as does rifabutin, though to a lesser degree. Patients requiring rifampin treatment for tuberculosis and receiving buprenorphine therapy are likely to require an increase in buprenorphine dose to prevent withdrawal symptoms. Rifabutin administration is associated with decreases in buprenorphine plasma concentrations, but no clinically significant adverse events have yet been reported.
Key papers	Bullingham et al. (1980, 1982), Cone et al. (1984), Ohtani et al. (1995), Walter and Inturrisi (1995), Kuhlman et al. (1996), Iribarne et al. (1997), Mendelson et al. (1997), Kobayashi et al. (1998), Oesterheld (1998), Nath et al. (1999), Schuh and Johanson (1999), Zubieta et al. (2000), Moody et al. (2002), Cowan (2003), Greenwald et al. (2003), Elkader and Sproule (2005), McCance-Katz et al. (2011)

Figure 5.22 Standard packaging of pharmaceutical buprenorphine, 2 mg tablets.

Physicians who prescribe buprenorphine therapy are required to maintain a log of all patients using Subutex and Suboxone and the amount that has been prescribed to them. Their medical records are subject to periodic DEA and FDA review. More than 1700 physicians or group practices in the United States, with nearly half located in the Northeast, are certified to prescribe buprenorphine. Initially they were not permitted to care for more than 30 patients at any one time (the limit also applied to group practices), but in January of 2007 the FDA increased the limit to 200.

French law was changed in 1996 to allow general practitioners to prescribe buprenorphine to heroin addicts. As in the United States, French law limits methadone prescribing to special government-controlled centers. When the French law was first changed, there were concerns that toxicity and lethal outcomes might be more frequent with buprenorphine than methadone. The results of recent studies indicate quite the opposite; death rates with methadone replacement therapy are nearly three times higher than with buprenorphine (Auriacombe et al., 2001).

5.11.1.2 Pharmacology

As with morphine, but unlike most other opioids, the major metabolic pathway for buprenorphine is glucuronidation, not oxidation. Microsomal oxidation does occur (via cytochrome P450 3A) but only modest amounts of norbuprenorphine are produced (Cone et al., 1984). Women have a significantly higher area under the plasma concentration curve (AUC) and also exhibit higher maximum plasma concentrations of buprenorphine, norbuprenorphine, and norbuprenorphine-3-glucuronide than do men. Gender-related differences also exist in buprenorphine pharmacokinetics but the clinical significance of this observation is not clear (Moody et al., 2011).

Except in the presence of end-stage liver disease, glucuronidation of buprenorphine is generally not impaired by liver disorders, which means that buprenorphine pharmacokinetics are not affected either (Tegeder et al., 1999). It follows that buprenorphine can be given safely to patients with renal failure (Boger, 2006). Free buprenorphine is not detected in urine, although the glucuronides can be detected in the urine for up to 4 days. Both of the glucuronides can be detected in feces, after either oral or sublingual administration, for as long as a week after ingestion, the result of extensive enterohepatic circulation (Cone et al., 1984).

Buprenorphine can be administered by any route, but very great differences in interindividual bioavailability mean that resultant peak plasma concentrations may vary widely (51.4% and 27.8% for sublingual and buccal routes, respectively) (Kuhlman et al., 1996; Lindhardt et al., 2000), occurring anywhere from 5 to 50 min after administration.

Plasma concentrations of 0.5 ng/mL are sufficient to produce surgical analgesia (Amani et al., 1997). In three studies, opioid naïve healthy male subjects received Subutex tablets (buprenorphine 2 and 8 mg [$n = 27$] and 16 mg [$n = 27$]) or two different formulations of Suboxone tablets (buprenorphine 8 mg/naloxone 2 mg [$n = 36$]) sublingually after first having been given naltrexone so that buprenorphine pharmacokinetics and tablet disintegration times could be evaluated. Maximum plasma values ranged from 1.6 to 6.4 ng/mL and T_{max} from 0.5 to 3 h. Large fluctuations in plasma levels after eating were observed, strongly supporting the role of enterohepatic recirculation in buprenorphine users. The terminal half-life in these studies was approximately 26 h (range, 9–69). The results of earlier work had suggested that, during daily buprenorphine maintenance therapy, plasma concentrations need to be greater than 0.7 ng/mL to be effective. More recent studies suggest that the number may be significantly lower.

Interaction with HIV medications is possible. *N*-dealkylation of buprenorphine is mediated by P450 CYP3A4 (Cowan, 2003). With each year it becomes increasingly clear that interactions do occur and that ARVs and buprenorphine compete for the same enzyme. In vitro buprenorphine inhibits CYP3A4 and other CYP enzymes as well. The concurrent administration of buprenorphine with some protease inhibitors can lower levels of buprenorphine (Iribarne et al., 1998a; Bruce and Altice, 2006). Indinavir can prevent more than 85% of buprenorphine's dealkylation (Iribarne et al., 1998a). As a consequence, concentrations of buprenorphine may be greatly increased when it is given with an ARV.

Ketoconazole and other imidazole derivatives are also well-known inhibitors of buprenorphine dealkylation (Iribarne et al., 1997), as are some antidepressants, especially selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and fluvoxamine (Regier et al., 1990; Iribarne et al., 1998b), and though quantitative differences in blood levels have not been identified, it has long been suspected that benzodiazepines enhance buprenorphine toxicity (Reynaud et al., 1998). There have been reports of fatal interactions between buprenorphine and some benzodiazepines (Reynaud et al., 1998; Tracqui et al., 1998a; Gaulier et al., 2000), especially diazepam and flunitrazepam. It is believed that when opiate users take both together the possibility of respiratory depression is enhanced, though this has never been proven in a controlled trial.

The clinical effectiveness of any opioid maintenance regimen for heroin dependence is believed to be a function of a medication's ability to decrease μ-opioid receptor availability, preventing heroin (or other opioids) from binding to them and preventing, or at least attenuating, withdrawal symptoms. These findings suggest that high-dose buprenorphine maintenance produces near-maximal μ-receptor occupation, and μ-receptor availability correlates well with plasma levels; buprenorphine-related opioid symptoms and antagonist blockade exhibit concentration–effect relationships. Other studies have shown that buprenorphine, unlike morphine, does not depress immune responses or activate the hypothalamic–pituitary–adrenal axis (Gomez-Flores and Weber, 2000).

In animal studies, buprenorphine treatment caused a statistically significant but clinically insignificant change in pulse and blood pressure (Martinez et al., 1997). In patients undergoing open-heart procedures with cold cardioplegia, pretreatment with

buprenorphine is cardioprotective; postoperatively there is improved metabolism and higher cardiac output. The protective effect is in some way related to activation of μ-opiate receptors (Boachie-Ansah et al., 1989), and there is interest in the use of this drug as an adjunct during heart surgery. Some studies show that drugs with δ-opiate activity confer myocardial protection, which is additive to cardioplegia, and buprenorphine along with fentanyl, morphine, and pentazocine are all, to one degree or another, cardioprotective. The use of δ-opiate drugs in this context may have important clinical implications (Benedict et al., 1999).

5.11.1.3 Detection

Buprenorphine is quickly cleared from the urine and it is many times more potent than morphine, which means that detection could be problematic. Earlier methodologies using radioimmunoassays could not separate parent drug from metabolite and were unreliable. A number of reagent makers now produce enzyme-linked immunosorbent assay (ELISA) kits, though these kits are expensive. Buprenorphine concentrations down to at least 1 ng/mL are easily detected with tandem MS. Buprenorphine can usually, but not always, be detected in hair samples, at concentrations ranging from 6 to 597 ng/g (mean, 137 ng/g) (Tracqui et al., 1998a). It is very stable in refrigerated blood samples, with recovery rates of more than 70% after 6 months of storage (Hadidi and Oliver, 1998). Exhaled breath is being developed as a possible specimen for drug testing based on the collection of aerosol particles originating from the lung fluid, and clinical testing has shown that buprenorphine can be demonstrated by this route (Beck, 2014).

5.11.1.4 Drug Concentrations

When measured in postmortem blood, concentrations of buprenorphine and its primary metabolite, norbuprenorphine, range from 1.1 to 29.0 ng/mL (mean, 8.4 ng/mL) and 0.2 to 12.6 ng/mL (mean, 2.6 ng/mL), respectively. As is true for morphine and heroin, these concentrations overlap those that have been reported in clinical settings where there is no evidence of toxicity. Somewhat surprisingly, given the high degree of buprenorphine protein binding, extensive tissue distribution occurs. Buprenorphine (like morphine) accumulates in bile, where concentrations may reach values of more than 75 mg/L. Norbuprenorphine seems to have the same pattern of distribution as the parent compound, although measured concentrations are generally very much lower than those of the parent compound.

Only a handful of reports describing cases of suicidal overdose have been reported. One was a 25-year-old male drug addict. Buprenorphine and norbuprenorphine were found in all tissues. Exceptionally high concentrations of buprenorphine and norbuprenorphine were found in blood (3.3 and 0.4 mg/L, respectively), urine (3.4 and 0.6 mg/L), bile (2035 and 536 mg/L), and brain (6.4 and 3.9 μg/g). The high concentration of buprenorphine (899 mg/L) and the absence of norbuprenorphine in gastric liquid suggested that intake had been oral. High concentrations of 7-amino-flunitrazepam, the main metabolite of flunitrazepam, were also found in blood, urine, and gastric liquid. This benzodiazepine may have been a contributory factor in the toxic effects of buprenorphine (Gaulier et al., 2000). These deaths rarely occur in individuals who are taking prescribed buprenorphine, but like methadone, most of the deaths are attributed to illicitly distributed forms of the two drugs. In any event, deaths from either buprenorphine or methadone account for only a fraction of the deaths caused by heroin (Wikner et al., 2014).

5.11.1.5 Maternal/Fetal Considerations

There is general consensus that buprenorphine produces a milder withdrawal syndrome than methadone (Johnson et al., 2003). Exposure of infants to buprenorphine is minimal, even though milk concentrations are similar to those in plasma. The explanation appears to be that oral bioavailability in the infant is poor (Elkader and Sproule, 2005). This may explain why there have been no reported cases of neonatal abstinence symptoms following the cessation of breast-feeding (Johnson et al., 2003). Several studies relevant to buprenorphine opiate replacement patients have been published. In one study, concentrations of buprenorphine and norbuprenorphine were measured in 10 random breast milk samples collected over 4 successive days. Concentrations of the parent drug ranged from 1.0 to 14.7 ng/mL, while those of norbuprenorphine ranged from 0.6 to 6.3 ng/mL. The authors of the study concluded "drug exposure of the infant may be considered to be low" (Grimm et al., 2005). A recent Australian study compared well-being in infants born to addicted mothers maintained on buprenorphine. No adverse effects were detected in infants exposed to buprenorphine via breast milk up to 4 weeks postnatally (Gowers et al., 2014). A separate study published in 2012 found that buprenorphine produced a much milder form of neonatal abstinence syndrome than did methadone (Jones et al., 2012b).

In a second study involving only one woman, buprenorphine concentrations on day three were the same in plasma and breast milk (0.52 ng/mL). On day 6 they were still nearly identical (0.72 and 0.64 ng/mL, respectively) (Johnson et al., 2003). A case report published in 1997 described a pregnant addict who took 4 mg/day of buprenorphine for 5 months. Twenty hours after her child's birth, plasma buprenorphine concentrations were higher in the infant than in the mother's serum just before birth, though the reverse was true for norbuprenorphine. Based on measurements made in breast milk at age 4 weeks, the authors of that study calculated that the infant would have received a total of 3.28 μg of buprenorphine and 0.33 μg norbuprenorphine over a 24 h period (Marquet et al., 1997).

In a 2009 study buprenorphine samples for analysis were collected from the urine of six infants of mothers taking buprenorphine together with breast milk, blood, and urine from their mothers during a 24 h period in the week after birth. One mother–infant pair was studied when the infant was 9 months of age. Buprenorphine and norbuprenorphine were found in low levels in the infants' urine, and it was calculated that breast-fed infants were exposed to a buprenorphine dose per kg bodyweight less than 1%, with an average milk/plasma area under the curve of 1.7 (range, 1.1–2.8) for buprenorphine and 0.7 (range, 0.4–1.2) for *n*-BUP. Such low concentrations are of minimal concern (Lindemalm et al., 2009).

There is now sufficient evidence to conclude that buprenorphine poses no significant risk to the fetus. There is no relationship between the total or cumulative third trimester dose of buprenorphine, or dose at delivery, and free or total buprenorphine or norbuprenorphine concentrations in the meconium of the neonate, nor does total buprenorphine dosage correlate with the neonatal abstinence score.

Variability in fetal metabolism may also influence buprenorphine and metabolite concentrations in meconium. CYP3A4 and CYP2C8 account for 95% of the conversion of buprenorphine to norbuprenorphine, although cytochrome P450 3A5 (CYP3A5), cytochrome P450 3A7 (CYP3A7), cytochrome P450 2C18, and cytochrome P450 2C19 also contribute to the process. CYP3A7 is the principal fetal hepatic enzyme, with CYP3A7 and CYP3A5 expression becoming evident as early as 42 days after fertilization. CYP3A7

expression decreases during gestation, and hepatic metabolism shifts primarily to CYP3A4 within days of birth. Large interindividual variations in CYP expression have been observed, and the data available on fetal hepatic uridine diphosphoglucuronosyltransferase expression and activity are limited (Kacinko et al., 2008).

5.11.1.6 Psychiatric Use

There have been fewer than a dozen studies into the use of buprenorphine in treatment-resistant depressed patients, with fewer than 100 patients having been studied. Every study has reported positive results, with rapid and significant improvements. Clearly, some endorphin–dopamine interaction is occurring in these patients, and the interaction is central to mood regulation, but just what that relationship might be is not known (Tenore, 2008).

5.11.1.7 Postmortem Considerations

Buprenorphine appears to be relatively stable with greater than 70% recovery after a year (Hadidi and Oliver, 1998). Several autopsy series have been published, mostly from France where buprenorphine was first used for replacement therapy. Nearly 100,000 opiate addicts have been enrolled in French programs to date. The first French fatalities were reported almost as soon as the drug was licensed in 1996 (Tracqui et al., 1998a,b; Kintz, 2001, 2002). Since then fewer than 60 additional reports have been published. Virtually all the decedents were polydrug abusers, and postmortem concentrations of buprenorphine and norbuprenorphine were not very different from those seen in living substitution patients. Very similar results were reported in a 2012 Swedish study. Concentrations of both buprenorphine and its metabolite were generally low, ranging from 0.4 to 27 ng/mL, and there was total overlap between cases where buprenorphine was the cause of death and cases where it was an incidental finding. In decedents who had clearly died of overdose, norbuprenorphine was absent from the urine (indicating acute intake). As in all previously reported studies, polydrug use was evident in three quarters of decedents (Seldén et al., 2012).

Seldén's report was interesting because it compared blood concentrations in 21 cases of buprenorphine-related deaths. Six of the cases had died from natural causes, and measurement of their blood buprenorphine levels ranged from undetectable in blood (detected in urine) to 3.2 ng/mL in plasma (mean 1.4 ng/mL). Twelve cases were attributed directly and indirectly to mixed drug poisoning. Blood buprenorphine levels ranged from undetected, except in urine, to 17 ng/mL (mean 3.2 ng/mL). Nineteen cases showed concurrent abuse of buprenorphine and benzodiazepine, diazepam being the most frequently detected, followed by nitrazepam and midazolam (Lai et al., 2006b). Reported autopsy findings do not appear to be different from any other sort of opiate overdose, with pulmonary congestion and track marks the only consistent findings.

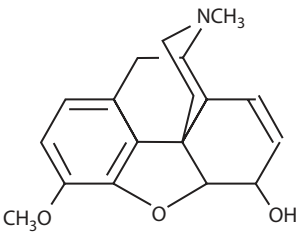
The same results were supported by a more recent study, also of Swedish origin. Ninety-seven cases where buprenorphine was detected at autopsy were divided into four groups: (1) clear intoxication ($n = 41$), (2) possible intoxication ($n = 24$), (3) unrelated to the cause of death ($n = 14$), and undetermined ($n = 13$), and five not classifiable. The concentrations of buprenorphine were low in all four groups, with medians between 0.4 and 2.7 ng/g. There was no significant difference in norbuprenorphine concentrations between the groups. However, 24 cases in the intoxication group were negative for norbuprenorphine indicating an acute intake, suggesting that lack of tolerance may have been a contributory factor. As in prior reports, no unique pathologic features were detected (Seldén et al., 2012).

5.11.2 Codeine (Table 5.11)

5.11.2.1 General Considerations

Codeine is one of several naturally occurring alkaloids found in opium. Depending on where the poppies are grown, the codeine content of raw opium ranges from 0.7% to 2.5%. Codeine was first isolated from opium by Robiquet in 1832, 27 years after Sertürner isolated morphine. Most of the codeine consumed in cough and analgesic mixtures is of semisynthetic origin, produced by the methylation of morphine. Since the Drug Abuse Warning Network (DAWN) report implemented its new reporting system, it is no longer possible to estimate the number of codeine-related fatalities in the United States. However, that number must be fairly small as the DAWN reports and the Treatment Episodes Data Set (TEDS) combines codeine-related hospital admission and codeine-related deaths under one category. The current incidence of codeine-related deaths is not known, but the

Table 5.11 Physiochemical Properties and Pharmacokinetics of Codeine

Chemical names	(5α,6α)-7,8-Didehydro-4-5-epoxy-3-methoxy-17-methyl-morphinan-6-ol
Physiochemical properties, structure and form	Available as hydrochloride, sulfate and phosphate salts, soluble in water CAS: 76-57-3 MW: 299.37 V_d: 2.6 L/kg p*K*a: 8.2 Protein binding: 7% 
Synonyms	Codeine anhydrous, codeine base, Codicept, *l*-codeine, methylmorphine, morphine monomethyl ether, norcodeine, *N*-methyl codeine, norcodeine
Pharmacokinetic parameters	Bioavailability: 30%–40% C_{max}: 30 mg orally, 66 ng/mL (34.0–119 ng/mL)
Common blood concentrations in drug users	Up to about 0.5 mg/L, about 10% of codeine or less is converted to morphine unless it is an ultrarapid metabolizer in which case it is much higher
Blood terminal elimination half-life	2–4 h
Metabolism	Partially converted by CYP2D6 to small amounts of morphine and its metabolites, but mainly to codeine metabolites, norcodeine, and codeine-6-glucuronide (by CYP3A4). The proportion of metabolites formed is determined by CYP2D6 polymorphisms. Some of the metabolites are psychoactive.
Urine excretion	Codeine: 5%–17% Norcodeine: 0%–5% Morphine: Trace Codeine glucuronides: 32%–56% Morphine glucuronides: 5%–13%
Postmortem artifacts	As for morphine
Interactions	Codeine is a prodrug and its pain-relieving abilities are derived from its conversion to morphine. Some CYP2D6 polymorphs convert too much codeine to morphine and toxicity may result. Chinese metabolize codeine less well than Caucasians. Yue et al. (1997) found a higher C_{max} and a slightly longer half-life in Chinese after a 60 mg dose of codeine, mostly because the Chinese cannot form glucuronides as readily.
Key papers	Quiding et al. (1986), Gerostamoulos et al. (1996), Yue et al. (1997), Lötsch et al. (2006)

2013 Emergency Room component of the new DAWN report lists 9927 visits occasioned by the ingestion of codeine-containing products, including 1772 attempted suicides and slightly more than 7000 cases of codeine abuse. The numbers are minuscule given the tons of codeine consumed annually in the form of prescription medications (SAMHSA, 2014).

5.11.2.2 Pharmacology

Two different enzymes metabolize codeine; both are in the P450 system. Cytochrome P450 is a heme-containing monooxygenase isoenzyme located in the smooth membranes of the endoplasmic reticulum of liver cells, but it can also be found along the mucosal surface of the intestinal tract. The P450 family of enzymes is involved in the metabolism of a host of different compounds, including endogenous steroids, prostaglandins, lipids, and the process of detoxification in general. The P450 enzymes are the first enzymes to come into contact with a molecule that is to be inactivated (called type I reactions). Type II enzymes conjugate (either via glucuronidation or sulfation) the product of the first reaction so that the kidney can excrete the conjugated drug. Codeine is metabolized by two members of the P450 family, namely, CYP2DA and CYP3A4 (Leppert, 2011; van der Vaart et al., 2011).

Codeine undergoes *O*-dealkylation to morphine (Figure 5.23). The conversion is catalyzed by polymorphic CYP2D6 (the same enzyme also converts dihydrocodeine [DHC], hydrocodone, and oxycodone) (Lotsch et al., 2004; Zanger et al., 2004; Kreek et al., 2005). Without specifically testing the patient, there is no way to predict how much morphine that individual will form from codeine; the amount converted will depend on how many copies of CYP2D6 they possess. Individuals entirely lacking CYP2D6 activity are called PMs (7%–10% of Caucasians) and are not likely to get much pain relief from codeine because they cannot convert enough of it into morphine. On the other hand, it is possible to have multiple duplications of CYP2D6, making the individual an UM (who converts up to 75%

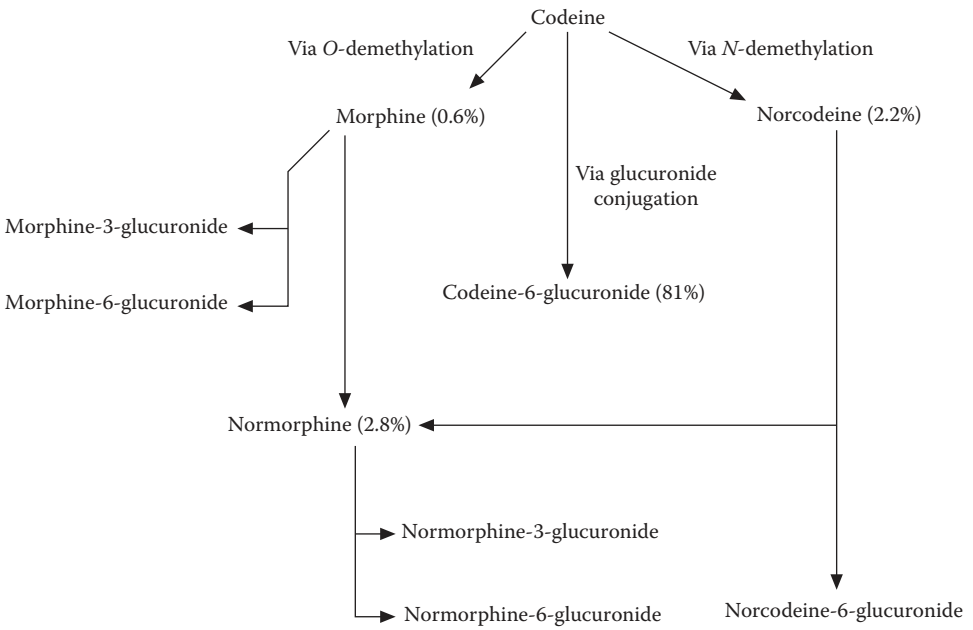


Figure 5.23 Basic elements of codeine metabolism.

of the codeine to morphine), capable of transforming abnormally large amounts of codeine into morphine. Advances in genotyping methods now make it possible to divide the population into four phenotypes: PM, intermediate metabolizer (IM), extensive metabolizer (EM), and UM (UM). However, it is important to note that the *CYP2D6* genotype does not fully predict phenotype, especially if a CYP2D6 inhibitor is present. In that case, someone who is an IM may be misidentified as a PM. Obviously, various permutations are possible depending on what drugs are taken. In addition to *CYP2D6*, other studies suggest that polymorphisms in *UGT2B7*, *ABCB1*, and *OPRM1* all may play a role in codeine metabolism (Lam et al., 2014).

Anyone carrying two copies of the same gene might experience an overdose even though only a modest dose of codeine is taken. In fact, this situation has been reported (Kirchheiner et al., 2006). Duplication might also explain the finding of very high levels of codeine in postmortem blood samples, even though the clinical history indicated that very little drug had been taken. Once the excess conversion to morphine has taken place, morphine is further metabolized by *N*-demethylation (by CYP3A4) and glucuronidation, just as it would have been if it had been ingested primarily.

Codeine is also *N*-demethylated by CYP3A4 and then conjugated by UGT2B4 and UGT2B7 to form codeine-6-glucuronide (Caraco et al., 1996; Yue and Sawe, 1997). The latter is another highly polymorphic enzyme (Court et al., 2003), but since two different enzymes are capable of making the same conversion, genetic variation in this phase of codeine metabolism seems to be irrelevant. Not all of codeine's psychoactivity is attributable to morphine formation that, in normal metabolizers, is not very great. Very small amounts may be converted to norcodeine, which is believed to be psychoactive (Fraser et al., 1960). There is also evidence that codeine-6-glucuronide itself may be psychoactive (Lötsch et al., 2006). All of these compounds are excreted in the urine, where somewhat less than 90% of a single dose of codeine can be recovered within 48 h, mostly as codeine-6-glucuronide (Chen et al., 1991).

The amount of each metabolite formed after codeine administration has been determined. In one randomized, placebo-controlled, double-blind study, the analgesic effects of 170 mg codeine were compared to the effects of 20 mg of morphine administered orally (clinically considered comparable doses) and also to the effects of placebo in PM and EM. Following administration of codeine, analgesia was observed in EMs but not in PMs. The finding makes sense because the PMs could not metabolize codeine to morphine while the EMs converted increased amounts of codeine to morphine. When oral morphine was given, plasma concentrations were similar in PMs (C_{max} 13 ± 4 ng/mL) and EMs (C_{max} 14 ± 4 ng/mL). However, following the administration of 170 mg codeine, only traces of morphine could be detected in plasma (C_{max} 0.5 ng). The percentage of the codeine dose converted to morphine and its metabolites was 3.9% in EMs and only 0.17% in PMs (Eckhardt et al., 1998). A further extension of this work was published in 2009, demonstrating that it was possible to predict the degree of pain relief based solely on knowledge of a subject's P450 CYP2D6 metabolizer subtype (non, slow, normal, fast, ultrafast) (Lötsch et al., 2010).

Codeine, like morphine, may also be converted to hydrocodone. However, the latter conversion seems to occur only in individuals treated with large amounts of morphine. In one study, 10 of the 13 patients treated with morphine excreted minor amounts of hydrocodone in their urine (120–1400 ng/mL). Morphine concentrations in these patients were very high (>10,000 ng/mL), suggesting that the conversion constitutes only a minor pathway

(Cone et al., 2006). Allegations of illicit hydrocodone use have been made against individuals taking physician-prescribed oral codeine who strenuously deny ever taking hydrocodone but who nonetheless have positive urine tests for that drug. Generally, the hydrocodone concentrations in these cases are quite low, on the order of 100 ng/mL or less (Oyler et al., 2000).

It had been thought that small amounts of morphine were metabolically converted to codeine (Boener and Abbott, 1973) but in fact that is not the case (Yeh, 1974). The codeine detected in urine after giving morphine is present because it exists as a contaminant even in pharmaceutical-grade morphine preparations (Vaughan and Dennis, 1979). Thus, the presence of codeine should not be presumed to be evidence for anything but the ingestion of codeine. The presence of trace amounts of morphine, on the other hand, is accounted for by the metabolic conversion of codeine to morphine.

5.11.2.3 Routes of Administration

The radioimmunoassays used in early studies could not discriminate between codeine and its metabolites and thus yielded spuriously high concentrations of codeine in the plasma and urine (Chen et al., 1991). With current technology, morphine, codeine, and each of their main metabolites can be measured simultaneously (Musshoff et al., 2006). Peak concentrations of codeine in healthy volunteers (50 mg orally) occur within 1–2 h and the plasma half-life is on the order of 2.4–3.2 h (Chen et al., 1991), depending upon metabolizer status. The peak concentration of codeine in the saliva is nearly three times that measured in the blood, even though the half-life in both fluids is approximately 3.2 h (Chen et al., 1991). After oral dosing, plasma levels of codeine glucuronide reach concentrations 5–10 times higher than those of codeine (Chen et al., 1991; Lafolie et al., 1996). Chronic administration of codeine does not appear to alter codeine kinetics.

5.11.2.4 Codeine Tissue Disposition

Data on the tissue distribution of codeine are sparse. The results of the few studies that have been performed suggest that when codeine is clearly the cause of death, total and free codeine concentrations completely overlap concentrations in cases where codeine is an incidental finding. Hair morphine concentration measurements can be used to assess morphine tolerance after death (Tagliaro et al., 1998), but it is not clear whether hair codeine measurements can be used in the same fashion, though it is not difficult to measure (Musshoff et al., 2005). Blood morphine/codeine ratios greater than one should be taken as strong evidence that heroin, not codeine, had been used prior to death (Stefanidou et al., 2010).

Simultaneous measurements of blood and bile concentrations in codeine-related deaths disclosed a mean blood morphine concentration of 0.29 mg/L (range, 0.10–0.89 mg/L) with a mean concentration of 38 mg/L in bile (range, 3.3–112 mg/L). Codeine concentrations measured at the same time were 0.06–6.4 mg/L (mean, 1.5 mg/L) in blood and 0.22–89 mg/L (mean, 24 mg/L) in bile (Crump et al., 1994). Results were not very different in a second study of 107 codeine-related deaths. Codeine was considered the actual cause of death in only six. Of these, the mean concentration of total codeine in femoral blood was 4.0 $\pm$ 2.3 mg/L (range, 2.1–8.0 mg/L), while the mean concentration of free codeine was 1.3 $\pm$ 0.9 mg/L (range, 0.4–2.8 mg/L). Free and total codeine concentrations were not significantly different in cases where other drugs were present and where codeine was not deemed to be the cause of death (Gerostamoulos et al., 1996; Svensson et al., 2007).

5.11.2.5 Forensic Considerations

The importance of CYP2D6 polymorphisms cannot be overemphasized. A case report published in the *Lancet* describes a breast-feeding mother being treated with moderate doses of codeine whose child died unexpectedly 12 days after birth. Because the child was looking ill and not feeding, the mother saved all of her milk production on day 10. Analysis of the milk showed an astonishing 70 ng/mL concentration of morphine. Genotype analysis was done for *CYP2D6*, the enzyme catalyzing the *O*-demethylation of codeine to morphine. The mother was found to be heterozygous for a *CYP2D6*2A* allele with *CYP2D6*2°–2* gene duplication and was classified as a UM (Koren et al., 2006).

A report published in 2009 (Ferreirós et al., 2009) described two cases of codeine intoxication occurring in 3-year-old monozygotic twin brothers being treated with codeine. One of the children died of gastric aspiration at home; the other survived a hospital admission. The concentrations of codeine and morphine found in the dead child exceeded the highest reported therapeutic levels seen in living persons. Genetic analysis showed that each of the twins had the characteristics of EMs. Concentration measurements for each child are shown in Table 5.12.

A recent Norwegian study of codeine-related deaths illustrates the forensic significance of the problem. Of 111 cases with detectable amounts of codeine in femoral blood, 34 had blood concentrations exceeding 0.3 mg/L, the toxicity threshold suggested by The International Association of Forensic Toxicologists (TIAFT). When the cases were retrospectively reviewed, a high degree of variability in individual morphine to codeine concentration ratios (*M*/*C* ratios) was found, and it was also evident that morphine levels could not be predicted from codeine concentrations, even when CYP2D6 genotype was known. In 13 cases codeine concentrations exceeded the TIAFT threshold for possibly lethal serum concentrations (1.6 mg/L). In one case, morphine as well as M6G and M3G concentrations were below the limit of detection, proving that the decedent had been totally unable to convert codeine to morphine, that is, there was a complete CYP2D6 deletion. Clearly, toxicology testing in suspected codeine-related fatalities should include quantification of morphine and morphine metabolites. Ideally, CYP2D6 genotyping should be performed when unexpectedly high or low *M*/*C* ratios are encountered (Frost et al., 2012).

Table 5.12 Summarized Data from a Pair of Twins, One of Whom Died; Both Were Extensive Metabolizers

	Surviving Child			Deceased Child			
Compound	Serum (ng/mL)	CSF (ng/mL)	Urine (μg/mL)	Serum[a] (ng/mL)	Serum[a] (ng/mL)	Urine (μg/mL)	Brain (ng/g)
Codeine	174.0	79.1	10.1	436.3	461.2	18.5	541.6
Norcodeine	7.6	5.1	1.1	20.5	20.6	3.1	ND
Codeine-glu	449.8	20.4	52.3	610.9	663.6	82.8	32.4
Morphine	25.6	9.7	2.7	138.7	153.9	6.2	70.8
Normorphine	30.0	ND	3.0	66.6	80.0	6.9	3.9
M6G	23.5	ND	3	39.5	58.4	3.4	ND
M3G	154.3	ND	15.3	134.8	167.6	18.7	ND

Source: Adapted from Ferreirós, N. et al., *Int. J. Legal Med.*, 123(5), 387, 2009.

Note: M6G, morphine-6-glucuronide; M3G, morphine-3-glucuronide; Codeine-glu, codeine glucuronide; ND, not detectable (LLOQ: 5 ng/mL for M3G, morphine, norcodeine, and codeine-glu; 10 ng/mL for M6G and normorphine; and 2 ng/mL for codeine).

[a] Serum derived from blood collected from the left chamber of the heart.

5.11.3 Desomorphine

Desomorphine, popularly known as "krokodil," is a morphine analog. It was first synthesized in the United States (U.S. Patent 1980972, 1934), but never brought to market. In 2003 an illicitly prepared version was first encountered in Russia (Grund et al., 2013), but since then it has been used mainly as a cheaper heroin substitute. It is still not a very common drug in the United States, but clusters of deaths have been reported from across the United States, the United Kingdom, and the European Union (E.U.) (Gahr et al., 2012).

Desomorphine is 4,5-α-epoxy-17-methylmorphinan-3-ol, dihydrodesoxymorphine. Its formula is $C_{17}H_{21}NO_2$, with a molecular weight of 271.35 g/mol and a melting point of 189°C. It binds to opiate receptors but its structure differs from that of morphine because both the 6-hydroxyl group and the 7,8 double bond of morphine have been reduced. It is an organic base like morphine and the protonated form has an estimated p*K*a of 9.69. As a consequence, it is nearly 100% ionized within physiologic pH ranges and easily crosses the BBB, a feature that makes it popular with abusers (Weill and Weiss, 1951).

Clandestine synthesis is a simple, inexpensive, two-step procedure that can be carried out in one vessel, using a process not that different from making methamphetamine. Any codeine-containing compound can be used as a precursor. A strong alkali (such as sodium hydroxide) is used to dissolve codeine from a convenient OTC or prescription product. Then any available organic solvent (in Russia, more often than not gasoline) is used to liberate codeine from the mixture. This mixture is then acidified with hydrochloric acid, thereby converting the codeine base into its water-soluble salt that separates in the aqueous layer. Finally the extracted codeine is reduced with iodine, hydrochloric acid, and red phosphorus to first form desocodeine, which is then demethylated to desomorphine. What makes this compound particularly toxic is that a large number of byproducts, not to mention the original medications that were mixed with the codeine, are included in the final product. If multiple extractions are performed, the product becomes purer and purer, though this is rarely the practice among users (Veronese, 2011).

Except for anecdotal reports that have been published in newspapers throughout the world, very little is known about the effects of this drug, partly because many of the effects reported are due to contaminants in the street drug, not desomorphine itself. The newspaper accounts often mention green, scaly looking skin accompanied by skin ulcerations. Many believe these changes, when they occur, are simply the effect of gasoline carried over from the synthesis, when street drug is injected intravenously. Case reports are now beginning to appear in the literature. Newspaper accounts are so sketchy that nothing really can be inferred about the specific toxic effects. To the extent that desomorphine is present in the product ingested, morphine-like effects can be expected. The published case reports are even fewer than the newspaper accounts.

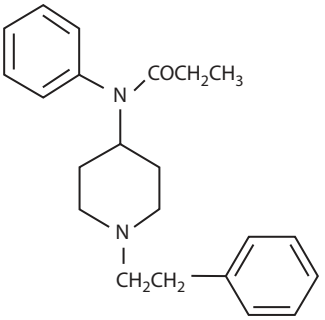
There are only three published studies concerning its analytic detection in biological fluids: Savchuk et al. (2008), Su et al. (2011), and a more recent study that found that desomorphine could be detected in blood for a few hours after injection and for several days in the urine (Hayashi et al., 2013). There are no data on intoxicated users (Hayashi et al., 2013). Save for the fact that users are subject to osteonecrosis of the jaw (Poghosyan and Avetisyan, 2014).

5.11.4 Fentanyl (Table 5.13)

5.11.4.1 General Considerations

Fentanyl is a synthetic μ-agonist, a phenylpiperidine derivative with a structure closely related to that of meperidine (Demerol®, i.e., the basic structure consists of a phenyl group coupled to

Table 5.13 Physiochemical Properties and Pharmacokinetics of Fentanyl

Chemical names	*N*-Phenyl-*N*-[1-(2-phenylethyl)-4-piperidinyl]propanamide
Physiochemical properties, structure, and form	Available as citrate salt, soluble in water CAS: 437-38-7 MW: 336.5 V_d: 3 L/kg p*K*a: 8.4 Protein binding: 84% 
Synonyms	China white, Actiq (Abbot Laboratories), and in combination with the neuroleptic drug droperidol (Astra USA). Alfentanil hydrochloride is available as Alfenta (Janssen Pharmaceuticals), and sufentanil is sold as Sufenta (Janssen Pharmaceuticals). Actiq (citrate), Remifentanil. A new effervescent buccal form has been introduced. It has higher bioavailability and more rapid onset.
Pharmacokinetic parameters	C_{max}: IV: After 200 μg, 4.6 ± 1.87 h IM: Not known Oral: After 15 mg, 4 h Sublingual: 0.24–2.51 ng/mL Transdermal: 2.6 ± 1.3 h Effervescent: 800 μg dose, 1.59 ± ng/mL Transmucosal: 800 μg dose applied as buccal film Half-life: IV: 219 ± 10 min; 3.7 ± 0.04 IM: not known Oral: after 200 μg, 200 min Sublingual: 3.2–6.4 h Transdermal: 21.9 ± 8.9 Effervescent: 800 μg, 11.70 h T_{max}: IV: 0.4 h (35 ± 15 h) IM: Not known Oral: 39–57 min Sublingual: After 200 μg, 40 ng/mL (range, 20–120) Transdermal: 20–40 h Buccal: 800 μg, 35–45 min
Metabolism[a]	Piperidine *N*-dealkylation with the formation of norfentanyl, CYP3A4, but single-nucleotide polymorphisms within the *CYP3A4* gene may contribute to the variability of analgesic efficacy and despropionylfentanyl formation.
Excretion	10%–20% as unchanged drug in urine
Postmortem artifacts	Substantial increases occur postmortem.
Interactions	Volume of distribution almost doubles in patients with burns. Cautious use is warranted when fentanyl and diltiazem are used together, as this can potentiate fentanyl toxicity. Other CYP3A4 inhibitors include ketoconazole, erythromycin, nefazodone, ritonavir, delavirdine, aprepitant, and imatinib. Probably all should be avoided, and the dose of fentanyl minimized

(*Continued*)

Table 5.13 (*Continued*) Physiochemical Properties and Pharmacokinetics of Fentanyl

Key papers	McClain and Hug (1980), Portenoy et al. (1993), Hung et al. (1995), Olkkola et al. (1999), Lennernas et al. (2005), Darwish et al. (2006), Mystakidou et al. (2006), Nitsun et al. (2006), Han et al. (2007), Levin et al. (2010), Vasisht et al. (2010), Dong et al. (2011)

[a] Fentanyl-derived designer drugs are metabolized in much the same manner as fentanyl itself. The main metabolic steps involve *N*-dealkylation to the respective nor-metabolites, namely, nor-fentanyl in the case of alpha-methylfentanyl and nor-3-methylfentanyl in the case of 3-methylfentanyl; however, more specific information is not available.

a piperidine group). Fentanyl was first synthesized in 1960 by chemist Paul Janssen, who later founded the Belgian pharmaceutical firm Janssen Pharmaceutica. Janssen produced fentanyl by reacting *N*-phenethylpiperidone with aniline to create 4-anilino-*N*-phenethylpiperidine. The product was then reacted with propionyl chloride to give pure fentanyl. On a weight-for-weight basis, fentanyl is 50–100 times more potent than morphine.

Two other clinically important fentanyl analogs were subsequently introduced by the manufacturer: alfentanil (Alfenta®), an ultra-short-acting (5–10 min) agent, and sufentanil (Sufenta®), which is 5–10 times more potent than fentanyl but also short acting. The latter is used mainly in heart surgery (Schwartz et al., 1994). The Duragesic patch is a fentanyl transdermal patch used for the management of chronic pain. Actiq is a lozenge formulation of fentanyl citrate designed for transmucosal absorption. It is intended mainly for opiate-tolerant patients and for cancer patients with breakthrough pain, though it is used "off label" in other chronic pain syndromes.

An effervescent tablet intended for the same purposes is also available. Carfentanil (Wildnil) is a fentanyl analog with an analgesic potency 10,000 times that of morphine. It is used in veterinary practice to immobilize certain large animals. In addition to the conventional forms of fentanyl, at least 12 different illicit fentanyl analogs are known to exist (Sorkin et al., 1994). Alpha-methylfentanyl is the form most frequently used to adulterate heroin, an increasingly common practice.

Since late 2005 clandestinely produced fentanyl has been linked to hundreds of fatal and nonfatal overdoses all across the United States, particularly in the Midwest, Northeast, and Mid-Atlantic areas. From 1990 through 2005 at least nine clandestine fentanyl laboratories were seized in the United States, and it appears that Mexico is the chief provider of illicit fentanyl. In May of 2006 authorities in Lerma, Mexico, raided a laboratory alleged to have produced and shipped about 22 lb of fentanyl into the United States. (Detroit Free Press, 2007). Shortly afterward, laws were passed that restricted the sales and manufacture and distribution of *N*-phenethyl-4-piperidone, or NPP, the preferred precursor used by clandestine laboratories for synthesizing fentanyl (DEA, 2010).

Whatever the reason, very few clandestine fentanyl laboratories are ever found in the United States, or anywhere else in the world for that matter, and reports of fentanyl-adulterated heroin seem to be decreasing. This may be a consequence of the fact that the de novo synthesis of fentanyl and its derivatives is an expensive process costing much more money than heroin. Since the net effect on the body is the same, it is hard to see why anyone would want to spend the extra money to produce fentanyl although, because of its potency, it is much easier to transport.

Fentanyl undergoes oxidative *N*-dealkylation to nor-fentanyl catalyzed by CYP3A4, and genetic polymorphisms can lead to wide interindividual variability. The situation

appears to be more marked in Asia, where it has been demonstrated that *CYP3A4*1G* polymorphism is related to the pharmacokinetics of fentanyl: patients with *CYP3A4*1G* variant A allele have a lower metabolic rate of fentanyl (Yuan et al., 2011b).

Unchanged fentanyl is detectable in urine almost immediately after administration. It remains detectable for approximately 72 h. Nor-fentanyl, the principal metabolite, is detected in all patients for at least 48 h and up to 96 h in some (Silverstein et al., 1993).

Illicit forms of fentanyl are formed by methylation of both the alpha position of the phenethyl group (alpha-methylfentanyl) and the 3 position of the piperidine ring (3-methylfentanyl). These drugs have essentially the same activity and potency as fentanyl itself. When they first appeared on the illicit market, they were referred to as "China White," even though the illicit fentanyls have nothing to do with the form of heroin once referred to as "China White" (Kram et al., 1981). Illicit alpha-methylfentanyl first appeared as a street drug in California in 1979. In 1984 3-methylfentanyl was identified and it was implicated in clusters of overdoses that occurred in California from 1984 to 1985. The *cis*-(+)-isomer of 3-methylfentanyl is approximately 7000 times as potent as morphine, while the *trans*-(±)-isomer is approximately 1000 times as potent.

Clustered outbreaks of fentanyl-related deaths have occurred on a regular basis ever since the mid-1980s (Wahaba and Winek, 1989; Henderson, 1991; Hibbs et al., 1991; McGee et al., 1992; Smialek et al., 1994; Kronstrand et al., 1997; Anderson and Muto, 2000; Kuhlman et al., 2003; Lemos et al., 2004), but mostly fentanyl-related deaths are sporadic and, if the drug is used intravenously, usually involve medical staff who have access. This pattern appears to be changing and there is a thriving black market in fentanyl patches, usually stolen from, or even worn by, a dying family member. Patch abuse can prove to be very dangerous, not so much because of fentanyl's inherent toxicity but because of the way the drug is used. Some abusers have died inhaling the vapors from a heated patch (Oechsler et al., 2009), others have died after intravaginal insertion, and still others have aspirated the patch while trying to avoid arrest and died while trying to hide the evidence from investigators (Carson et al., 2010). One recent study showed that between 1997 and 2007, there had been a 525% increase in legal sale of this drug (Manchikanti et al., 2010). Many deaths have now occurred in Australia due to abuse of fentanyl patches, including death from intravenous injections of patch contents (Reeves and Ginifer, 2002; Drummer, unpublished observations). The death of at least one child has occurred after accidental oral ingestion. Toxicologic analysis by LC-MS/MS with positive electrospray ionization yielded fentanyl and norfentanyl concentrations in the peripheral blood of 5.6 and 5.9 ng/mL, heart blood 19.0 and 8.9 ng/mL, and liver 235 and 26 ng/g, respectively (Teske et al., 2007).

Until the early 1990s, it was assumed that doctors became addicted to fentanyl simply because they had easy access, which made the drug tempting (Storr et al., 2000; Trinkoff et al., 2000). However, the results of recent studies suggest that aerosolized fentanyl is detectable within operating rooms, possibly making operating room workers more susceptible to addiction (McAuliffe et al., 2006). This notion has become an issue of contention (Law et al., 2010). Still, pathologists are increasingly confronted with cases where fentanyl is the cause of death and not simply an incidental finding. As is true of all opiates, both tolerance (Albrecht et al., 1997; Bot et al., 1998) and redistribution (sometimes on a massive scale) occur (Anderson and Muto, 2000). It follows that isolated blood fentanyl concentrations cannot be used to make the diagnosis of fentanyl toxicity.

5.11.4.2 Pharmacology and Pharmacokinetics

Fentanyl acts at the μ-receptor producing the same adverse effects as any other opioids: sedation, nausea, vomiting, and constipation. In addition to the anticipated side effects, fentanyl can cause rigidity of the chest wall muscles, resulting in a condition known as *wooden chest syndrome* (Jackson, 1994). Unexpected increases in muscle tone may make ventilation difficult and pose a significant danger during endoscopic procedures, a situation in which fentanyl is often given. The results of animal studies suggest that increased muscle tone is in some way related to altered serotonergic transmission (Jaros and Kolasiewicz, 1995). Other studies have shown that Type 1A 5-HT receptors are involved (Guenther et al., 2003). Fentanyl both promotes 5-HT efflux and, at the same time, also binds to 5-HT(1A) receptors, activating somatodendritic autoreceptors. In short, fentanyl is a 5-HT(1A) receptor agonist. This property may be a contributory factor in cases of fentanyl overdose (Tao et al., 2003; Fox et al., 2009; Kirschner and Donovan, 2010). Accordingly, persons consuming other serotonin-active drugs (e.g., tramadol, SSRIs, MDMA) are at risk of developing serotonin toxicity.

Treatment with fentanyl causes modest increases in intracranial pressure and small decreases in both mean arterial blood pressure and cerebral perfusion pressure, which is much the same reaction observed after treatment with intravenous morphine. In studies of high-dose anesthesia for cardiac surgery, plasma fentanyl concentrations between 34 ± 7 and 48 ng/mL were observed (Lunn et al., 1979), but respiratory depression may be detected at levels as low as 1–5 ng/mL (Fung and Eisele, 1980; Andrews et al., 1983). The plasma level required to produce effective analgesia in general surgical patients is 1–3 ng/mL, but with very considerable interpatient variability (Gourlay et al., 1988). Values in the 1–3 ng/mL range can also be associated with severe respiratory depression. Depression is observed in human volunteers at plasma concentrations between 2 and 3 ng/mL (Cartwright et al., 1983). When fentanyl patches are used to treat cancer patients, steady-state serum concentrations of 2.6 (± 1.3 ng/mL) are approached by the time the second patch has been applied, and the kinetics of the drug remain stable for the duration of treatment.

5.11.4.3 Routes of Administration

Abusers may take fentanyl by any route. No matter the route, CYP3A4 genetic variability guarantees that there will be wide interindividual variability in resultant plasma concentrations (Jin et al., 2005). Evidence also exists that some of the variability in the metabolism of alfentanil may be related to CYP3A5 polymorphisms (Klees et al., 2005). After an oral dose of 15 μg/kg, peak plasma concentrations in healthy volunteers average 3.0 ng/mL. Peak plasma levels after administration of 15 μg/kg intravenously are nearly 10 times higher. The difference is attributable partly to first-pass effects in the liver and partly to the fact that fentanyl is metabolized in the gut wall. However, the terminal elimination half-life is approximately 7 h after either intravenous or oral administration (Streisand et al., 1991).

Outside of the operating room, fentanyl is most often given by transdermal application. Duragesic patches (Figures 5.24 and 5.25) have four layers. A polyester backing overlies a drug reservoir composed of fentanyl and an alcoholic gel. A release membrane in direct contact with the skin controls the drug's rate of release, and the product is held in place by a final layer of adhesive. Four different sized patches are available (25, 50, 75, 100 μg/h). All of the patches release fentanyl at the same rate, but the larger the patch, the more fentanyl there is to release. Once the patch is applied, drug is absorbed into

Figure 5.24 Duragesic patch. Fentanyl is extremely lipophilic, more so than any other currently available opiate. The mean apparent partition coefficients of oxycodone, morphine, and fentanyl in *N*-octanol at pH 7 are 0.7, 0.5, 10.5, and 399, respectively, giving fentanyl a 570-times greater affinity for fat than morphine (Poyhia and Seppala, 1994). The patches are sold in four different strengths (2.5, 5, 7.5, and 10 mg per patch). A transmucosal form was introduced in 2003.

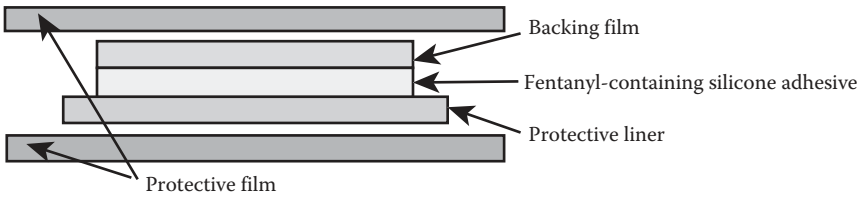


Figure 5.25 Schematic of Duragesic patch.

the upper layers of the skin and none appears in the systemic circulation for at least 2 h. By the time 8–12 h have elapsed, plasma concentrations approximate those seen when fentanyl is given intravenously (Calis et al., 1992). Blood concentrations reach steady state by 24 h. Because of the lag in absorption and delayed onset of analgesia, the patches are not used for immediate or postoperative pain relief (Fiset et al., 1995). Plasma fentanyl concentrations gradually decline over the second and third day after application, but not enough to lose effectiveness. Because a depot has been formed in the skin, fentanyl continues to be absorbed into the systemic circulation even after a patch has been removed, at least for 12 h (Grond et al., 2000).

Lozenges containing fentanyl citrate have been used to premedicate children before surgery (Fentanyl Oralet), and they are also used in the elderly (Actiq, Cephalon Corporation; Figure 5.26). When fentanyl is given by this route, plasma concentrations peak in 20 min and may reach levels of 3–4 ng/mL (Mystakidou et al., 2006). In children the estimated fentanyl bioavailability via this route (mean ± SD) is low (36.1% ± 0.4%), as are the peak plasma concentrations (1.03 ± 0.31 ng/mL), suggesting that many children swallow a large fraction of the dose, which probably explains why peak concentrations occur a relatively long time after the drug has been administered (53 ± 40 min) (Wheeler et al., 2002). Other workers have reported slightly lower peak levels (C_{max} 0.39–2.51 ng/mL) (Mystakidou et al., 2006).

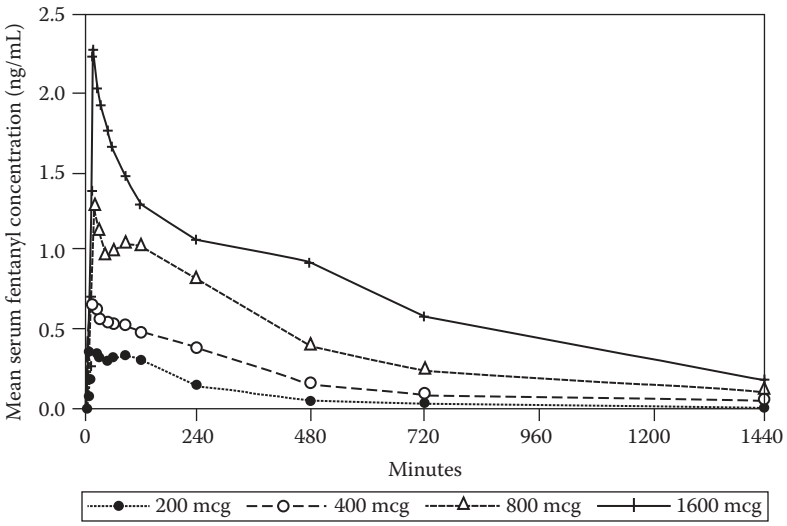


Figure 5.26 Actiq is a lozenge formulation of fentanyl citrate designed for transmucosal absorption. It is intended mainly for opiate-tolerant patients and for cancer patients with breakthrough pain, though it is used *off label* in other chronic pain syndromes. The graph displays the mean serum fentanyl concentration (ng/mL) in adult subjects comparing four doses of Actiq.

As with adults, great variation exists in the plasma concentration finally achieved, and very little relationship exists between drug concentrations and pain relief (Dsida et al., 1998). The important issue with oral and transmucosal formulations is that absorption via this route avoids hepatic first-pass metabolism, greatly increasing bioavailability and, presumably, effect (Mystakidou et al., 2006).

Very recently effervescent fentanyl tablets have been introduced to the market (Cephalon®). The tablets, which are intended for insertion between the gum and lip, come in various strengths. They are intended for use mainly in patients with cancer, particularly those undergoing chemotherapy or radiotherapy and therefore prone to develop oral mucositis. In one study of 16 patients, 8 with and 8 without oral mucositis, the median C_{max} was essentially the same no matter whether oral inflammation was present or not: 1.1 ng/mL (range, 0.3–2.7 ng/mL) in patients with mucositis and 1.2 ng/mL (range, 0.2–2.3 ng/mL) in patients without mucositis. The T_{max} was the same in both groups: median T_{max} was 25 min (range, 15–45 min) in patients with and without mucositis and so was absorption. In a well-designed phase III trial in opioid-tolerant patients with cancer, a single dose of fentanyl buccal tablets 100–800 μg provided clinically significant decreases in pain intensity within 15–60 min after drug administration. Single doses of fentanyl buccal tablets of 100–800 μg are generally well tolerated, at least among those who are already tolerant. Abuse of this particular formulation seems fairly unlikely, but it still would be prudent to swab the gum area in suspect cases.

Reports of individuals ingesting whole patches as a suicide gesture exist. A 2014 report describes a 42-year old man who committed suicide by ingesting the entire center of the patch being used to treat lymphoma pain. The patient was last seen awake and normal 1.3 h prior to being found apneic and cyanotic. During the resuscitation attempt, a small square-shaped film was removed from the patient's oropharynx. Femoral blood was collected

0.5 and 2 h postmortem, and the measured fentanyl concentration increased from 1.6 to 14 ng/mL. The authors of the case report take pains to emphasize that the values measured do not necessarily represent those found in life; the pH will have dropped after death, resulting in increased solubility and redistribution (Moore et al., 2014).

Abusers have found a surprising number of ways to obtain fentanyl from the Duragesic patch. Suicides may attach multiple new (or used) patches to their body. Depending on the amount of fentanyl remaining in the patch, results may or may not be fatal (Flannagan et al., 1996; Tharp et al., 2004). Addicts may simply attach multiple used patches (often taken from cadavers). Anecdotal reports describe users extracting residual fentanyl from the patches and injecting it intravenously (Lilleng et al., 2004; Tharp et al., 2004), chewing whole patches (Liappas et al., 2004) that greatly enhances absorption, inserting new or used patches vaginally and rectally (Coon et al., 2005), and even using them for tea bags (Barrueto et al., 2004). An analysis of fentanyl patches worn by hospice patients disclosed that 0.7–1.22 mg still remained in the 2.5 mg patches and 4.46–8.44 mg remained in the 10 mg patches after they had been removed (Marquardt and Tharratt, 1994; Flannagan et al., 1996; Yerasi et al., 1997; Kramer and Tawney, 1998). If respiratory depression does occur, merely removing the patch will do nothing to stop further fentanyl absorption, since a depot will have already been deposited in the skin; continuous administration of naloxone may be required.

Heating the patches to liberate fentanyl vapor is not uncommon, and deaths from respiratory depression have occurred (Marquardt and Tharratt, 1994). Systemic absorption after inhalation is extremely fast. A 1994 case report describes an individual who collapsed after only one inhalation. The concentration was 2.6 ng/mL in femoral blood, 3.3 ng/mL in the vitreous, and 122 ng/g in the liver. The pharmacokinetics of nasal insufflation has not been studied, but clinical trials with hospice patients have shown that effective relief of breakthrough pain can be achieved via this route (Zeppetella, 2000). In a recent double-blind, double-dummy study, there was no difference in the efficacy of intranasal fentanyl and intramuscular and intravenous morphine in controlling postoperative pain and emergence delirium in children undergoing insertion of myringotomy tubes. The intramuscular route is the simplest and avoids the potential for delays to establish vascular access for intravenous therapy and the risks of laryngospasm if intranasal drugs pass through the posterior nasopharynx and irritate the vocal cords (Hippard et al., 2012).

5.11.4.4 Metabolism and Excretion

After initial rapid uptake by lung and fat, fentanyl is slowly released back into the circulation. Metabolism occurs mainly in the liver, but when fentanyl citrate is given orally, it is subject to not only first-pass metabolism in the liver but also metabolism by the same P450 3A4 microsomes located in the duodenum. Piperidine *N*-dealkylation with the formation of norfentanyl is fentanyl's predominant metabolic pathway; however, small amounts of fentanyl undergo amide hydrolysis to form despropionylfentanyl and alkyl hydroxylation to form hydroxyfentanyl. Secondary metabolites are also formed; small amounts of hydroxynorfentanyl undergo *N*-dealkylation to yield hydroxyfentanyl (Labroo et al., 1997). Metabolism does not appear to be altered by age (Kharasch et al., 2007), but obese individuals will have lower blood levels than predicted for a given dose.

It has been known for some time that serum fentanyl concentrations and the ratio of norfentanyl to fentanyl vary considerably between cancer pain patients being treated with transdermal fentanyl patches. *CYP3A4*22* and *CYP3A5*3* genotypes, and multiple clinical

factors, combine to influence transdermal fentanyl pharmacokinetics, but even so, polymorphism also accounts for only a small proportion of variability that has been observed. Identification of the remaining factors determining serum fentanyl concentrations, and their relationship to efficacy and adverse effects, may aid in improving the safety and effectiveness of transdermal fentanyl. It is known that even the delivery rate of the drug can alter its effectiveness, as can the sex of the patient, comedications, kidney disease, body mass index, and even serum albumin (Barratt et al., 2014).

Studies of surgical patients have shown that unchanged fentanyl appears in the urine shortly after administration and that it persists there for up to 24 h. By 72 h, fentanyl is undetectable. Norfentanyl appears in the urine almost as quickly as fentanyl but in much higher concentrations. Norfentanyl is detectable in the urine of all surgical patients for 48 h and in half of these patients for periods as long as 96 h. Neither fentanyl nor its metabolites are consistently detectable in saliva (Silverstein et al., 1993).

5.11.4.5 Tissue Concentrations

All of the fentanyls are highly lipid soluble and are distributed widely throughout the body (Hess et al., 1972). When administered intravenously, 3%–4% of the dose will be secreted into the gastric juice, where there is minimal reabsorption (Stoeckel et al., 1979). Thus, the detection of fentanyl in gastric contents does not imply oral administration. In the series of 112 fentanyl-related deaths described by Henderson (1991), fentanyl concentrations in blood ranged from 0.2 to >50 ng/mL, and urine concentrations ranged from 0.2 to >800 ng/mL. If the few individuals with extremely high levels are excluded, then the mean fentanyl level at autopsy was 3.0 ± 3.1 ng/mL in the blood and 3.9 ± 4.3 ng/mL in the urine. In the handful of deaths due to fentanyl citrate (the pharmaceutical-grade product used as an intravenous anesthetic), blood concentrations have ranged from 3 to 27 ng/mL (Garriott et al., 1984; Matejczyk, 1988).

In 2013 the effects of fentanyl postmortem redistribution were compared in blood and liver in a series of 59 cases collected from four different medical examiner offices in the U.S. Fentanyl concentrations ranged from <2 to 15 μg/L in non–drug-related deaths ($n = 5$), <2 to 22 μg/L in cases of mixed drug toxicity ($n = 26$), and 3.7 to 56 μg/L where fentanyl toxicity ($n = 33$) was deemed the cause of death. At the same time, liver fentanyl concentrations ranged from 11 to 104, 6 to 235, and 18 to 365 μg/kg, respectively, with only a modest blood to liver concentration ($r = 0.67$), and the ratio decreased as the postmortem interval lengthened. The findings suggest that, depending on the postmortem interval, measuring liver fentanyl concentrations might be the preferred route. The authors of that particular study suggest that liver fentanyl concentrations best define therapeutic use when measured at concentrations of less than 23 μg/kg and fatal toxicity at 56 μg/kg, because no substantial overlap was found in blood fentanyl concentrations (Palamalai et al., 2013).

Blood and tissue concentrations have also been described in fentanyl patch users. One series described 25 decedents wearing transdermal patches (Table 5.14). While the observed blood concentrations ranged from 1.8 to 139 ng/mL, the mean blood concentration in eight decedents being treated for cancer was 3.6 ng/mL (range, 2–7 ng/mL). Concentrations in abusers were generally much higher (Anderson and Muto, 2000).

Note that in Figure 5.27 showing plasma concentrations achieved with the 25 μg fentanyl patch, the peak plasma level attained was 0.6 ng/mL after 24 h. Given the relatively high V_{ss} of fentanyl, it can safely be assumed that the concentration would have been substantially higher if measured at autopsy. This suspicion has been confirmed by

Table 5.14 Blood and Tissue Fentanyl Concentrations in 25 Decedents Wearing Transdermal Fentanyl Patches

Organ/Tissue	Concentration Range (ng/mL)
Heart blood	1.8–139
Femoral blood	3.1–43
Vitreous	<2.0–20
Liver	5.8–613
Bile	3.5–262
Urine	2.9–895
Gastric content	0–1200
Spleen	7.8–79

Source: Anderson, D.T. and Muto, J.J., *J. Anal. Toxicol.*, 24(7), 627, 2000.

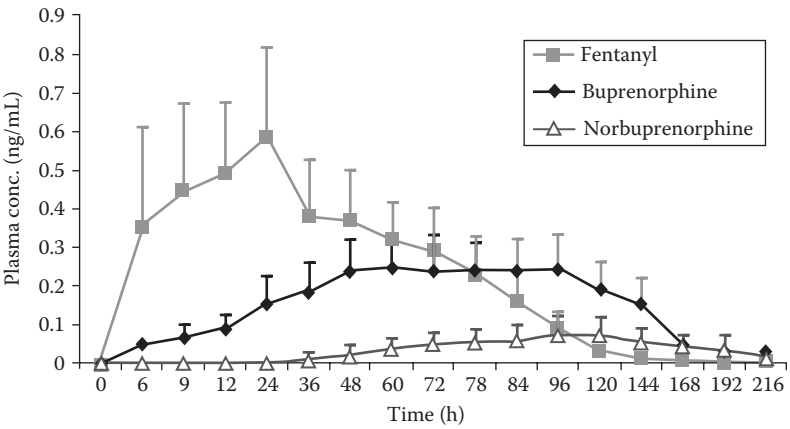


Figure 5.27 The comparative plasma levels seen in healthy volunteers, $n = 22$, after the application of either a 20 μg/h buprenorphine patch versus the plasma concentrations seen in the same volunteers 72 h after a μg/h fentanyl patch. (Adapted from Anderson, M.E. et al., *Paediatr. Anaesth.*, 21(3), 280, 2010.)

the results of animal studies. Postmortem redistribution of fentanyl in the rabbit was investigated after application of the 50 μg/h Duragesic pain patch. Patches were applied for 48 h. Two cycles of patch administration were used before characterization of the postmortem redistribution. Fentanyl showed marked redistribution into the femoral and pulmonary veins of the rabbit 48 h after the animals had been sacrificed and the patches removed. The plasma concentration was 2.34 ng/mL in the femoral blood before killing the animals, and it had increased 5.6-fold by 48 h to 13.2 ng/mL after the patch had been removed (Ceelen et al., 2010).

McGee et al. (1992) compared blood and tissue fentanyl levels in seven anesthetized patients who died at surgery (Table 5.15). As is the case with transdermal patches themselves, overdose deaths cannot be linked to a particular concentration. Though deaths in abusers tend to be associated with concentrations 5–10 times higher than those observed in anesthetic and/or surgical deaths, that is not always the case. A report from Germany describes two polydrug abusers who intravenously injected the contents of fentanyl patches. In one case the fentanyl concentration in postmortem blood was 2.7 ng/mL, while in the second it was 13.9 ng/mL (Lilleng et al., 2004).

Table 5.15 Comparison of Blood Levels in Fentanyl-Related *Overdose* Deaths and Levels Seen in Anesthetized Patients Dying of Surgical Complications

	Deaths from Fentanyl Overdose	Deaths at Surgery
Blood	11–233 ng/mL	5–45 ng/mL
Brain	20–194 ng/mL	18–85 ng/mL
Liver	28–1000 ng/mg	41–158 ng/mg

Source: McGee, M., Fentanyl related deaths in New York City, in: *44th Annual Meeting of American Academy of Forensic Sciences*, New Orleans, LA, 1992.

Because of fentanyl's lipophilicity and rapid tissue absorption, changes of extraordinary magnitude can occur between the immediate antemortem period and the time of death. In an American study, fentanyl concentrations were measured in postmortem specimens collected in 20 medical examiner cases from femoral blood, heart blood, heart tissue, liver tissue, and skeletal muscle. In seven of these cases, femoral blood was obtained at two postmortem intervals, shortly after death (FB1) and at the time of autopsy (FB2). The mean collection times of the first and second samples were 4.0 and 21.6 h after death. Fentanyl concentrations in both the first and second samples ranged from undetectable to 14.6 μg/L (mean, 4.6 μg/L) and 2.0 to 52.5 μg/L (mean, 17.3 μg/L), respectively. Astonishingly, in four of the cases, fentanyl was not detected in the first femoral blood specimens but was present in substantial quantities (58.8 ng/mL in one case) in the second. Corresponding mean heart blood, liver tissue, and heart tissue fentanyl concentrations were 29.8 μg/L, 109.7 mg/kg, and 103.4 mg/kg, respectively (Olson et al., 2010).

In fact, with the publication of every new study, the extreme nature of postmortem fentanyl redistribution becomes more obvious (Krinsky et al., 2014). German researchers analyzed whole blood from 118 decedents who had been receiving therapeutic morphine and compared them with serum concentrations measured in 27 living patients who were also wearing fentanyl patches. They found that fentanyl concentrations in autopsy blood were as much as nine times higher than in the living (Andresen et al., 2012). There is a reason for the variability. The movement from high concentration tissue stores into the blood is likely after death and the decrease in pH of postmortem blood, which is an expected component of the decomposition process, results in an increase in the ionized fraction of fentanyl. Any increase in ionized fentanyl concentration causes ion trapping within the vasculature. As a result, postmortem blood fentanyl concentrations cannot be taken as directly reflective of antemortem concentrations (Palmer, 2010). Clearly, postmortem blood concentrations cannot be directly compared with in vivo serum levels. In the forensic setting, it makes absolutely no sense to speak of therapeutic and toxic *ranges* or *threshold* fentanyl concentrations.

As is true in all opiate-related deaths, other drugs are frequently detected. Ethanol is frequently detected, often in high concentration. In 20% of the decedents in Henderson's case series, cocaine was also detected (Henderson, 1991). In deaths associated with the use of the transdermal patch, other drugs are almost inevitably present (Anderson and Muto, 2000). There appears to be no correlation between dosage (patch size) and urine fentanyl concentration or analgesia. Indeed, concentrations in specimens from legitimate chronic pain patients are often far less than those recorded in cases of obvious overdose (Poklis and Backer, 2004).

Fentanyl can also be detected in hair (Wang et al., 1993). Moore et al. (2008) used radioimmunoassay to analyze fentanyl in hair from 13 patients who had received 1–6 μg of fentanyl during surgical anesthesia. Hair concentrations ranging from 13 to 48 pg/mg were identified. A case report published in 1995 described the findings in a criminalist suspected of stealing fentanyl patches from cadavers; his hair concentration was 20 pg/mg (Selavka et al., 1995). When tandem MS was used to test hair samples from a registered nurse suspected of narcotic theft and abuse, fentanyl concentrations ranging from 20 to 93 pg/mg were detected (LaBeau et al., 2002). Kintz et al. (2005b) reported on their analytic findings in four anesthesiologists suspected of fentanyl abuse. Two were using only fentanyl and had hair concentrations of 101 and 644 pg/mg, respectively. One decedent was chronically abusing multiple drugs. Analysis of cardiac blood revealed an acute overdose of alfentanil (45 ng/mL), but ethanol (1.32 g/L) was also detected; in the hair alfentanil (2 pg/mg) and fentanyl (8 pg/mg) were also detected.

5.11.4.6 Maternal/Fetal Considerations

The concentration of fentanyl in the breast milk of women undergoing surgery has been studied, and the amount of fentanyl transferred by this route is so negligible (0.0006%–0.073% of a given dose) that women who receive fentanyl at surgery are not advised to discontinue breast-feeding (Nitsun et al., 2006). No additional information has ever been published to contradict these findings.

5.11.4.7 Opiate Cardioprotection

There is evidence that opioids protect the myocardium from ischemic injury. The μ-opioid receptor located on cardiac myocytes appears to mediate opioid-induced cardioprotection. It is believed that the stimulation of the μ-opioid receptor causes activation of the potassium–ATP channels leading, in turn, to release of an as yet uncharacterized low molecular weight circulating factor (Shimitzu et al., 2009). This effect has been demonstrated both in human atrial tissue and in animal models. The effect is also clear and reproducible when morphine is given prior to bypass surgery and it had been assumed that fentanyl would offer the same protection. The issue is still under debate, but the evidence that does exist suggests that morphine is the more effective of the two (Murphy et al., 2006). In vitro studies show that continuous administration of remifentanil and sufentanil induces cardioprotection in human myocardium (Lemoine et al., 2011).

5.11.4.8 Autopsy Findings

The autopsy findings in fentanyl-related deaths are the same as in heroin overdose: pulmonary and cerebral edema. If licit transdermal patches are present, nothing prevents the continuous release of fentanyl after death. In such cases and in instances where multiple patches have been applied, postmortem concentrations may be well over 50 ng/mL (Edinboro et al., 1997). At least one study has addressed the problem of continuing release of fentanyl from patches after death, and the authors concluded "the antemortem dose cannot be reliably predicted based on the postmortem concentration" (Gill et al., 2013). It is quite conceivable that a patient with a painful injury, being treated with a fentanyl patch, might die from their original injury while wearing a fentanyl patch. If absorption from the patch continues after death, and there is no reason to assume that the patch will stop releasing fentanyl at the time of death, high fentanyl concentrations observed at autopsy might mistakenly be considered the cause of death.

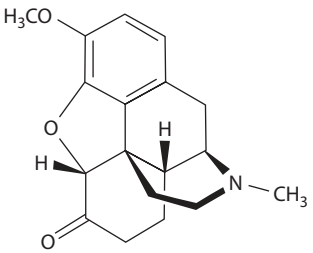
Because there is no question that fentanyl redistribution is certain to occur, measurement of fentanyl in postmortem blood samples is a relatively valueless exercise. If a cadaver is seen to have a fentanyl patch placed directly over the heart, or even near to it, diffusion from the patch into the bloodstream should be anticipated. The result may be another reason for a spuriously high reading that bears no relationship to fentanyl plasma concentrations at the time of death. In such situations, measurement of hair fentanyl concentrations can be extremely helpful, as this will reveal patterns of abuse and may allow some inferences about tolerance. Finally, the issue of P450 heterogeneity (Jin et al., 2005) may well be relevant, particularly in Asians, but genetic resequencing is unlikely to be worth the expense, as discussed earlier, given fentanyl's enormous propensity for postmortem redistribution. It is unlikely that any single polymorphism could ever clarify the final diagnosis.

5.11.5 Hydrocodone (Table 5.16)

5.11.5.1 Clinical Considerations

Hydrocodone is a semisynthetic narcotic and a potent antitussive, very widely prescribed for the treatment of cough, especially by oncologists (Homsi et al., 2002). It is a poorly studied drug, and information about human toxicity is limited mainly to the fact that, as

Table 5.16 Physiochemical Properties and Pharmacokinetics of Hydrocodone

Chemical names	4,5-Epoxy-3-methoxy-17-methylmorphinan-6-one
Physiochemical properties, structure, and form	Available as HCl, phosphate and tartrate salts CAS: 125-29-1 MW: 299.4 V_d: 3–5 kg/L p*K*a: 8.3 
Synonyms and trade names	Dihydrocodeinone, Hycodan tablets and syrup, Hycotuss, Vicodin, Vicoprofen, Zydone, Hycet, Lorcet, Lortab, Maxidone, Norco, Pneumotussin, Repexain, Tussafed, Tusso
Pharmacokinetic parameters	Bioavailability: >80% C_{max}: 18–32 ng/mL (mean 23 ng/mL) after 10 mg p.o. T_{max}: 1.5 h
Blood terminal elimination half-life	Approximately 4 h
Metabolism	*Hepatic*: Metabolized by *O*- and *N*-demethylation and 6-keto reduction to produce 6α and 6β hydroxy metabolites, hydromorphone (HM), norhydrocodone, and hydrocodol. The last exists as a stereoisomer. One of the isomers is dihydrocodone.
Excretion	Renal, 26% in 72 h. Only 12% excreted unchanged, 5% norhydrocodone, 4% conjugated hydromorphone, 3% 6-hydrocodol, and 0.1% as conjugated 6-hydromorphol. Hydrocodone is a minor metabolite of codeine.
Postmortem artifacts	Unknown but likely to be similar to morphine
Interactions	Other opioids; any drug metabolized by CYP2D6
Key papers	Barnhart and Caldwell (1977), Anon (2004), Hutchinson et al. (2004), Holstege (2005), Jenkins et al. (2009), and Meyer and Maurer (2011)

a μ-receptor agonist, it can, if taken in sufficiently large doses, produce respiratory depression. Injectable formulations are not produced, but addicts solubilize pills (and syrup) and then inject it. Hydrocodone's antitussive actions are thought to be the result of medullary depression, but the mechanism has never been addressed. Randomized controlled trials have shown that its analgesic properties are comparable to those of oxycodone (Marco et al., 2005). The main active metabolite of hydrocodone is hydromorphone, produced by *O*-demethylation (CYP2D6). The hydromorphone metabolite has a much greater affinity for the opioid receptor. The other major hydrocodone metabolite is produced by *N*-demethylation catalyzed by CYP3A4. Approximately 40% of hydrocodone is metabolized via alternative pathways including reduction at the 6-keto position, as well as fecal, biliary, intestinal, and renal elimination (Hutchinson et al., 2004).

Small amounts of morphine are metabolically converted to hydromorphone. One group studied 73 urine specimens that had already screened positive for morphine with morphine concentrations ranging from 131 to 297,000 ng/mL. Hydromorphone was present at a concentration ≥5 ng/mL in 36 of these specimens at concentrations ranging from 0.02% to 12% of the morphine concentration. Hydrocodone was not detected in these specimens at the assay detection limit of 25 ng/mL. In other words, the detection of hydromorphone in urine specimens does not necessarily mean that exogenous hydromorphone or hydrocodone has been used (McDonough et al., 2008).

5.11.5.2 Postmortem Data

In seven cases of fatal hydrocodone mono-intoxication, the mean and median hydrocodone concentrations were 0.53 and 0.40 mg/L, respectively. The range was 0.12–1.6 mg/L, with 11 cases (65%) less than 0.5 mg/L (Spiller, 2003). Because hydrocodone has a relatively large volume of distribution, significant redistribution should be anticipated. Hydrocodone can also be measured in hair but no apparent correlation with blood concentrations has ever been demonstrated. In hair specimens collected from 24 volunteers administered hydrocodone, the range of concentrations observed was extremely wide (130–15,933 pg/mg). On the other hand, concentrations as high as 1400 ng/mL can be observed in the blood of perfectly healthy living individuals. Hair from the volunteers was also found to contain hydromorphone (range, 59–504 pg/mg) (Moore et al., 2006).

In 2011 researchers undertook a retrospective review of death where hydrocodone had been detected at autopsy. Cases were divided into three groups: those where hydrocodone was clearly the cause of death, cases where it was clearly an incidental finding, and those where hydrocodone was found after the individual had been arrested for impaired driving, The average hydrocodone concentration in the cases where hydrocodone caused death was 0.47 mg/L (median 0.38 mg/L). The average hydrocodone concentration in cases where it was incidental to death was 0.15 mg/L (median 0.08 mg/L). The average hydrocodone concentration in the driving under the influence cases was 0.09 mg/L (median 0.08 mg/L). While three distinct groups appear to be represented, it would be unwise to overrely on them. As the authors of this particular study observe, and as has been repeatedly stated in this book, "Analysis showed the possibility of postmortem redistribution as well as significant overlap of the concentrations noted in the different groups. Given that no definitive lethal concentration could be delineated, it is recommended that each hydrocodone case encountered be assessed individually to include a thorough medical record review to accurately interpret hydrocodone concentrations" (Molina and Hargrove, 2011).

5.11.5.3 Maternal/Fetal Considerations

One case report described the findings in two breast-feeding women who were taking hydrocodone. Pumped milk was analyzed for hydrocodone. All of the milk that was present was analyzed, which is important because earlier measurements of breast milk drug concentration analyzed only the milk expressed initially (called foremilk), which has a very different composition from what follows it (hindmilk); analysis of the entire specimen is actually necessary to gain a full picture of any drug's excretion pattern. The infants of these two women received an estimated 3.1% and 3.7% of the maternal weight-adjusted dosage. The absolute hydrocodone dosages were 8.58 and 3.07 μg/kg/day because of the differences in the dosages ingested by their mothers. The findings suggest that moderate dosages of hydrocodone do no harm to breast-feeding children, though the maximum safe dosage has yet to be determined (Anderson et al., 2007). Additional studies conducted since then confirm that lactating women can use hydrocodone (and hydromorphone) safely. A study of 30 postpartum women receiving hydrocodone for postpartum pain analyzed timed breast milk samples and they were measured for both drugs. It was found that neonates being only fed breast milk received 1.6% (range, 0.2%–9%) of the maternal weight-adjusted hydrocodone. When combined with the hydromorphone produced by hydrocodone metabolism, the total median opiate dosage received from the breast milk was 0.7% of what is considered a therapeutic dose. The results suggest that the standard postpartum dosage of hydrocodone now in use is perfectly safe for use in women nursing newborns, although long-term use may be problematic (Sauberan et al., 2011).

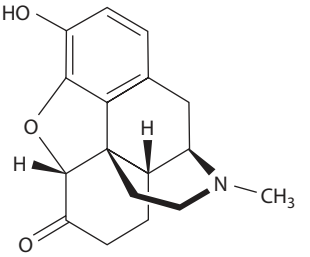
5.11.6 Hydromorphone (Table 5.17)

5.11.6.1 General Considerations

Hydromorphone is a hydrogenated ketone derivative of morphine that is increasingly used for the management of patients with chronic pain, especially those with cancer. Like the other semisynthetic opiates, it is a powerful μ-opioid agonist and produces the same effects as any other potent μ-agonist. It is 3–7.5 times more potent than morphine (Coda et al., 2003). For a short time an extended-release version of hydromorphone, called Palladone, was sold in the United States, but it has now been replaced with a slightly different version called osmotic-controlled release oral delivery system (OROS). OROS hydromorphone (Jurnista™, Janssen-Cilag), an effective opioid analgesic that is used for the long-term treatment of severe chronic pain, contains hydromorphone. The OROS tablet uses a different technology than Palladone. The tablet has a rigid water-permeable jacket with one or more laser-drilled small holes. As the tablet traverses the gastrointestinal tract, the osmotic pressure of water entering the tablet pushes the active drug through the opening in the tablet. Palladone was voluntarily withdrawn from the market after a July 2005 FDA advisory warned of a high overdose potential when taken with alcohol, though it remains for sale in other countries, including Australia (Stepanovic et al., 2011). In 2010 the FDA approved sales of Exalgo for the treatment of moderate to severe pain. The drug is taken orally and delivered via an osmotic "push–pull" mechanism similar to that used in OROS.

Very little has been written about OROS, but drug maker filings with the FDA indicate that after an 8 mg oral dose of the immediate-release formulation, C_{max} was 186 (±36) ng/mL, reached within 15 min of administration; with the Exalgo's formulation the C_{max} was less than a nanogram but the half-life was over 18 h (FDA, 2010a).

Table 5.17 Physiochemical Properties and Pharmacokinetics of Hydromorphone

Chemical names	4,5-α-Epoxy-3-hydroxy-17-methyl morphinan-6-one
Physiochemical properties, structure, and form	Available as base and HCl CAS: 466-99-9 MW: 285.338 V_d: 3 L/kg Protein binding: 7% 
Synonyms and brand names	Dihydromorphinone, Hydal, Dimorphone, Sophidone LP, Dilaudid, Hydrostat, Hydromorfan, Hydromorphan, Hymorphan, Laudicon, Hymorphan, Opidol, Palladone
Pharmacokinetic parameters	Bioavailability: Oral: after one dose, 50.7% ± 29.8% Rectal: 33% ± 22% T_{max}: 2 mg IV (n = 24) = 0.167 (range, 0.083–0.25) h C_{max}: IV: 242 ng/mL after 2 mg bolus 332 Oral: 11.8 ± 2.6 (4 mg to 6 subjects) 18–27 ng/mL, mean 22 Intranasal: (1 mg) 17 ng/mL
Blood terminal elimination half-life	About 2.5 h
Metabolism	CYP3A and, to a lesser extent, CYP2C9 catalyze hydromorphone *N*-demethylation in humans
Excretion	6% excreted unchanged in urine
Postmortem artifacts	Likely to be similar to morphine
Interactions	Troleandomycin and ketoconazole prevent breakdown to norhydromorphone
Key papers	Vallner et al. (1981), Parab et al. (1988), Moulin et al. (1991), Coda et al. (2003), and Benetton et al. (2004)

Some have questioned whether the enzyme (UGT2B7*2) might play a specific role in OROS metabolism, but in controlled studies this hypothesis has been disproven (Vandenbossche et al., 2014).

5.11.6.2 Clinical Considerations

Long-term treatment may produce a neuroexcitation syndrome with agitation, myoclonic activity, and even seizures (Juba et al., 2013). Occurrence of this complication is not related to gender or age. It has been suggested that these symptoms are a result of the accumulation of hydromorphone-3-glucuronide, a metabolic product of hydromorphone (Dean, 2004); however, the situation is hardly proven. In a study of hydromorphone-treated hospice patients, the only factor that seemed to matter was age and renal function, not the malignancy itself (Kullgren et al., 2013). In patients with renal compromise, the half-life may be greatly prolonged, possibly to 40 h or more (Dean, 2004). Different considerations apply to the management of chronic pain as opposed to the management of chronic pain secondary to malignancy, and there is concern in many circles that chronic treatment of noncancer pain may lead to abuse and diversion into the black market.

5.11.6.3 Postmortem Data

There are no diagnostic lesions and no way to differentiate hydromorphone-related deaths from those produced by any other narcotic. A handful of postmortem measurements have been reported. Deaths from hydromorphone are uncommon. In 2006 a paper was published reviewing the findings in an examination of 251 hydromorphone-positive cases that had occurred in the Canadian province of Ontario from 1985 to 2003. Thirty-three of these cases were reviewed in detail. In four cases in which hydromorphone was the sole drug detected and death was attributed to hydromorphone toxicity, concentrations ranged from 77 to 2684 ng/mL. Hydromorphone concentrations ranged from 21 to 441 ng/mL in 28 cases in which at least one other drug was detected. In five deaths attributed to natural causes, blood hydromorphone concentrations ranged from 75 to 423 ng/mL (Wallage and Palmentier, 2006).

Jenkins et al. (2009) described their findings in 64 hydrocodone-related deaths. The range of hydrocodone concentrations was 9–3039 ng/mL in heart blood (n = 43) and 42–12,353 ng/mL in urine (n = 21). Concentrations of DHC (a metabolite of hydrocodone) in these cases ranged from 3 to 243 ng/mL in heart blood and 5 to 1842 ng/mL in urine.

Another recent report describes the case of a 15-year-old who committed suicide by taking an unknown amount of timed-release hydrocodone. No other drugs or alcohol were involved, and the cause of death was given as acute aspiration-related bronchopneumonia secondary to hydromorphone ingestion. Hydromorphone and hydromorphone-3-glucuronide were quantified and the hydromorphone concentrations in the peripheral blood, urine, and vitreous humor were 57, 4460, and 31 ng/mL, respectively. The hydromorphone-3-glucuronide concentrations in the corresponding three fluids were 459, 36,400, and 40 ng/mL. On the basis of this, and previous studies, the proposed minimum lethal hydromorphone blood concentration in the nontolerant user is generally considered to be in the vicinity of 60 ng/mL (Meatherall et al., 2011).

The importance of genetic testing should not be underestimated. One case report describes a developmentally delayed child aged 5 years 9 months who was inadvertently administered high doses of hydrocodone for a respiratory tract infection. The concentration of hydrocodone in the postmortem blood was in the range associated with fatality; however, hydromorphone is catalyzed by CYP2D6, none of which was detected when the sample was analyzed with MS. Subsequent genetic analysis showed that the child had a reduced capability to metabolize hydromorphone via the CYP2D6 pathway (CYP2D6*2A/*41). The result would not have been catastrophic except that the child was also receiving clarithromycin, which is a potent inhibitor of CYP3A4, the other enzyme that could have metabolized the hydromorphone. Unfortunately the child was also taking valproic acid for seizures, and the presence of this drug further prevented hydromorphone elimination from the body (Madadi et al., 2010).

5.11.7 Kratom

5.11.7.1 General Considerations

Very little is known about either the human pharmacology or toxicology of this ancient herbal remedy. Nonetheless, it can be abused, and in vitro studies show that at least one of its components, mitragynine, is a potent opiate agonist, capable of producing analgesia comparable to that of morphine. One of the minor kratom metabolites, 7-hydroxymitragynine, is actually thought to be more potent than morphine (Assanangkornchai et al., 2007; Babu et al., 2008). Figure 5.28 shows fresh kratom leaves. Figure 5.29 depicts the chemical formula.

Figure 5.28 Photograph of kratom leaves. (From Anon, Special intelligence brief: Kratom (*Mitragyna speciosa*), *Microgram Bull.*, March 2006. With permission.)

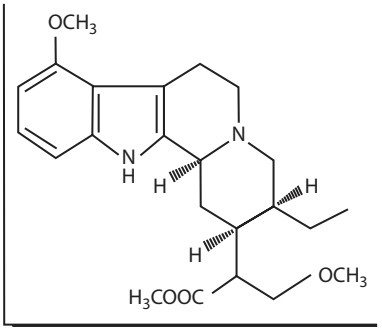


Figure 5.29 Chemical formula for mitragynine, the active ingredient in kratom. (From Anon, Special intelligence brief: Kratom (*Mitragyna speciosa*), *Microgram Bull.*, March 2006. With permission.)

5.11.7.2 Active Agents

Mitragynine only accounts for about 70% of the active alkaloid found in kratom leaves. Many other indole alkaloids have been identified (paynantheine, speciogynine, speciociliatine, 7-α-hydroxy-7H-mitragynine, and rhynchophylline), and it has been established that mitragynine and its primary metabolites are P-gp substrates (Manda et al., 2014). The results of animal binding studies demonstrate the affinity of kratom to μ-, δ-, and κ-opioid receptors and to dopamine D_1 receptors, strongly indicating that, in animals at least, kratom's analgesic effect is mediated via κ-opioid receptors (Stolt et al., 2014).

Clinical reports suggest that at lower doses chewing the plant leaves produces effects reminiscent of those produced by cocaine or any other stimulant. There is no evidence that the leaves ever produce hallucinogenic effects, but the results of animal studies suggest that kratom's interactions are not confined to opiate receptors. An as yet to be identified component of the plant interacts with the serotonergic and adrenergic systems. Kratom is beginning to gain popularity in the United States as a recreational drug. Internet advertisements promote it as a legal psychoactive herb (which it is).

Mitragynine may be a useful anti-inflammatory agent. In vitro studies show that it suppresses production of prostaglandin E_2 (PGE_2) by inhibiting release of cyclooxygenase enzymes (Utar et al., 2011). Mitragynine is an indole alkaloid isolated from kratom. A 9-demethyl analog of mitragynine, 9-hydroxycorynantheidine, is synthesized from mitragynine. It is also a potent μ-agonist, but not nearly as potent as mitragynine. Possible explanations for the plant's stimulant effects have never been determined, but there is ample evidence that these effects are quite real. In fact, until the recent changes in Thai law, kratom extracts were included in commercial energy drinks that were especially popular among construction workers (Pichainarong et al., 2004).

5.11.7.3 Clinical Information

Possible negative effects include dry mouth, increased urination, loss of appetite, and constipation, but none of these is likely to take a person to the emergency room. Unlike many other indoles, mitragynine itself is unlikely to produce nausea or vomiting—as a rule, inexperienced users just become sleepy. A UN report published more than a quarter of a century ago described the kratom alkaloids as addictive. When kratom is fed long term to experimental animals, both food and water intake are reduced, but the animals do not develop tolerance to the drug's effects. Kratom is used by the Thailand military for its stimulant properties.

Information on the illicit use of kratom in the United States is anecdotal, and it would not be detected by any of the present immunoscreening systems used in medical examiner laboratories (mitragynine is easy enough to detect with GC/MS, but only if an effort is made to find it). No clinical studies have been published, but there is one case report that describes a young man who presented with cholestatic jaundice and pruritus after 2 weeks of kratom ingestion (Philipp et al., 2011). A report of kratom-related seizures has been published. A 64-year-old male was witnessed to have had a seizure at home immediately following kratom consumption and had a second seizure on arrival at the hospital, though he did survive. A urine specimen collected shortly after admission had a urine mitragynine concentration of 167 ± 15 ng/mL, but blood mitragynine was not measured (Nelsen et al., 2010). A 2013 case report described a 17-year-old white man found dead in bed. The autopsy was nondiagnostic, but the toxicology report showed a mitragynine blood concentration of 0.60 ng/mL (Neerman et al., 2013).

Based on information posted on the Internet, it appears that kratom is mainly being abused orally as a tea, but chewing kratom leaves is another method of consumption. Doses in the range of 2–10 g are said to produce the desired effects. In London, kratom dealers promote it as an "herbal speedball." In Malaysia, kratom (known as ketum) juice preparations are sold in stores (Jansen and Prast, 1988). More recently kratom leaves have been sold over the Internet with *O*-desmethyltramadol (μ-agonist) (Krypton) added as an adulterant. One episode resulted in the death of nine persons (Kronstrand et al., 2011). Blood concentrations of mitragynine and *O*-desmethyltramadol were 0.02–0.18 and 0.4–4.3 μg/g, respectively.

In 2005, five Thai distributors of "ketum" juice were arrested. In that same year a BBC report claimed that young Thai troops were required to drink a "4 × 100," a kratom formula designed to help them stay alert during military missions. The product "4 × 100" is a mixture of boiled kratom leaves, mosquito coils, and cola or a mixture of boiled cough syrup and whole kratom leaves served with ice. In Thailand it is available in local coffee and teashops. As of this writing kratom was still not a controlled substance in the United States.

However, sales are controlled in Thailand, Malaysia, and Myanmar. In 2004, mitragynine and kratom were both placed in Schedule 9 (the most restrictive level) of the Australian National Drugs and Poisons Schedule (Assanangkornchai et al., 2007).

In Southern Thailand it is called the "kratom cocktail," where kratom is mixed with other substances to produce a drink with more psychoactive effects. Samples of the cocktail have been analyzed. The concentrations of mitragynine, codeine, caffeine, chlorpheniramine, and phenylephrine in "kratom cocktail" were 90.021, 234.174, 73.986, 7.053, and 1.486 mg/L, respectively (Chittrakam et al., 2012).

Precise identification of the plant product may be problematic, because routine GC/MS methods may fail to separate mitragynine from related metabolites because of their similar tandem mass spectra (Wang et al., 2014). On the other hand, direct analysis in real time-mass spectrometry (DART-MS) can be used to rapidly identify *Mitragyna speciosa* plant material found at any death scene (Lesiak et al., 2014).

5.11.8 Methadone (Table 5.18)

5.11.8.1 Introduction

Methadone is a derivative of diphenylpropylamine. Drugs in this class have a general formula that looks quite different from the basic morphine molecule. But on closer examination it is apparent that methadone contains the same basic structures common to all morphine analgesics (see structure in Table 5.18 and Figure 5.30). Methadone is supplied as a racemic mixture, but almost all of the opiate activity derives from the *l* form. Unfortunately, the *d* form, while lacking any opiate activity, avidly binds to the hERG potassium receptor and may cause sudden death, particularly in women (Yang et al., 2010). There is good reason for supposing that many methadone deaths previously thought to be a result of respiratory depression were in fact the result of malignant ventricular arrhythmias induced by the *d* form of methadone (Eap et al., 2007).

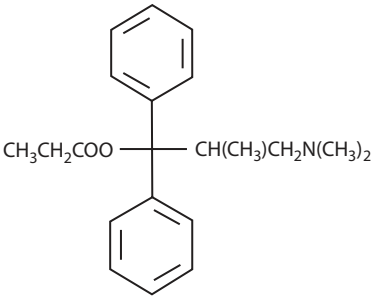
In the 1999 DAWN survey, only 643 methadone-related deaths were reported to the federal government, amounting to 5.5% of all reported narcotic deaths for that year (Kissin et al., 2000). That number has increased drastically in the last decade.

In the United States there has been a continuous increase in drug-related deaths that began in 1999, especially deaths involving opiates. In 1999 that number was 4030, but it increased to 15,597 in 2009 and 16,651 in 2010 (the last year for which statistics are available). In 2010, nearly 60% of the drug overdose deaths (22,134) involved pharmaceutical drugs. Opioid analgesics, such as oxycodone, hydrocodone, and methadone, were involved in about three of every four pharmaceutical overdose deaths (16,651), confirming the predominant role opioid analgesics play in drug overdose deaths in the United States today (CDC, 2013a).

Until 1999, the absolute number of poisoning death reports mentioning methadone was far fewer than deaths attributable to cocaine or other opioids such as oxycodone. Since 1999 the number has increased continuously and substantially. Between 73% and 80% of poisoning deaths mentioning methadone have been classified as unintentional (3701 such deaths in 2005), with an additional 11%–13% classified as being of undetermined intent, and 5%–7% as suicides (CDC, 2010).

In 1965 methadone was introduced for the treatment of heroin addiction. One of methadone's attractive features is its half-life, which can exceed 50 h, a property that makes outpatient management feasible. Since 1965 new agents such as *l*-α-acetylmethadol

Table 5.18 Physiochemical Properties and Pharmacokinetics of Methadone

Chemical names	6-Dimethylamino-4,4-diphenyl-3-heptanone
Physiochemical properties, structure, and form	Available as HCl salt CAS: 76-99-3 MW: 309.4 V_d: 3–5 L/kg p*K*a: 8.6 Protein binding: >70% 
Synonyms and brand names	Dolophine, Methadose, Physeptone, Eptadone, Mephenon, Metasedin, Symoron
Pharmacokinetic parameters	Bioavailability: 41%–95% C_{max}: Methadone 0.12–1.3 and 0.01–0.3 mg/L for ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and the steady state concentrations, 0.065–0.63 and 0.005–0.055 mg/L, respectively, after chronic methadone hydrochloride (mean, 60 mg; range, 10–225 mg)
Blood terminal elimination half-life	15–72 h, dependent on pH
Metabolism	Hepatic CYP3A4 metabolizes methadone in vitro, but in vivo CYP2D6 and CYP2C19 perform most of the conversion. Variants of *CYP2D6* and *OPRM1* gene predict methadone-related deaths
Urine excretion	Methadone: 5%–50% EDDP: 3%–25%
Postmortem artifacts	Moderately elevated in postmortem femoral blood
Interactions	*CYP3A4 inducers*: Barbiturates, carbamazepine, dexamethasone, efavirenz, felbamate, hypericum, nelfinavir, nevirapine, oxcarbazepine, phenytoin, phosphophenytoin, rifampin, risperidone, ritonavir, topiramate *CYP3A4 inhibitors*: Cimetidine, ciprofloxacin, clarithromycin, diltiazem, erythromycin, fluconazole, fluoxetine, fluvoxamine, grapefruit juice, josamycin, ketoconazole, nefazodone, norfloxacin, norfluoxetine, paroxetine, protease inhibitors, venlafaxine *CYP2B6 inducers*: Glucuronyltransferase (UGT), rifampin, UFT1A6, phenobarbital, quercetin, as well as numerous agrochemicals
Key papers	Meresaar et al. (1981), Nilsson et al. (1982), Inturrisi et al. (1987), de Vos et al. (1995), Foster et al. (1999), Rostami-Hodjegan et al. (1999), Ferrari et al. (2004), Rodriguez-Rosas et al. (2005), van de Kerkhof et al. (2008), and Bunten et al. (2010)

(L-LAAM) and buprenorphine have been introduced to replace methadone. Both of the new agents were thought to offer advantages over methadone. Instead, L-LAAM turned out to be a potent hERG channel blocker with serious proarrhythmic effects (Kang et al., 2003), even greater than those of methadone (Ehret et al., 2006). As a result, L-LAAM is no longer used in clinical practice. Buprenorphine is thought to be safer than methadone and much less likely to be abused, but it is also much more expensive than methadone and so methadone remains the drug of choice for the treatment of heroin addiction. Methadone is now also prescribed for cancer patients and for the management of patients with intractable pain (Sandoval et al., 2005). Depressed immune function is a common problem in

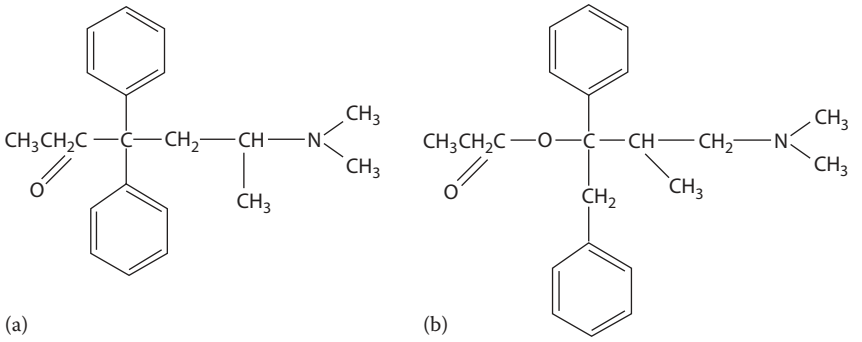


Figure 5.30 (a) Methadone and (b) propoxyphene. Even though it is not obvious, the methadone molecule contains the same basic structures as all the other morphine-related analgesics. Propoxyphene is a derivative of morphine.

heroin addicts, because chronic opiate exposure is known to be associated with down-regulation of G-protein–coupled opioid receptor gene expression in human lymphocytes (Toskulkao et al., 2010). There is a body of laboratory and clinical evidence strongly suggesting that methadone replacement therapy normalizes immune function (Alonzo and Bayer, 2002) but it is difficult to understand how one opiate could reverse the immune depression caused by another. If methadone does reverse long-term heroin-related immune suppression, it may have more to do with improved lifestyle and a decrease in the number of intravenous injections rather than with any direct effect of the drug itself (McLachlan et al., 1993; Radkowski et al., 1996). This possibility is reinforced by the recent observation that methadone, along with oxycodone and diamorphine, inhibits the production of IL-6 by IL-2-stimulated peripheral blood mononuclear cells (PBMCs) (Boland et al., 2014).

5.11.8.2 Epidemiology

In the United States, during the 4 years from 2004 to 2008, the estimated number of emergency room visits attributable to the nonmedical uses of prescription narcotics increased by 111%, rising from 144,644 in 2004 to 305,885 in 2008 (CDC, 2010). Almost all of the increase was attributed to the use of three prescription drugs: oxycodone, which rose by 152%; hydrocodone, which rose by 123%; and methadone, which rose by 73%. In total this amounted to 63,629 visits. The number of methadone-related visits in 2008 represents almost a 10-fold increase since the early 1990s (SAMHSA, 1992). Many of the methadone-related visits are attributed to the use of methadone that has been diverted from legitimate sources, though not a few cases seem to be the result of therapeutic misadventure.

Data from medical examiner offices located in a limited number of individual U.S. states also confirm that methadone mortality rates are rising. Of the three opioids responsible for most of the increase in the use of illicit prescription drugs, methadone-related deaths remain the most problematic. In the past, methadone-related deaths occurred chiefly among addicts already enrolled in methadone maintenance treatment (MMT) programs (Dole and Nyswander, 1965) and patients being treated for chronic pain syndromes, which is an increasingly common and accepted practice. However, as the numbers of both types of patient have increased, so too has the amount of drug reaching the black market, adding to the total numbers of methadone-related deaths and emergency room visits, particularly among naïve users.

Physicians expert in treating chronic pain syndromes, or in addiction medicine, are accustomed to managing patients with methadone. They are well acquainted with its properties and know how to use it safely and to maximal effect. But, except for these two groups, most physicians have little experience with methadone and remain unaware of its complex actions. Physicians have a recognizable tendency to think of all opioids as one drug group with more or less the same properties. However, methadone is not just another synthetic opiate. Physicians who practice forensic medicine and pathology often find that the unique properties of methadone can pose difficult diagnostic problems. The most important of these are discussed in what follows.

5.11.8.3 General Pharmacology

Population studies consistently demonstrate extreme interindividual variation and unpredictability in methadone kinetics. The *d* form of methadone exerts little narcotic effect but pure *l* isomer is expensive to make, so racemic mixtures are used in most countries. This makes results difficult to compare because the different methadone isomers have different binding affinities for μ-receptors (the *d* form has very little affinity). Almost all of methadone's respiratory depressant effects are the result of *l*-methadone binding to opiate receptors, but not all methadone-related deaths are a consequence of respiratory depression. Blockade of the hERG channel occurs with methadone use, which may result in a lethal arrhythmia. If blockade is more extreme, death from QT prolongation and torsades de pointes can occur, solely due to the binding of the hERG channel with *d*-methadone (Kristensen et al., 1995).

Still, much of the interpatient variability in the effects caused by methadone is more apparent than real: methadone and its principal metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) are chiral molecules, and the pharmacokinetics of each isomer is different. The plasma half-life and volume of distribution (V_d) of the minimally active, but much more dangerous, *d*-methadone are entirely different from those of the potent narcotic *l* form (53 vs.33 h and 3.8 vs. 4.0). Although these differences are quite real, the pharmacokinetic difference between the racemates seems to have little clinical impact.

Much of the data on clinical methadone pharmacokinetics were generated before chiral separation was routinely available and before the role of genetic polymorphism in methadone metabolism was recognized. CYP3A4 is the main enzyme involved in methadone metabolism, but other members of the P450 family also play a significant role (Bunten et al., 2010). Not all individuals produce the same amount of hepatic CYP3A4 (and this P450 enzyme can also be found in many other cells besides those of the liver) so that the rate of conversion of methadone to EDDP may vary widely from individual to individual. Smaller amounts of methadone are also oxidized by cytochrome P450 2B6 (CYP2B6). All of these enzymes are polymorphic and some of the polymorphic forms can greatly disrupt methadone metabolism.

The overlapping excretion rates and different degrees of tissue distribution largely explain why plasma concentrations in the living, and whole blood concentrations in the dead, are so highly variable. Reported therapeutic concentrations are said to range anywhere from 570 to >1000 ng/mL in stabilized methadone replacement patients taking 100–200 mg/day (Inturrisi and Verebely, 1972) and blood levels may be 0.1 to 2.0 mg/L in the dead (Wolf et al., 2004). The reported range of concentrations, both in the living and the dead, is so wide as to effectively render isolated measurement of total methadone concentration valueless in cause-of-death determinations, and not much more helpful in attempting to determine just what constitutes a therapeutic concentration in the living.

One might suppose that, because *l*- and *d*-methadone have such different pharmacokinetic properties, chiral separation might simplify postmortem interpretation. But when the hypothesis was finally put to the test, it turned out not to be the case, probably because there are so many other factors affecting patient response, including tolerance (Buchard et al., 2010). This is partly because both isomers, and isomers of the metabolite, undergo redistribution (Jantos and Skopp, 2013). Analysis was carried out in a small group of methadone-related deaths ($n = 6$). In three of the cases, only *l*-methadone was detected, even though a racemic mixture had been administered, making it fairly simple to rule out any role of the inactive *d*-isomer. The results suggest that determining the isomer ratios might be a useful exercise when confronted with a methadone-related death.

Some of the interindividual differences may be accounted for by the fact that the degree of binding is itself polymorphic (Meresaar et al., 1981); it is also influenced by other exogenous and endogenous factors. Not only is free *l*-methadone the only isomer that exerts any significant narcotic effect, the kidneys can only excrete free methadone. The methadone half-life measured in any given individual depends on how much of the methadone in their serum is free and how much protein bound, as well as how much is *l* form and how much is *d* form. These realities may explain why it has been shown, many times, that postmortem methadone measurements do not have any relationship to what concentrations were in life (Caplehorn and Drummer, 1999).

The typical pattern of distribution of methadone and EDDP enantiomers in 19 MMT patients was analyzed in a paper published in 2005. Mean values for total methadone concentrations were 110 ng/mL for *l*-methadone, 114 ng/mL for *d*-methadone, 1.2 ng/mL for *l*-EDDP, and 1.7 ng/mL for *d*-EDDP. The measured concentrations of free methadone were 37, 18, 0.3, and 0.4 ng/mL for *l*- and *d*-methadone and EDDP, respectively. Similar concentration distributions were observed all 19 patients studied (Rodriguez-Rosas et al., 2005), but interindividual concentrations of free methadone varied substantially and unpredictably.

Basic drugs such as methadone bind to the acidic alpha-1 acid glycoprotein, while acidic and neutral molecules bind to albumin. The distinction matters to methadone patients because many different medical disorders, especially liver disease, may alter serum albumin concentrations, as well as those of alpha-1 acid glycoprotein (AAG), and there may not be enough protein available to bind methadone. In healthy volunteers given methadone, the measured free fractions for racemic methadone, *l*-methadone, and *d*-methadone and a racemic mixture of *RS*-methadone were, respectively, 12.7% ± 3.3%, 10% ± 2.9%, and 14.2% ± 3.2% (mean ± SD). A significant correlation was observed between the binding ratio for *dl*-methadone and the total AAG concentration ($r = 0.724$; $p < 0.001$) (Eap et al., 1990). Thus it would not be an exaggeration to argue that the concentration of AAG predicts the occurrence of methadone toxicity every bit as much as absolute dose of methadone administered, if only because the amount of protein present determines the amount of methadone that is bound and the amount that is free.

Finally, there is the issue of the methadone to EDDP ratio. When urine EDDP concentrations are corrected for creatine concentration (*normalized*), the urinary methadone to EDDP ratio can be used as a measure of compliance (Johansen and Linnet, 2008). Some clinicians rely on this fact but others do not, and many feel that serum measurements, either of methadone or its metabolites, are not particularly useful either in managing patients or determining the cause of death (Plummer et al., 1988).

Polymorphic forms of AAG exist (Klaassen and Aleksunes, 2010). At least three main phenotypes of AAG have been identified and each one has a different affinity for methadone (Callaghan and Riordan, 1993). The effects of liver disease on AAG production are equally well recognized. Compared to normal individuals, circulating alpha-1-acid glycoprotein levels in cirrhotic patients and patients with chronic active hepatitis are reduced (Arima et al., 1977). As a result, when methadone is given, plasma concentrations of free methadone are increased, increasing the probability of an untoward event.

Individuals who abuse heroin tend to abuse other drugs as well. A German survey found that 51.7% of the entire population of MMT patients had a comorbid axis-I disorder, with a higher prevalence in females ($p = 0.05$). In this study, comorbid patients tended to have higher levels of benzodiazepines, alcohol, cannabis, and cocaine abuse, but not of heroin (Wedekind et al., 2010).

The exact number of heroin addicts who are cirrhotic, either as a consequence of chronic hepatitis C (virtually all intravenous drug takers in California are infected) or alcoholism, is not known, but a patient stabilized on methadone might quickly become destabilized after a bout of heavy drinking or some unrelated illness that exacerbated their preexisting liver disease, thereby decreasing production of AAG. This scenario is far more likely to cause fatal respiratory depression in long-term MMT patients than any more complex genomic scenario or hERG-associated arrhythmia.

Now that it is possible to routinely measure each of the methadone enantiomers simultaneously, their metabolism and interrelationships have become clearer. Taiwanese researchers showed that for each 1 mg of methadone administered per day, there was an average increase of 5.7 ng/mL of *l*-methadone, 4.6 ng/mL of *d*-methadone, 9.6 ng/mL of *l*-EDDP, and 1.9 ng/mL of *d*-EDDP (Wang et al., 2010). They also observed that there was a 10-fold interindividual difference in the concentrations of the *l* and *d* serum isomers. Even greater interindividual differences of up to 200-fold were observed for the *l* isomer of EDDP and 42-fold for the *d* isomer.

5.11.8.4 Methadone Toxicogenetics

5.11.8.4.1 hERG Nearly all of methadone's analgesic and respiratory depressant actions are produced by the free *l* form of the drug. The *d* form is minimally narcotic and basically inert, except for one unique feature: it binds to and blocks the cardiac hERG potassium channel, but only when it is present in very high concentrations—concentrations not seen in patients unless they are polymorphic for CYP2B6 (*slow metabolizers*). Toxic levels of *d*-methadone can accumulate in individuals who are slow metabolizers. When the hERG channel is blocked, heart muscle cells cannot depolarize properly, and the action potential is prolonged. The result is an acquired form of the LQT syndrome, and its occurrence explains why high-dose methadone treatment (the highest doses are generally seen in cancer and chronic pain patients) occasionally leads to QT prolongation and sudden death from a form of ventricular tachycardia known as torsades de pointes. See Figure 1.42 for an image of hERG's molecular structure; Table 5.19 lists hERG inhibitors.

The connection between high-dose methadone therapy, especially intravenous, and this syndrome was first recognized in 2002 (Krantz et al., 2002). It is now clear, however, that QT prolongation occurs even with relatively low-dose methadone therapy (less than 80 mg/day) (Huh and Park, 2010). In one study of methadone maintenance, where patients served as their own controls, racemic methadone was replaced with half the dose

Table 5.19 hERG Inhibitors

Inhibitors

Strong
- Protease inhibitors
  - Ritonavir
  - Indinavir
  - Nelfinavir
  - Saquinavir
- Some macrolide antibiotics
  - Clarithromycin
  - Telithromycin
- Chloramphenicol (antibiotic)
- Some azole antifungals
  - Ketoconazole
  - Itraconazole
- Nefazodone (antidepressant)

Moderate
- Aprepitant (antiemetic)
- Some calcium channel blockers
- Verapamil
- Diltiazem
- Some macrolide antibiotics
  - Erythromycin
- Some azole antifungals
  - Fluconazole
- Bergamottin (constituent of grapefruit juice)

Weak
- Cimetidine (H2-receptor antagonist)
- Buprenorphine (analgesic)
- Cafestol (in unfiltered coffee)

Unspecified potency
- Amiodarone (antiarrhythmic)
- Ciprofloxacin (antibiotic)
- Dithiocarbamate (functional group)
- Voriconazole (antifungal)
- Imatinib (anticancer)
- Mifepristone (abortifacient)
- Norfloxacin (antibiotic)
- Non-nucleoside reverse transcriptase inhibitors
  - Delavirdine
- Gestodene (hormonal contraceptive)
- Mibefradil (in angina pectoris)
- SSRIs
  - Fluoxetine/norfluoxetine
  - Fluvoxamine
- Star fruit
- Milk thistle

d-methadone for a 2-week period. The corrected QT interval (calculated using the Fridericia correction formula or QTcF) decreased by a mean of 7.8 ms after 2 weeks, only to increase by 9.4 ms 2 weeks later when the racemic formulation restarted (Ansermot et al., 2010). There may be several genetic explanations for this phenomenon. Methadone, in addition to interacting with hERG, also interacts with sodium channels (specifically Na(v)1.5), the same channel responsible for the cardiotoxicity associated with local anesthetics (Schulze et al., 2014). Another factor is potassium channel polymorphism, specifically the Lys allele at codon 897 of *KCNH2* (Hajj et al., 2014). When QT prolongation is seen with low-dose methadone therapy, it is a result of the confluence of many interacting factors.

Slow metabolizers do not have any difficulty in metabolizing *l*-methadone. Since hERG interactions with the *l* form of methadone do not occur, even a *slow metabolizer* will not experience QT prolongation and will not be at risk for sudden death. Of course, if *l*-methadone concentrations did increase to toxic levels, there still would be an increased risk for respiratory depression, which is why the dose of methadone administered is always increased slowly (Srivastava and Kahan, 2006). The American Association of Interventional Pain Physicians recommends initiating treatment with no more than 40 mg/day (Manchikanti et al., 2012); starting doses of 10 mg twice a day are not uncommonly used.

It matters a great deal, however, if a *slow metabolizer* is treated with racemic methadone, even in low doses. Then concentrations of *d*-methadone, which does interact with hERG, might rise sufficiently to cause sudden death (Eap et al., 2007). Interestingly, women are much more prone to the development of torsades de pointes than men. It has been estimated that 68% of cases of torsades de pointes, no matter the cause, occur in women

(Drici et al., 1998). This situation is somewhat counterintuitive, since QT intervals in normal women under the age of 50 years are shorter than in normal men (sex steroid hormones have an impact on cardiac repolarization) (Yang et al., 2010).

5.11.8.4.2 Opiate Receptor Polymorphisms A group of different genes acting together code for the opiate receptor (OPRMI), and it is thought that mutations within one or more of these genes increase the risk of becoming opiate dependent (Crettol et al., 2008). If a structurally abnormal μ-receptor is present, then methadone binding to the receptor is affected. Two genes in particular seem to be related to the effectiveness of MMT. If methadone cannot bind the abnormal μ-receptor, then MMT treatment will be ineffective. In one study, SNP variants in several candidate genes and regions were genotyped in a group of 116 MMT patients and the results correlated with the success or failure of MMT. Based on the number of positive urine tests, 83 of the patients were judged to be responders, and 33 were judged to be treatment failures. A positive association was observed between response to methadone and two variants in the gene *MYOCD* and the gene known as *GRM6* that is also involved in production of the μ-receptor (Fonseca et al., 2010).

Put another way, nearly one-third of the addicts enrolled in MMT programs seem to have fail treatment simply because they carried an abnormal form of the μ-receptor gene. This result also suggests that as many as one-third of the patients treated with methadone will not get adequate pain relief, even though they take normal doses of that drug. This anomaly also raises the interesting question of whether an individual carrying *MYCOD* and *GRM6* mutations might not be protected from overdose because the methadone cannot bind effectively to the μ-receptor. At this point, there is no answer.

5.11.8.4.3 β-Arrestin-2 Members of this protein class interact with G-coupled protein–type receptors, including catecholamine (Shenoy et al., 2008) and opiate receptors (Li et al., 2009). When β-arrestin-2 binds to a receptor, it decreases the response of that receptor so that the effect of methadone binding is diminished. Individuals who are polymorphic for the gene that produces this protein are resistant to MMT treatment and are much more prone to treatment failure (Oneda et al., 2010). Although there are no studies directly addressing the issue, it would follow that, given equal doses of methadone, individuals who were polymorphic for β-arrestin-2 would experience less analgesia and, perhaps, less respiratory depression. The frequency of these polymorphisms has not been established in any large population studies, but even the mere fact that such connections are known to exist should give the clinician pause before rejecting patient complaints of inadequate pain relief from the dosage of methadone they are receiving.

5.11.8.4.4 P-Glycoprotein The *MDR1* gene encodes the drug transporter P-gp. If too little of this glycoprotein is present, as is often the case in alcoholics with liver disease, then concentrations of free methadone, the only active form of methadone, rise, sometimes to lethal levels. One might speculate that an individual who was polymorphic for this protein might, like someone with liver disease, have increased concentrations of free methadone. Only one study has addressed the issue (Buchard et al., 2010). *MDR1* polymorphisms certainly exist, but the *MDR1* polymorphism did not seem to have any clinical significance. In this respect the P-gps are similar to members of the cytochrome P450 family—polymorphic forms exist but, with the exception of CYP2B6 and *d*-methadone, their existence has little or no clinical significance.

5.11.8.5 Clinical Considerations

Tolerant individuals may take doses of methadone that would induce fatal respiratory depression in naïve users, and so plasma concentrations, taken in isolation, are poor predictors of toxicity. In several independent studies, heroin addicts treated with methadone doses ranging from 180 to 260 mg/day have experienced no ill effects (Crettol et al., 2005). Plasma methadone values in hospitalized patients have been reported to range from 20 to 1308 ng/mL with a mean concentration of 451.4 ± 306 ng/mL (Loimer and Schmid, 1992).

Deaths have been reported in addicts who were just beginning methadone maintenance, receiving a mean dose of only 57 mg/day (Drummer et al., 1992). These deaths are thought to be a consequence giving too high a dose of methadone to nontolerant persons and/or advancing the methadone dosage too quickly, leading to fatal respiratory depression (Wu and Henry, 1990; Caplehorn and Drummer, 1999). This notion is supported by the fact that the relative risk of respiratory depression is nearly seven times higher in patients starting therapy than in untreated heroin addicts and 97.8 times higher than for methadone maintenance patients who have been in maintenance for more than 2 weeks. However, in light of what is now known, it seems likely that a significant proportion of these deaths occur in individuals who are CYP2B6 polymorphs, unable to metabolize the *d* form of methadone. And, of course, tolerance must also be considered when trying to evaluate any individual death, along with a detailed medical history that must be obtained before certifying the cause of death (Foster et al., 1999, 2000).

Individual responses to methadone also depend on a very long list of independent variables including sex, weight, use of concomitant medications, duration of methadone treatment, previous exposure to other opioids (and other drugs), as well as the pharmacogenetic factors discussed previously. If death does not occur at the initiation of therapy, and is not a consequence of illegal drug diversion, it almost certainly is the result of an underlying medical problem or of an unrecognized drug interaction. Cases of torsades de pointes seem to occur mostly in individuals receiving high-dose intravenous treatment (Romach et al., 1981; Inturrisi et al., 1987; Wilkins et al., 1997; Almehmi et al., 2004). Levels of AAG fluctuate depending on the underlying health of the individual. Another confounding factor is that AAG levels increase when there is significant stress. As stress levels rise, as they well might during abstinence, the amount of free (and therefore active) methadone decreases (Wilkins et al., 1997).

5.11.8.6 Metabolism and Pharmacokinetics

Information about the clinical toxicology of methadone is largely derived from studies of healthy volunteers given single doses of methadone or cancer patients injected with methadone intravenously. Studies of drug addicts suggest that measurements made in the chronically ill falsely underestimate methadone's terminal half-life and volume of distribution (Kristensen et al., 1996; Wolff et al., 1997). Naïve users take much longer to clear methadone from their circulation, which is one reason why they are at greater risk for overdose.

Some clinicians rely upon measurement of the plasma methadone-to-EDDP ratio as an indicator of safe and effective dosing. Various reports have placed the normal value for the methadone-to-EDDP ratio at between 18 and 22 (de Vos et al., 1996), depending on whether pure *l*-methadone or racemic methadone has been administered. The ratio seems to be much lower in postmortem blood samples (mean value of 13.6:1 in 38 methadone-related fatalities) (Karch and Stephens, 2000), probably because of redistribution (methadone has a very large volume of distribution, 6/7 L/kg), while that of EDDP has never been measured.

Table 5.20 Drugs Known to Interfere with CYP3A4 Metabolism

Strong Inhibitors	Moderate Inhibitors	Weak Inhibitors
Boceprevir, clarithromycin, conivaptan, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir/ritonavir, mibefradil, nefazodone, nelfinavir, posaconazole, ritonavir, saquinavir, telaprevir, telithromycin, voriconazole	Amprenavir, aprepitant, Atazanavir, ciprofloxacin, darunavir/ritonavir, diltiazem, erythromycin, fluconazole, fosamprenavir, grapefruit juice, imatinib, verapamil	Alprazolam, amiodarone, amlodipine, atorvastatin, bicalutamide, cilostazol, cimetidine, cyclosporine, fluoxetine, fluvoxamine, ginkgo, goldenseal, isoniazid, nilotinib, oral contraceptives, ranitidine, ranolazine, tipranavir/ritonavir, zileuton

Source: U.S. Food and Drug Administration, http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm080499.htm, last accessed September 10, 2014.
Note: This enzyme alone accounts for more than one-half of phase I drug metabolism.

Reported values for the terminal half-life of methadone are very wide, ranging from 13 to 58 h (Inturrisi et al., 1987; Goldstein and Herrera, 1995). In opioid addicts, methadone kinetics is best described by a single-compartment model. The volume of distribution is high (6.7 L/kg) and the clearance rate is low (3.1 mL/min/kg). The observed elimination half-life of 26.8 h in addicts appears to be substantially lower than earlier estimates had suggested (Wolff et al., 1993).

Methadone clearance is altered in the presence of other drugs. When alcohol and methadone are taken at the same time, the metabolism of each is enhanced and withdrawal symptoms can ensue (Tong et al., 1981). When methadone and cocaine are taken together, methadone plasma levels decrease, apparently because the cocaine accelerates methadone excretion (Tennant and Shannon, 1995). Because CYP3A4 activity is involved with the metabolism of so many drugs, numerous interactions are possible. Drugs that inhibit CYP3A4 metabolism can be expected to increase blood methadone concentrations. Some of the best-known CYP3A4 inhibitors include itraconazole, ketoconazole, clarithromycin, erythromycin, nefazodone, ritonavir, and grapefruit juice. Many of the drugs used to treat HIV are metabolized by CYP3A4 (Fromm et al., 1997; Iribarne et al., 1997; Heelon and Meade, 1999). Table 5.20 contains a complete list of known CYP3A4 inhibitors.

5.11.8.7 Maternal/Fetal Considerations

There is general agreement that infants born to mothers being treated with methadone are significantly less mature and lower in weight than control infants. More than half of all such infants can be expected to develop neonatal abstinence syndrome, and most stay in the hospital longer than infants not exposed to drugs (Kelly et al., 2000). However, there is also general agreement that the exposure of infants to methadone through their mothers' breast milk is minimal. Women using methadone for the treatment of opioid dependence should not be discouraged from breast-feeding. The benefits of breast-feeding largely outweigh any theoretical minimal risks (Glatstein et al., 2008). Still, the drug is not without effect. At peak methadone concentrations, fetuses displayed less motor activity, and the integration between heart rate and motor activity was attenuated. Maternal heart rate and skin conductance were unchanged, but methadone administration was associated with lower maternal respiratory rate and the occurrence of sinus arrhythmias, an indicator of increased parasympathetic tone (Jannsson and Dipietro, 2005).

During the first day of life, maternal plasma methadone levels correlate significantly with neonatal plasma methadone levels. It also has been observed that the severity of CNS signs of withdrawal correlates with the rate of decline in the infant's plasma methadone levels. In 21 neonates with symptoms of withdrawal, the mean maternal methadone level 16 h after delivery was 183 ± 118 ng/mL, while the mean plasma level in samples drawn from the infants at the same time was 26 ± 8 ng/mL. Methadone levels decreased in the infants at the average rate of 0.2 ± 0.3 ng/mL/h (Doberczak et al., 1993).

In a second study, blood and milk samples were obtained from 12 breast-feeding women who were taking methadone in daily doses ranging from 20 to 80 mg/day, and blood was obtained from 8 of their infants who were also observed for withdrawal symptoms. The mean (95% CI) milk/plasma ratio was 0.44 (0.24–0.64). Exposure of the infants (calculated on the assumption that the average milk intake was 0.15 L/kg/day and that bioavailability was 100%) was 17.4 μg/kg/day (10.8–24 μg/kg/day). The mean infant dose expressed as a percentage of the maternal dose was 2.79% (2.07%–3.51%). In seven of the infants, methadone concentrations were below the limit of detection, while one infant had a plasma methadone concentration of 6.5 μg/L (Wojnar-Horton et al., 1997). The other maternal/fetal consideration that cannot be ignored is childhood exposure. There have been instances where some mothers have attempted to sedate irritable infants with their methadone, with a predictably bad outcome (Kintz et al., 2005a). Other deaths have occurred in toddlers who find their parents' take-home dose of methadone (Inselman, 1971; Klupp et al., 2000; Palmiere et al., 2010).

The difficulty inherent in each of the case reports and small series that have been published is the very high rate of additional opioid and drug use by the mothers. Estimates suggest that nearly half of the MMT mothers are continuing to use other drugs, and it may not be possible to tease out which agent caused what complication (Delano et al., 2013).

5.11.8.8 Hospice and Pain Patients

Hospice patients and those with chronic pain syndromes are often maintained on very high doses of either morphine or methadone. A study of 13 terminally ill hospice patients disclosed an average steady-state methadone concentration of 1970 ng/mL (980–379 ng/mL) for the *l* isomer and 2720 ng/mL (550–3780 ng/mL) for the *d* isomer, and only half of these individuals had achieved adequate pain relief. At these very high levels, the apparent volumes of distribution for the *d* and *l* forms were 6.5 and 4.8 L/kg, respectively. The half-lives also differed: 53 h for the *l* form and 32 h for the *d* form. By comparison, when this same group was treated with morphine (mean dose 178 mg/day), the mean plasma morphine concentration was 290 ng/mL (111–750 ng/mL) (Auret et al., 2006). It should be apparent from these very high concentrations that tolerance occurs on a massive scale and any attempt at relating plasma concentration to outcome would be futile and misleading.

5.11.8.9 Routes of Administration

Methadone oral absorption is excellent and, when compliance is good, a high degree of correlation exists between the dose administered and plasma levels. Over the range of 3–100 mg, plasma methadone concentrations increase by 263 ng/mL for every mg of methadone/kg of body weight. A similar, nearly linear, concentration increase is also observed in saliva, although the peak levels are somewhat higher and the half-life somewhat longer (Wolff et al., 1992). In cancer patients, a 10 mg intravenous injection produced a peak

plasma level slightly above 500 ng/mL, falling to below 100 ng/mL at 1 h (Inturrisi et al., 1987). Blood and plasma concentrations after the less common routes of administration have not been measured.

5.11.8.10 Compliance Monitoring

The lack of venous access makes monitoring MMT patients difficult. When blood is available, the ratio of methadone to its principal metabolite is considered an effective measure. However, compliance can also be monitored by testing alternative matrices: urine, sweat, and hair. A study of established replacement patients found that methadone and EDDP were present in very high concentrations in the urine samples but that the methadone concentration in the hair samples was scattered in the range of 9.5–80.8 ng/mg, while EDDP was detected in the range 2–6.25 ng/mg. The methadone concentration in the sweat samples from the same group of patients ranged from 120 to 2160 ng/patch, with EDDP concentrations ranging from 25 to 535 ng/patch. It is noteworthy that none of the measurements in any of the individuals' hair or sweat correlated with the dose given, but the methadone/EDDP ratio in every case fell between 0.1 and 0.3, which is the same ratio that is observed in testing the blood of living replacement patients (Fucci and De Giovanni, 2007).

5.11.8.11 Autopsy Findings

Methadone maintenance patients are likely to have some cutaneous stigmata of past intravenous heroin abuse. They are also very likely to be infected with hepatitis C virus (HCV), even if histologic changes are not evident. Table 5.21 lists the most frequent abnormalities detected at autopsy in a series of 38 methadone-related deaths (Karch and Stephens, 2000). Many of the autopsy findings, such as terminal aspiration and pneumonia (15.6%), are known complications of intravenous opiate abuse. QT interval prolongation in hospitalized methadone maintenance patients is not rare, even if no anatomic changes are identifiable in the heart. Whether or not an arrhythmia will occur depends not only on cardiac anatomy but also the dose of methadone, the presence of cytochrome P450 inhibitors, the antemortem potassium level, and liver function (Ehret et al., 2006). If no EKG tracing is available to death investigators, the diagnosis of torsades de pointes cannot be made at autopsy, although gene resequencing might well disclose abnormalities

Table 5.21 Autopsy Findings in Methadone Users

Diagnosis	Number	Percent (%)
Track marks	13	34.2
Coronary artery disease	9	21.0
Cirrhosis	7	18.4
Pneumonia	6	15.7
Hepatic fibrosis	5	13.1
Fatty liver	4	10.5
Necrotizing fasciitis	4	10.5
Birefringent crystals	4	10.5
HIV	3	7.8

Source: Based on an analysis of 38 cases investigated by the Office of the San Francisco Medical Examiner, San Francisco, CA; Karch, S.B. and Stephens, B.G., *West. J. Med.*, 172(1), 11, 2000. With permission.

consistent with that diagnosis. If the metabolizer status were known, or if the decedent's DNA was resequenced, slow metabolizer 2B6 status could be considered a biomarker for torsades de pointes, but few medical examiners have the equipment required for such testing (Eap et al., 2007).

5.11.8.12 Postmortem Blood Concentrations

Because methadone has a very high volume of distribution, concentrations can be expected to rise after death. Indeed, fourfold increases have been reported (Levine et al., 1995; Milroy and Forrest, 2000). Postmortem methadone concentrations are also site dependent and, for unexplained reasons, the increases appear to be greater in men than women (Caplehorn and Drummer, 2002). In every published series methadone blood concentrations in fatal cases completely overlap those found in methadone maintenance program participants (Table 5.22). Similarly, levels in cases of overdose are indistinguishable from those in decedents where

Table 5.22 Postmortem Methadone Concentrations

Author	*n*	Year	Median (mg/L)	Range (mg/L)	Cause of Death
Buchard	90	2010	0.62	0.011–8.0	Not given (Buchard et al., 2010)
Albion	11	2010	0.41	0.2–3.0	Methadone (Albion et al., 2010)
Johansen	27	2008			Johansen and Linnet (2008)
R-methadone			0.47	0.005–2.27	Mainly overdose
S-methadone			0.34	<LOD–1.51	
Johnson	10	2008	0.41	0.006–1.23	Multiple causes (Johnson et al., 1990)
Chugh	22	2008	0.48	0.1–0.9	SCD vs. OD (Chugh et al., 2008)
Johansen	10	2008			Johansen et al. (2006)
R-methadone			0.41	0.006–1.235	Multiple causes
S-methadone			0.33	0-0.794	Multiple causes
Shields	176	2007	0.535	0.02–40	Multiple causes (Shields et al., 2007)
Wolf		2004			Wolf et al. (2004)
R-methadone	23		0.559	0.114–1.90	Methadone
S-methadone	42		0.411	0.050–1.90	Mixed OD
Pirnay	9	2004	0.352	0.208–1.04	Buprenorphine and methadone (Pirnay et al., 2004)
Gagajewski	96	2003			Gagajewski and Apple (2003)
R-methadone			1.17 (mean)	0.180–3.99	Methadone
S-methadone			0.65 (mean)	0.180–3.0	Not methadone
Caplehorn	33	2002	0.60	—	Methadone (Caplehorn and Drummer, 2002)
Perret	21	2000			Methadone (Milroy and Forrest, 2000)
Milroy	111	2000			Methadone (Milroy and Forrest, 2000)
R-methadone	55		0.435		Methadone
S-methadone	56		0.584	0.049–2.44	Mixed methadone
Karch	38	2000	0.957 (mean)	—	Mixed drug (Karch and Stephens, 2000)
Cooper	6	1998	0.32	0.09–0.42	Methadone (Cooper and Oliver, 1998)
Levine	15	1995	0.55	0.1–2.70	Trauma/drugs (Levine et al., 1995)
Drummer	10	1992	0.726(mean)	0.30–2.80	Mixed drugs (Drummer et al., 1992)

Note: This table lists the concentration ranges for nearly all reported studies to date. While reported median values fall roughly into the same range, the spread between highest and lowest reported doses is enormous.

death is due to trauma, where the presence of methadone was simply an incidental finding (Karch and Stephens, 2000; Milroy and Forrest, 2000; Gagajewski and Apple, 2003; Pirnay et al., 2004; Wolf et al., 2004). In a study of 38 decedents where methadone was detected, the mean blood methadone concentration was 975 ± 681 ng/mL, the mean blood EDDP was 253 ± 529 ng/mL, and the mean blood methadone-to-EDDP ratio was 13.6. Urine concentrations of methadone ranged from 5 to 6 mg/L and were the same in individuals where methadone was an incidental finding and in those where it was the cause of death. The mean blood methadone-to-EDDP ratio for the entire group of 38 patients was 13.5 ± 17.4, but the range was so wide, from 0.572 to 60, that determination of the ratio was of no diagnostic value (Karch and Stephens, 2000). Because of the overlap in observed concentrations, it makes no sense to speak of toxic or therapeutic ranges. As with other drugs, central samples are likely to show greater increases than samples taken from the periphery. However, if the testing facility is equipped to perform enantioselective methods, measurement of the methadone/EDDP and calculation of their *d:l* ratios might prove helpful as significantly higher MTD *d:l* ratios in femoral and heart blood were present in individuals having participated in an MTD maintenance program (Jantos and Skopp, 2013).

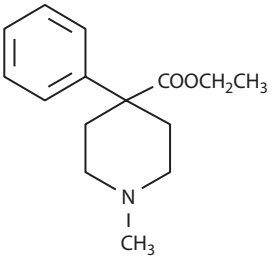
Attempts were made to determine whether small nucleotide polymorphisms existed in the *MDR1* gene responsible for encoding the drug transporter P-gp, but no significant difference could be found. Still, in spite of a succession of negative studies, a significant association between high methadone concentrations and the *CYP2B6*6* allele (i.e., slow metabolizer phenotype) has been identified and it is possible that methadone-related deaths may be partly determined by the *CYP2B6*6* allele. A significant correlation has also been found to exist between postmortem benzodiazepine concentrations and the *OPRM1 A118G* allele GA, at least in the cases of suspected methadone-related deaths (Bunten et al., 2011).

5.11.9 Meperidine (Table 5.23)

5.11.9.1 General Considerations

Meperidine metabolism can follow one of two different metabolic routes. The primary route is conversion to an inactive metabolite called meperidinic acid. This conversion is performed by liver carboxylesterases. Meperidinic acid is then conjugated and excreted by the kidneys. The pathway of most clinical significance is the *N*-demethylation by hepatic P450 enzymes. Meperidine is a synthetic phenylpiperidine derivative. Like morphine it binds to P-gp, an efflux transporter protein, in order to cross the BBB and exert its opioid effects. It was first developed as an anticholinergic agent and introduced into clinical medicine during the 1930s, created in the hope that it might be effective in the management of patients with gallstones and pancreatitis, where cholinergic-linked spasm of the sphincter of Oddi was thought to be the primary etiology of gallstone pancreatitis. In spite of widespread use during the 1970s and 1980s, there still are no studies directly comparing the effects of meperidine and morphine on sphincter pressure, no comparative studies in patients with acute pancreatitis, and no outcome-based studies comparing the effectiveness of meperidine and morphine in patients with acute pancreatitis. Many clinicians feel that morphine may be of more benefit than meperidine because it offers longer pain relief with less risk of seizures (Thompson, 2001). These observations explain why use of meperidine is falling into disfavor, not just among palliative care experts (Beckwith et al., 2002) but within the general medical community as well (Vermeulen et al., 1997; Raymo et al., 2007; Benner and Durham, 2011). Indeed, many institutions have limited or even banned its use,

Table 5.23 Physiochemical Properties and Pharmacokinetics of Meperidine

Chemical names	1-Methyl-4-phenyl-4-piperidine-carboxylic acid ethyl ester
Physiochemical properties, structure, and form	Available as hydrochloride, salt MW: 247.3 V_d: 2–4 L/kg p*Ka*: 8.6 Protein binding: 70% 
Synonyms and brand names	Pethidine, Demerol, Dispadol, Dolantine, Dolantin(a), Dolestine, Dolosal, Mefedina, Pethoid, and as an ingredient in Mepergan and Pamergran P10
Pharmacokinetic parameters	Bioavailability: 41%–60% C_{max}: 100 mg oral dose 0.17 mg/L at 1.3 h 100 mg IM injection 0.3 mg/L at 1 h $T_{1/2}$: Normal: 3–9 h IV: 3.93 ± 0.33 h IM: 3.25 ± 0.71 h PO: 3.49 ± 0.37 h Cirrhotic: 8.3–18.7 h Sickle cell: 2.5 mg/kg IV or IM; 3.18 h, 5.1 respectively
Blood terminal elimination half-life	Pethidine: 2–6 h Norpethidine: 15–30 h
Metabolism	Normeperidine and meperidinic acid, hepatic (CYP2B6 and CYP3A4, a minor contribution from CYP2C19) isoenzymes
Excretion	Pethidine: 5%–25% Norpethidine: 15%–25% Meperidinic acid glucuronide: 40% Normeperidinic acid glucuronide: 20%
Postmortem artifacts	Likely to be similar to methadone
Interactions	Selegiline and linezolid induce serotonin syndrome; propofol induces antimuscarinic syndrome, care with other serotonin-active drugs
Key papers	Mather et al. (1975), Mather and Tucker (1976), Stambaugh et al. (1976), Mather and Meffin (1978), Pond et al. (1981), Boreus et al. (1983), Yang et al. (1995a,b), Ramirez et al. (2004), Goodnick (2007), Snow et al. (2007), and Das et al. (2008)

both for fear of toxicity and the fact that many other agents are just as effective, but safer. Nonetheless, meperidine is still very widely used in PCA devices (Seifert and Kennedy, 2004). There is evidence that the use of meperidine has decreased significantly; it is no longer even mentioned in national or international drug monitoring studies.

5.11.9.2 Metabolism (Figure 5.31)

Although meperidine is mostly converted to inactive meperidinic acid, the pathway of most clinical significance is *N*-demethylation by hepatic P450 enzymes to form normeperidine (Eisendrath et al., 1987). Substantial racial and intraindividual variation exists due to polymorphisms of the P450 system, and these differences may explain some

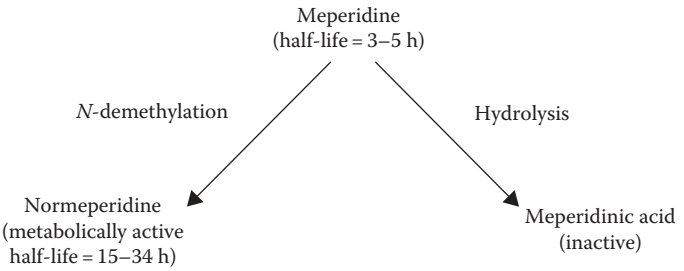


Figure 5.31 Meperidine metabolism. The metabolite normeperidine has about half the analgesic potency of meperidine and is also neurotoxic. The half-life of normeperidine is much longer than that of meperidine, and toxic levels may accumulate in individuals with renal impairment. (Adapted from McDonald, J.R., *Infect. Dis. Clin. N. Am.*, 23, 643, 2009.)

variations in the clinical effects observed (Braenden et al., 1955; Houghton et al., 1992). Normeperidine has modest analgesic properties, but more importantly, it is a potent CNS stimulant and can cause seizures and possibly even serotonin syndrome, even when given in low doses (Guo et al., 2009). Normeperidine is subsequently metabolized to normeperidinic acid and excreted by the kidneys. P450 microsomes also form small amounts of *N*-hydroxynormeperidine that also undergoes renal excretion (Dahlstrom et al., 1979). The existence of these polymorphisms may, perhaps, explain why there is very little correlation between meperidine blood levels and pain relief.

Meperidine is well absorbed by all routes of administration but absorption is significantly slowed in the presence of cirrhosis and renal disease. Kidney disease prevents the excretion of both meperidine and normeperidine, but the clearance of the metabolite decreases to a much greater degree than that of the parent, and toxic levels of normeperidine may accumulate. After oral administration there is extensive hepatic first-pass metabolism so that only 50%–60% of a given dose reaches the systemic circulation (Chan et al., 1975a; Edwards et al., 1982).

Another consequence of oral administration is that more normeperidine will be generated in the liver, increasing the possibility of neurotoxicity, especially when patients are given large doses or when a patient is chronically treated with the oral form of the drug (Pond et al., 1981). Interestingly, the probability of toxicity is greater in normal individuals than in cirrhotics; cirrhotics are less able to form normeperidine (Pond et al., 1981). The amount of normeperidine excreted in the urine is the same after intramuscular and intravenous dosing, but much greater after it is taken orally, another proof of increased hepatic metabolism (Stambaugh et al., 1976).

When healthy individuals are given a single intramuscular dose of meperidine, the half-life is approximately 3.5 h. In healthy volunteers, intravenous and subcutaneous administration produces peak concentrations within 10 min. However, after oral administration peak concentrations are not reached until 45 min later (Schmitt et al., 1994). Peak plasma concentrations vary from individual to individual and do not correlate in any predictable way with analgesic effects. Meperidine's volume of distribution increases with age (3.8 L/kg in the young, but up to 4.5 in the elderly, and over 5.0 L/kg in those with cirrhosis) and some diseases. Evidence suggests that chronic heroin users metabolize meperidine more slowly than non-drug users and, as a result, they may develop higher blood levels (Houghton et al., 1993). Because substance abuse is a frequent finding among trauma victims (McLeod et al., 1999), the potential for meperidine toxicity should not be ignored.

5.11.9.3 Toxicity

Clinical signs of toxicity are not uncommon but reports of death are rare. Two types of toxicity may be distinguished: direct respiratory depression secondary to excessive accumulation of the parent compound and indirect neurotoxicity where seizures and/or serotonin syndrome occurs secondary to the accumulation of normeperidine, the principal metabolite. Normeperidine exerts different effects than the parent compound, and the difference can have important clinical consequences. Like any other opiate, meperidine binds to the μ-receptors and causes respiratory depression comparable to that of morphine (to a lesser degree it also binds to 5-HT, norepinephrine, and dopamine transporters). Accordingly, meperidine's respiratory effects are reversed by naloxone (Lomenzo et al., 2005).

Normeperidine does not bind to μ-receptors (Reifenrath et al., 1980) and therefore it is not displaced from its receptors by naloxone. Instead, normeperidine accumulates in the CNS where it causes seizures and symptoms of 5-HT excess (Jiraki, 1992). Seizure activity is especially probable in patients with underlying renal insufficiency (especially the elderly and patients with debilitating cancers) and even those with sickle cell disease and liver disease (McHorse et al., 1974; Danziger et al., 1994; Marinella, 1997; Simopoulos et al., 2002). Even in the absence of preexisting disease, normeperidine tends to accumulate with chronic dosing (Kaiko et al., 1983).

Case reports suggest that daily doses of meperidine in excess of 400–600 mg are sufficient to produce normeperidine accumulation and toxicity, especially in susceptible individuals (Szeto et al., 1977). Hypertension, hyperpyrexia, tachycardia, and seizures are the expected result (Austin et al., 1980; Chan et al., 1987; Fairlie et al., 1999) when excessive doses of meperidine are given. Meperidine should not be used if an opioid analgesic is required in a patient who is also receiving a monoamine oxidase inhibitor (MAOI) (Barlow and Lewis, 1951; Chan et al., 1975b). An older case study found concentrations in breast milk samples were 36.2–314 and 0–333 ng/mL for meperidine and normeperidine, respectively. Preliminary results suggest that postpartum pain medication with meperidine results in considerable levels of both meperidine and its active metabolite, normeperidine, in breast milk (Quinn et al., 1986). However, there are too few data to be sure and, in any case, the drug has been removed from the market.

Meperidine is also a negative inotrope. Intravenous administration causes a significant, but transient, decrease in blood pressure. Meperidine is rapidly taken up by the myocardium but just what effect it exerts on the myocardium to cause decreased output is not clear (Upton et al., 1999). Like morphine, meperidine causes histamine release and exerts atropine-like effects on heart rate (Bowdle, 1998).

The postmortem toxicology of meperidine has been poorly studied. Meperidine blood concentrations measured in one series of six autopsies ranged from 4,300 to 12,000 ng/mL (Siek, 1978). Hepatic drug concentrations were twice the blood concentrations of patients who were intravenous users but only one-half the blood concentrations if the individual had taken the meperidine orally (Holmberg et al., 1982). Clinical evidence of normeperidine toxicity has been reported with concentrations ranging from 425 to 1900 ng/mL and normeperidine-to-meperidine ratios of 0.79–5.4 (Szeto et al., 1977). The importance of preexisting renal disease in cases of meperidine toxicity is once again emphasized by a case report describing a heroin addict with end-stage renal failure; he was found to have a meperidine blood concentration of only 60 ng/mL, while at the same time the normeperidine concentration was 3000 ng/mL (Jiraki, 1992).

5.11.9.4 Drug Interactions

Serotonin syndrome (or more accurately serotonin toxicity) can occur if meperidine is administered at the same time as a serotonin-active drug such as dextromethorphan, pentazocine, tramadol, any MAOI, or SSRI. Symptoms include confusion, fever, shivering, diaphoresis, ataxia, hyperreflexia, myoclonus, and occasionally diarrhea. Serotonin syndrome occurs when excess 5-HT is available within the CNS and, in particular, when concentrations at the 5-HT1A-receptor sites are elevated. Serotonin syndrome is something of a rarity although it may well be that it is underdiagnosed. Symptoms are usually mild and self-limited, although the occurrence of hyperthermia signals a poor outcome and requires aggressive cooling measures (Sporer, 1995; Upton et al., 1998; Weiner, 1999). If large doses of meperidine are given, normeperidine accumulates in the plasma (Koska et al., 1981). In normal individuals the terminal half-life of normeperidine is from 15 to 34 h, but in the presence of renal impairment, clearance may require 3 or 4 days, and toxicity, when it occurs, may be prolonged (Szeto et al., 1977). In control studies, only 5% is excreted unchanged in the urine, while more than 25% is excreted as meperidinic acid or normeperidinic acid.

5.11.9.5 Patient-Controlled Analgesia Devices

The most serious complication of PCA is respiratory depression. The evidence for respiratory depression in the literature is derived mostly from the results of intermittent pulse oximetry sampling of oxygen saturation and respiratory rate. In 2007 a study was undertaken of 178 patients receiving PCA, 12% of whom experienced desaturation and 41% of whom experienced bradypnea (respiratory rate < 10) lasting 3 min or more. One patient required *rescue* with positive pressure ventilation but none required naloxone for opiate reversal. Patients over 65 years of age and the morbidly obese were at greater risk for desaturation. Patients over 65 years of age were also more likely to have bradypnea, whereas the morbidly obese and patients receiving continuous infusions were less likely to have bradypnea (Overdyk et al., 2007).

These results have not been confirmed (or disproven) in any study undertaken since, but it is not an observation that should be readily dismissed when investigating the hospital deaths of patients with these devices.

The results of animal studies, and occasional anecdotal case reports, suggest that large doses of meperidine given via PCA devices may result in generalized seizures. McHugh (1999) described the case of a 35-year-old woman weighing 47 kg who underwent elective laparotomy. She requested Demerol PCA and 23 h postoperatively experienced a generalized seizure but recovered without adverse sequelae. The cumulative meperidine dose was 3000 mg and the normeperidine level was 1.8 μg/mL (McHugh, 1999). The author of the report suggested that large cumulative doses given via this route should be avoided.

Both intrathecal and epidural patient-controlled devices are popular in obstetric care. Both routes appear effective, with resultant plasma concentrations ranging from 450 to 700 μg/L, and, in general, the associated normeperidine concentrations were lower than those known to produce neurotoxicity (Jung and Reidenberg, 2005). In a study of 20 women treated with meperidine delivered via an epidural device, dosages ranged from 124 to 140 mg (mean 575 mg) over 48 h with great interindividual variation in meperidine concentrations, though in every case meperidine levels were less than 0.46 μg/mL (Ngan Kee et al., 1996). Intrathecal meperidine is also given to control intractable cancer pain. In one study

of 10 cancer patients, plasma concentrations of parent drug and metabolite both increased rapidly and in some patients normeperidine concentrations actually exceeded those of meperidine. Plasma meperidine concentrations ranged from below 60 to 1840 ng/mL. Normeperidine concentrations increased very rapidly with concentrations ranging from below 40 to 423 ng/mL (Vranken et al., 2005).

Still another case report described a 36-year-old man admitted to the hospital for surgical treatment of a pancreatic cyst. He developed contrast-related renal failure after a CT scan but, because of a history of morphine abuse, he was placed on meperidine PCA. On days 3–11 he received varying doses of meperidine, ranging from a low of 319 mg/day to maximum doses of 940 mg. He had myoclonus for 4 days and then developed a respiratory arrest. Plasma concentrations of meperidine and normeperidine, drawn during resuscitation, were 500 and 9000 ng/mL, respectively (Geller, 1993).

In a controlled study of meperidine given in conjunction with intra-abdominal surgery, normeperidine concentrations never exceeded 500 ng/mL. How much higher concentrations may go before seizures occur is not known (Hartvig et al., 1982). In general, when normeperidine toxicity has been reported, plasma concentrations have been between 1.5 and 3.0 mg/L (Stone et al., 1993). In the most recently published survey, patients with severe pain required, on average, 16.9 mg/kg/day (14.7–19.2 mg/kg/day), and the authors recommended 10 mg/kg/day as the maximum safe meperidine dose to be given by an intravenous PCA device (Simopoulos et al., 2002).

5.11.9.6 Postmortem Issues

No matter the age or the underlying medical condition, meperidine has a very large volume of distribution, though no one is exactly sure just what it is. The greater the V_{ss}, the more likely it is that postmortem redistribution will occur. It should never be presumed that concentrations measured in postmortem plasma samples bear any meaningful relation to blood concentrations at the time of death, especially if the sample being analyzed was obtained from a central source; it is almost certain that the postmortem concentrations of both parent drug and metabolite will be higher than in life (Drummer and Gerostamoulos, 2002; Jung and Reidenberg, 2005; Yarema and Becker, 2005). Animal data suggest that the distribution pattern of parent drug and metabolite are very different, making it impossible to speculate about the situation obtained in life. Table 5.24 shows the tissue distribution of meperidine and normeperidine in near-term rhesus monkeys whose mothers had been injected with a 1.25 mg/kg bolus containing equal parts of meperidine and normeperidine just prior to Cesarean delivery. The samples were harvested 30 min later. Note that normeperidine concentrations in the brain stem were 35 times higher than in the blood.

Table 5.24 Fetal (Rhesus) Tissue Distribution of Meperidine and Normeperidine

Organ	Meperidine	Normeperidine
Liver	6.39 ± 2.42	40.26 ± 7.66
Gallbladder	4.90 ± 2.10	21.26 ± 6.10
Brain stem	12.15 ± 3.69	24.59 ± 4.59
Kidney	36.01 ± 5.24	39.74 ± 6.04
Muscle	26.42 ± 4.32	28.59 ± 5.43
Serum	2.23 ± 0.09	0.67 ± 0.42

Source: Data adapted from Morrison, J.C. et al., *J. Perinatol.*, 8(1), 24, 1988.

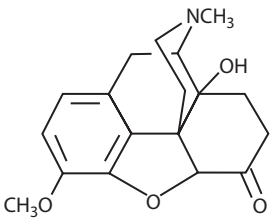
Concentrations of both parent drug and metabolite in muscle were essentially the same, confirming the belief of many that postmortem blood concentrations in muscle are quite similar to those found in blood. In vitro studies have shown that meperidine and normeperidine, like morphine, remain stable in stored frozen tissues. However, when tissue is stored in formalin, all three opiates leach into the storage solution; both tissue and storage fluid must be tested together to obtain a valid result (Xiang et al., 2001).

5.11.10 Oxycodone (Table 5.25)

5.11.10.1 History and Extent of Use

Oxycodone, a thebaine derivative, is a minor component of opium. Thebaine itself is not a narcotic but it is used as a precursor in the production of other opioids (Lenz et al., 1986). In 1917 oxycodone was first introduced as a pain reliever (Faulk, 1917), and it has been in use ever since (Figure 5.32). It has been administered intravenously (IV; Poyhia et al., 1991; Takala et al., 1997), intramuscularly (IM; Poyhia et al., 1992), intranasally (IN; Takala et al., 1997), subcutaneously (SC; Maddocks et al., 1996), rectally (Leow et al., 1995), epidurally (Backlund et al., 1997), and orally. Transdermal formulations have also been tested but oxycodone is not nearly as lipophilic as fentanyl and buprenorphine and the transdermal form of oxycodone is unlikely to have any clinical importance (Plummer et al., 1990).

Table 5.25 Physiochemical Properties and Pharmacokinetics of Oxycodone

Chemical names	(5α)-4,5-Epoxy-14-hydroxy-3-methoxy-17-methylmorphinan-6-one
Physiochemical properties, structure, and form	Available as hydrochloride, pectinate, and terephthalate salts CAS: 76-42-6 MW: 315.37 V_d: 3 L/kg p*K*a: 8.9 Protein binding: 45% 
Synonyms and brand names	Combunox, Dihydrohydroxycodeinone, Dihydrone, Dihydroxycodeinone, Dinarkon, Diphydrone, Endocet, Endonan, Endone, Eubine, Eucodal, Eutagen, Ossicodone, Oxanest, Oxicodona, Oxicoon, Oxycet, Oxycodeinone, Oxycodon, OxyContin, Percocet, Percodant, Roxicet, Supendol, Tecodin, Tylox
Pharmacokinetic parameters	Bioavailability: Oral: 50%–80% Rectal: 61.6% ± 30% Intranasal: 46% $T_{1/2\beta}$ (half-life): 3.4 h, range, 3–14 h C_{max}: 10 mg = 26 ng/mL at 68 min 15 mg = 36 ng/mL and 84 min 20 mg = 43 ng/mL at 51 min
Metabolism	*N*- and *O*-demethylation to noroxycodone and oxymorphone, catalyzed by CYP2D6, and conjugation
Urine excretion	Oxycodone and glucuronides 8%–14%
Postmortem artifacts	Likely to be similar to morphine
Interactions	SSRIs and perhaps other serotonin-active drugs, other opioids
Key papers	Leow et al. (1992), Takala et al. (1997), and Lalovic et al. (2006)

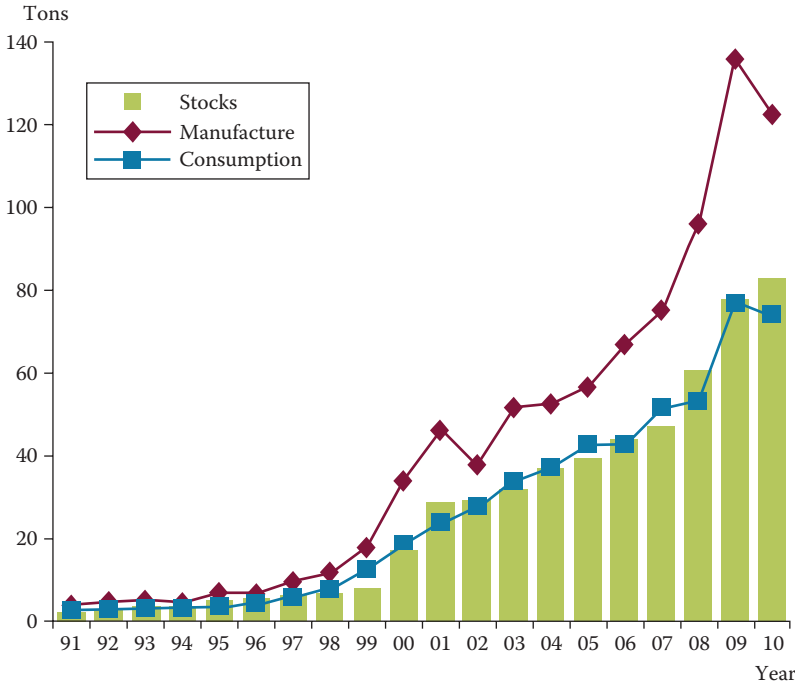


Figure 5.32 Global manufacture, consumption, and stocks of oxycodone, 1991–2010. (From INCB, Report of the International Narcotics Control Board for 2011, United Nations Publication, New York, 2012, Sales No. E.12.XI.5.)

In clinical studies of cancer patients, oral oxycodone and oral morphine appear to provide comparable pain relief. On a weight-for-weight basis, morphine administered intravenously is three times more potent than oral oxycodone (Zhukovsky et al., 1999).

OxyContin is the brand name of an oxycodone sustained-release formulation, first sold in 1996 by the Purdue Pharma LP. In 2000 reports of OxyContin abuse began to circulate and the extent of abuse only increased in 2004 when a generic version of OxyContin came to market. The Purdue formulation dominates the single-entity oxycodone market. According to the DEA, 7,185,000 prescriptions of single-entity oxycodone products were sold in 2000, and approximately 5.8 million (81.4%) of them were for OxyContin (DEA, 2006).

In the absence of what Purdue considered to be adequate federal monitoring, the company began its own large-scale surveillance program. The company's own results indicated that abuse of all oral prescription pain medications has become a problem over the last decade, with oxycodone formulations being the worst offender (followed by hydrocodone > other oxycodone > methadone > morphine > hydromorphone > fentanyl > buprenorphine) (Purdue Pharma, 2002; Cicero et al., 2005a,b). The White House Office of Drug Control Policy (National Office of Drug Policy, 2006; https://www.whitehouse.gov/ondcp) released similar findings suggesting that the situation is much worse than the DEA had suggested.

The FDA released a notice in July of 2012 observing that more people now die from accidental overdoses of OxyContin and other prescription opioid drugs than from heroin and cocaine combined. DEA intelligence reports suggest that the pattern is evolving, and that OxyContin is rapidly being replaced in popularity by oxymorphone (Opana®). In the fourth quarter of 2011, 226,000 prescriptions for Opana ER® were written in the United States, compared with

1,279,000 prescriptions for OxyContin (CDC, 2013a). The FDA released another statement in 2013 announcing that it had encouraged the makers of OxyContin to reformulate the drug in a form that made it more difficult to use (National Office of Drug Policy, 2013).

Some opioid abusers use prescription opioids or heroin or both, depending on the availability and the price of each drug. Heroin availability is increasing in many regions of both the United States and the E.U. Abusers tend to switch from oxycodone to heroin as they build tolerance to prescription opioids, leading to less and less of a "high." Conversely, they may well switch back to heroin because the price for black market OxyContin has increased so much. For example, oxycodone abusers with a high tolerance may ingest 400 mg of the drug daily (five 80 mg tablets) for an average daily cost of $400. These abusers could maintain their addictions with 2 g of heroin daily, at a cost one-third to one-half that of prescription opioids, depending on the area of the country and the purity of the heroin (U.S. Department of Justice, 2010).

5.11.10.2 Pharmacology

Unlike immediate-release oxycodone, the sustained-release form is absorbed biexponentially. During the first 37 min, 38% of the dose is released and blood levels rise rapidly. Following the first peak, a second peak occurs at 6.2 h, at which time the remainder of the drug is released. However, the bioavailability of both forms is the same.

The main known metabolic pathways for oxycodone are *O*-demethylation (cytochrome P450 2D6 [CYP2D6]) to oxymorphone and *N*-demethylation (CYP3A4 and CYP3A5 mediated) to noroxycodone (Weinstein and Gaylord, 1979; Poyhia et al., 1992). The latter predominates, and none of the metabolites appears to exert any important central effects in humans. Because concentrations of noroxycodone are higher in plasma and urine after oral administration, it seems likely that first-pass metabolism of oxycodone plays an important role. There are two phenotypes of CYP2D6 in the white population, with 5%–10% being PMs with decreased CYP2D6 activity and 25% having a decreased ability to metabolize oxycodone. Most oxycodone and noroxycodone is excreted unconjugated in the urine while oxymorphone is mainly excreted in the conjugated form (Poyhia et al., 1992). It has been suggested that oxycodone treatment might provide more pain relief if the dose was *personalized* by determining the patient's *CYP2D6* phenotype (Linares et al., 2014).

Oxycodone's volume of distribution is 2–3 L/kg, comparable to that of morphine, but it is more slowly eliminated. The $T_{1/2}$ is about 2–3 h after intravenous administration (Takala et al., 1997), 3 h after immediate-release formulation, and about 8 h after OxyContin administration (Mandema et al., 1996). Maximum plasma concentrations of oxycodone are reached within 25 min after intravenous administration, but not until 1.3 h after administration of immediate oral release formulations, and not until 2.6 h after giving the continuous-release formulation. Maximum plasma concentrations of oxycodone after immediate-release oxycodone are twice as high as those observed following an equivalent dose of continuous-release oxycodone (Mandema et al., 1996). There are important age and sex differences in metabolism: absorption is greatest in elderly women and lowest in young men, and it takes women 25% longer to clear the drug (Kaiko et al., 1996).

5.11.10.3 Drug Interactions

The conversion of fluoxetine, its nor-metabolite, and the conversion of most of the other SSRIs (Brosen, 1998; Fu et al., 2000), involves the P450 enzyme CYP2D6. All of the SSRIs seem to share the ability to both inhibit and induce 2D6 activity, with unpredictable results

(Daniel et al., 2006). It is not uncommon for cancer patients also to be taking antidepressants, which means that, deprived of the benefit of active metabolite formation, they may not achieve the same pain relief as those not taking SSRIs. Higher doses of oxycodone (with resultant higher blood concentrations of the parent compound) will be required. Conversely, SSRI–oxycodone interaction might explain an unanticipated episode of serotonin syndrome, which can be observed with the use of oxycodone (Karunatilake and Buckley, 2006).

The ability to metabolize oxycodone is reduced in patients with liver and/or kidney disease. Oxycodone pharmacokinetics has been studied in volunteers with end-stage liver disease, both before and after transplantation. Prior to transplantation, the median elimination half-life of oxycodone was 13.9 h (range, 4.6–24.4 h) (Tallgren et al., 1997). In patients with diminished renal function, the mean elimination half-life is prolonged because the volume of distribution is increased and clearance reduced (Kirvela et al., 1996). It follows that postmortem blood oxycodone concentrations in patients with renal or hepatic compromise are likely to be altered by postmortem redistribution.

Detection of oxycodone abuse is problematic for a number of reasons. Even under normal circumstances, oxycodone is cleared from the urine rapidly, and the window for detection (when using many immunoassay screening systems) is certainly less than 24 h. Genetic metabolizer status can also lead to surprising results. One case report described a man thought to be diverting his prescription oxycodone because his urine tests were consistently negative for the drug. Further investigation disclosed that the individual was a hypermetabolizer whose enzymes had been further induced by a concurrent prescription of rifampin; he was taking his medications but appeared not to be (Lee et al., 2006). No doubt, if additional investigation had not been undertaken, the patient would have been accused of criminal diversion.

5.11.10.4 Maternal/Fetal Considerations

Available data suggest that breast-feeding by oxycodone-using mothers is safe. In an older study, six postpartum women were administered either one or two capsules of oxycodone/acetaminophen every 4–7 h; maternal plasma oxycodone concentrations of 0.014–0.035 mg/L were associated with milk concentrations of <0.005–0.226 mg/L. The average milk to plasma concentration ratio was 3.4, but there were large variations in the ratio, and peak milk concentrations occurred 1.5–2 h after the initial dose (Marx et al., 1986). A case report describing the death of a 10-month-old child who experienced a cardiac arrest is of particular interest. It was written in 2004 before the polymorphic nature of the P450 enzymes was truly appreciated (Levine et al., 2004). The infant's autopsy was said to be unremarkable (though there is no mention of channelopathy testing or even microscopic examination of the heart, which can only mean that the autopsy was incomplete). The only remarkable finding was said to be the detection of oxycodone in the postmortem specimens: the blood and liver oxycodone concentrations were 0.6 mg/L and 1.6 mg/kg, respectively. The presence of the drug was arbitrarily attributed to breast-feeding and the medical examiner ruled homicide as the cause of death. The authors of the case report used the information provided in the earlier case study and concluded that total exposure could not have been nearly so great. Even if the original report had understated oxycodone excretion by an order of magnitude, the child's maximal exposure would have been on the order of 1 mg/feeding, an unlikely cause of death.

Most recently, researchers studied 50 breast-feeding mothers who were taking oxycodone. They collected blood and breast milk samples and measured oxycodone levels

at 24 h intervals afterwards. Forty-one neonates had additional blood samples taken at 48 h. Oxycodone was noted to be present in the milk of all mothers who had taken any dose in a 24 h period, and there was significant correlation between maternal plasma and milk levels. Over the subsequent 48 h, the relationship between plasma and milk levels weakened considerably ($R(2) = 0.59$) and there was a larger range of M:P levels with evidence of persistence of oxycodone in the breast milk of some mothers. Oxycodone levels up to 168 ng/mL were detected in breast milk (20% > 100 ng/mL), but oxycodone was detected in the plasma of only one infant. The measurements suggest that breast-fed infants may receive >10% of a therapeutic infant dose given to the mother but are at minimal risk because the intake of breast milk during the study period would have been quite small (Seaton et al., 2007).

5.11.10.5 Postmortem Issues

Little is known about the postmortem toxicology or pathology of oxycodone-related deaths, but it is clear that the drug undergoes extensive redistribution. In Anderson's study of 36 medical examiner cases, some individuals displayed large concentration differences between central and peripheral blood while others did not. The tissue distribution ranges of oxycodone in the 36 case examples were heart blood 0.12–46 mg/L (36), femoral blood 0.10–13 mg/L (35), liver 0.11–6.1 mg/kg (16), urine 2.5–122 mg/L (22), bile 0.19–49 mg/L (15), vitreous 0.24–0.82 mg/L (6), and gastric 0.06–119 mg total (21) (Anderson et al., 2002). No unique pathologic findings are recognized, but "ghost" pills (the matrix containing timed-release oxycodone) can occasionally be identified in the gastric contents. If, as is rarely the case, oxycodone is pulverized and injected, the possibility for angiothrombotic arteriopathy exists, and birefringent crystals may be detected in the lungs, retina, or kidney.

One case report described a schizophrenic woman with a massive oxycodone overdose (4000 mg based upon pill count); her blood level was 2600 ng/mL just after arrival in the emergency room. She was resistant to naloxone treatment and required three days of ventilator support before making a complete recovery (Schneir et al., 2002).

In a study of 9194 oxycodone-related decedents, most of them polydrug abusers, femoral blood concentrations were between 600 and 1400 ng/mL, with a mean of 900 ng/mL. The differences between blood concentrations in cases where oxycodone was the cause of death and cases where it was an incidental finding were sufficiently wide to suggest that, concerns about postmortem redistribution aside, postmortem blood concentrations of less than 600 ng/mL are an unlikely cause of death (Drummer et al., 1994). This assumption seems to have been borne out by the results of the Purdue Pharma studies of oxycodone-related deaths: in cases where multiple drug use was identified, the mean oxycodone concentration was 0.93 μg/mL ($n = 167$), but when oxycodone was the only drug identified, the mean concentration was between 1.55 and 1.70 μg/mL.

Two other large case series have been reported. Neither refers to any specific associated pathologic alterations, although a nexus between oxycodone use and thrombocytopenia has been suggested by research from the Centers for Disease Control (CDC, 2013b). Spiller (2003) analyzed postmortem blood in 24 cases where oxycodone was considered the sole cause of death. Mean and median postmortem oxycodone blood concentrations were 1.23 and 0.43 mg/L, respectively. The range was 0.12–8.0 mg/L, with more than half of the cases having blood concentrations of ≤0.5 mg/L (Spiller, 2003). In 2005 another study of 172 consecutive oxycodone deaths was published. It included 18 cases where death was attributed solely to oxycodone toxicity, 117 where death was attributed to combined

drug toxicity, another 23 to trauma, 9 to natural causes, and 5 to another drug or drugs. The postmortem blood concentrations of oxycodone overlapped among the groups. The mean blood oxycodone concentration among the cases of pure oxycodone toxicity was 0.69 mg/L, essentially the same as the combined drug toxicity group (0.72 mg/L) and the trauma group (0.62 mg/L). Concentrations were lower in cases of death attributed to natural causes and in those attributed to another drug or drugs (mean each 0.087 mg/L). Benzodiazepines were detected in 96 of the cases and cocaine in 41. The most frequently encountered benzodiazepine was alprazolam (Wolf et al., 2005). This overlap in drug concentrations has been confirmed in other recently published studies (Baker and Jenkins, 2008).

Finally, there is evidence that vitreous humor and postmortem blood concentrations are strongly correlated. The average vitreous humor/blood ratio was 1.16; it ranged from 0.12 to 3.26. However, unexplained disparities between vitreous fluid and blood oxycodone concentrations were seen in a few cases (Knittel et al., 2009).

5.11.11 Oxymorphone (Table 5.26)

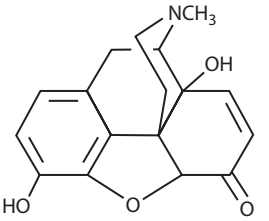
5.11.11.1 Pharmacology

Oxymorphone is an oxycodone metabolite but it is now sold in the United States under the name Opana and is a popular drug of abuse. It has a greater affinity for the μ-receptors and is nearly nine times as potent as morphine (Kalso, 2005). For many years it was sold in the United States (as Numorphan) for parenteral injection or use as a rectal suppository. Opana recently became available as an immediate- and extended-release oral formulation. Unlike most other opiate agonists (except tramadol), oxymorphone is not metabolized by the P450 system, though it is metabolized in the liver, either by glucuronidation to form inactive metabolites or by reduction, in which case some of the metabolites are psychoactive. This difference may explain why oxymorphone is displacing oxycodone on the illicit market—users claim that it produces more euphoria. Very little research has done on this drug.

Oxymorphone bioavailability is poor when taken orally, probably less than 10%. After oral administration it reaches peak concentrations in 30 min but even more quickly if liver or kidney disease is present, or if there is food in the stomach. Alcohol ingestion causes increased absorption. If the timed-release formulation is taken with alcohol, C_{max} can increase by as much as 100%. When there is food in the stomach, bioavailability is closer to 50%. The half-life is 7–10 h. Excretion and detectability studies have not been performed in humans. Much higher peak levels occur in the elderly but the mechanism is not understood and the drug should be given to the elderly only with great caution (Anon., 2007).

A minor pathway for the biotransformation of morphine to hydromorphone has been identified in humans. Urine specimens from chronic pain patients treated exclusively with high daily doses of morphine (n = 34) or hydromorphone (n = 26) were analyzed for oxymorphone, hydromorphone, and morphine (limit of detection, 25 ng/mL). Consistent with earlier reports, hydromorphone was detected in patients treated with high-dose morphine, but not in those treated with low doses. The ratio of hydromorphone to morphine ranged from 0.2 to 2.2. Oxymorphone was not detected in any specimen from high-dose morphine or high-dose hydromorphone patients. Thus, while small amounts of morphine may be converted to hydromorphone, none is converted to oxymorphone (Cone et al., 2008). It appears that oxymorphone is not a metabolite of morphine or hydromorphone, though it is an established metabolite of oxycodone.

Table 5.26 Physiochemical Properties and Pharmacokinetics of Oxymorphone

Chemical names	(5α)-4,5-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-one
Physiochemical properties, structure, and form	Available as HCl salt CAS: 76-41-5 MW: 301.3 V_d: 3 L/kg p*K*a: 8.5, 9.3 
Synonyms and brand names	Oxymorphone, Oxydimorphone, Numorphan (injectable, suppository), Opana oral tablets
Pharmacokinetic parameters	Oral bioavailability < 10%, but radically increases in the presence of food or alcohol $T_{1/2\beta}$ (half-life): Intravenous: 1.3 ± 1.07 h Oral: 5 mg = 11.30 ± 10.81 h 10 mg = 9.83 ± 5.68 h 20 mg = 9.89 ± 3.21 h C_{max}: 5 mg = 1.1 ng/mL 10 mg = 1.9 ng/mL 20 mg = 4.4 ng/mL
Metabolism	6-Hydroxy-oxymorphone or glucuronidation to form inactive metabolites; is also a metabolite of oxycodone
Excretion	Parent drug: <10% Oxymorphone glucuronide: 13%–82% 6-Hydroxy metabolite: 0.1%–3%
Postmortem artifacts	Unknown but likely to be similar to morphine
Interactions	CNS depressants
Key papers	Cone et al. (1983) and Adams et al. (2005)

5.11.11.2 Pharmacokinetics

A randomized three-way crossover study analyzed the effect of the immediate-release (IR) tablet following single- and multiple-dose administration in healthy volunteers (Adams et al., 2005). Following a single dose of 5, 10, or 20 mg, the immediate-release oxymorphone maximum plasma concentration (C_{max}) was 1.1, 1.9, and 4.4 ng/mL, respectively. Steady state was achieved within 3 days of 6-hourly administration. The median T_{max} was 0.5 h for all single doses of oxymorphone and, at steady state, the terminal elimination half-life ($T_{1/2}$) was approximately 7.3–9.4 h.

LC-MS/MS was used to study plasma concentrations in 19 healthy volunteers who were each given one 40 mg extended-release tablet. Blood samples were collected over 48 h. Concentrations peaked at roughly 4 ng/mL after hours, falling to under 1 ng/mL at 16 h (Figure 5.33) (Zha and Shum, 2012). LC-MS/MS was used to study urinary excretion. In a study of >30,000 samples, oxycodone excretion into urine was found to have a geometric mean of 1.93 mg/g of creatinine and oxymorphone had a value of 0.41 mg/g of creatinine. The greater the oxycodone concentration, the smaller the amount of oxymorphone excreted. This suggests that oxycodone metabolism is saturatable.

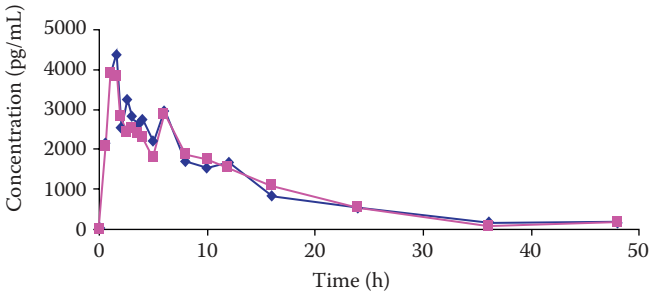


Figure 5.33 Plasma oxymorphone concentration after administration of one 40 mg extended-release tablet. (From Zha, A. and Shum, L., *J. Chromatogr. B: Anal. Technol. Biomed. Life. Sci.*, 902, 116, 2012. With permission.)

In the same study, urine samples containing oxycodone, but no oxymorphone, indicated that the proportion of PMs was 2.4% ± 2.1% in the population being studied. Conversely, the proportion of UMs in that same population was estimated to be 1.8% ± 1.1%. Samples with concentrations of oxycodone above 10 mg/g of creatinine showed a subpopulation of subjects with metabolic ratios roughly 100-fold less than urinary oxycodone with a geometric mean of 1.93 mg/g of creatinine and oxymorphone had a value of 0.41 mg/g of creatinine (Yee et al., 2012).

At autopsy medical examiners must seriously consider the causal role of this opiate when significant amounts of alcohol are detected in the blood or vitreous, particularly in the opiate naïve. When oxymorphone was only given by injection, there was little danger that the patient would take the drug with alcohol. Now that danger exists and it makes the possibility of unintentional overdose much more likely.

5.11.12 Propoxyphene (Table 5.27)

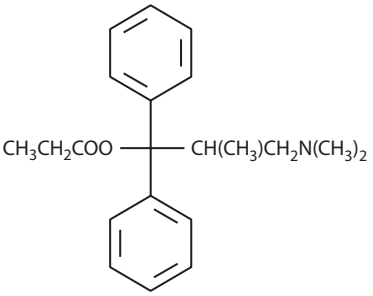
5.11.12.1 General Considerations

Propoxyphene was withdrawn from the U.S. market in early 2011, though it still remains in use in some parts of the world. The FDA banned sales because it was concerned, among other things, about the possibility that use of the drug might make patients develop torsades de pointes (Figure 5.34). Propoxyphene is a methadone derivative but, unlike methadone, it is a relatively weak μ-agonist and has only mild analgesic properties. In the past, propoxyphene overdose was common and the drug was associated with large numbers of fatalities (Soumerai et al., 1987). Propoxyphene is particularly toxic because, in addition to exerting the usual respiratory depressant effects common to all μ-agonist narcotics, its principal metabolites are local anesthetics with potent membrane-stabilizing activity.

Early formulations of propoxyphene contained a pellet of pure propoxyphene inserted at the end of a capsule containing aspirin or acetaminophen. It was a simple matter to remove the pellet and dissolve it in water for injection. That formulation has not been produced for many years.

Propoxyphene-related deaths are more common in Europe, particularly in Scandinavia, though there seems to be wide variation from country to country and city to city, and the drug has been withdrawn from the market in the United Kingdom, where it was known as co-proxamol. During the 6 years following the withdrawal of co-proxamol in the United Kingdom, there was a major reduction in poisoning deaths involving it,

Table 5.27 Physiochemical Properties and Pharmacokinetics of Propoxyphene

Chemical names	[*S*-(*R*,*S*)]-α-[2-(dimethylamino)-1-methylethyl]-α-phenylbenzenethanol propanoate ester
Physiochemical properties, structure, and form	Hydrochloride and napsylate salts CAS: 469-62-5 MW: 339.48 p*K*a: 6.3 V_d: 16 L/kg Protein binding: 78% 
Synonyms	Algafan, Antalvic, *d*-propoxyphene, Darvocet, Darvon, Darvon-N, Deprancol, Depromic, Dextropropoxyphene, Dextropropoxyphene-M, Dextroproxifeno, Dolene, Dolocap, Doloxen, Doloxene, Erantin, Femadol, Harmar, Kesso-Gesic, Propacet, Prophene 65, Propoxychel, Propoxyphene HCl, Proxagesic
Pharmacokinetic parameters	Bioavailability: 30%–70% C_{max}: 17–37 ng/mL (mean 23) after 130 mg orally T_{max}: 1–2 h
Blood terminal elimination half-life	Propoxyphene: 4–24 h Norpropoxyphene: 24–34 h
Metabolism	*N*-demethylation, de-esterification, hydroxylation and conjugation, CYP3A4 responsible for formation of norpropoxyphene
Urinary excretion	Propoxyphene: 1% Norpropoxyphene: 13%
Postmortem artifacts	Large increases in blood concentration postmortem: can be 10-fold even in peripheral blood
Interactions	*CYP3A4 competitors*: Carbamazepine, acetazolamide, macrolide antibiotics, isoniazid, metronidazole, verapamil, diltiazem, cimetidine, and some antidepressants
Key papers	Verebely and Inturrisi (1973), Hardman and Limbird (2001), Somogyi et al. (2004), and Barkin et al. (2006)

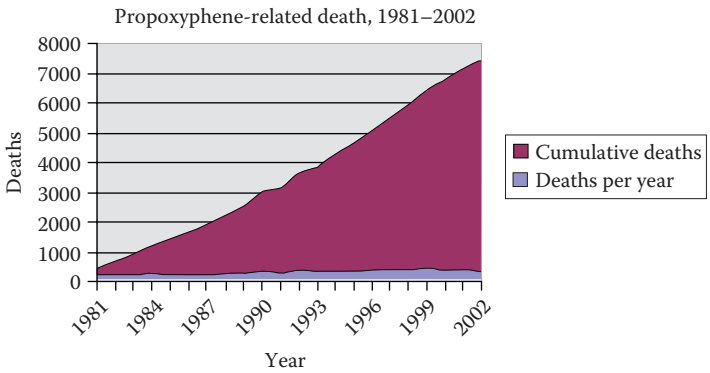


Figure 5.34 Trends in propoxyphene-related deaths; estimate prepared for FDA commission that ultimately removed the drug from the market.

without any apparent significant increase in deaths involving other analgesics (Hawton et al., 2012). The most recent papers on drug use in Scandinavia no longer even mention propoxyphene as a problem drug (Steentoft et al., 2005), nor does the current edition of the UN's World Drug Report even discuss the drug. Sporadic reports suggest that some propoxyphene formulations are still injected in America, but the origin of the propoxyphene used is not known.

5.11.12.2 Metabolism and Pharmacokinetics

Propoxyphene is absorbed rapidly from the gastrointestinal tract (Giacomini et al., 1980; Young, 1983; Flanagan et al., 1989). Peak plasma concentrations occur within 1 to 2 h after a single oral dose. It then undergoes extensive first-pass metabolism in the liver. In healthy volunteers, peak propoxyphene levels after a single 65 mg dose ranged from 260 to 900 ng/mL, with a mean of 590 ng/mL (Flanagan et al., 1989). In this same group of volunteers, the drug's half-life ranged from 6.4 to 26 h, with a mean of 13 h. Simultaneous measurements of nordextropropoxyphene showed peak levels that were much higher, ranging from 510 to 2140 ng/mL, with a mean of 1950 ng/mL. This may explain some of propoxyphene's toxicity, since it is the metabolite that is cardiotoxic. The metabolite also has a longer half-life, with a mean value of 22.2 h.

Propoxyphene is oxidized by CYP3A4 to form norpropoxyphene (Somogyi et al., 2004). At the same time, it appears that propoxyphene may be a CYP2D6 inhibitor, setting the stage for even more possible drug interactions. A recent case report described the occurrence of severe bradycardia in a 48-year-old man taking propoxyphene and metoprolol (which is metabolized by CYP2D6) (Marraffa et al., 2006). If propoxyphene is coadministered with other drugs that compete for CYP3A4, such as carbamazepine, acetazolamide, macrolide antibiotics, isoniazid, metronidazole, verapamil, diltiazem, cimetidine, and some antidepressants, then potentially dangerous plasma elevations of other drugs could result (Spina et al., 1996).

No metabolic differences between the sexes are detectable but age has definite effects on propoxyphene metabolism. The half-life of dextropropoxyphene in the young is only 13 h but it rises to over 35 h in the elderly. Similarly, the half-life for norpropoxyphene in young adults is approximately 22 h, rising to over 40 h in the elderly (Flanagan et al., 1989). Age-related changes in metabolism are not unique to propoxyphene. They can occur with almost any drug that undergoes hepatic oxidation followed by renal excretion. Excretion can be prolonged in individuals with liver impairment because first-pass oxidation is reduced in older individuals, and concentrations of propoxyphene in the circulation increase as a consequence.

Propoxyphene-induced respiratory depression is readily reversed by narcotic antagonists, but myocardial depression, which is a much more likely cause of death, is not reversed because it is not mediated by μ-receptors. Rather, it is the result of norpropoxyphene's effects on the myocardium. Norpropoxyphene accumulates in cardiac tissue, where it blocks not only the inward sodium current but also the potassium currents (Ulens et al., 1999). The situation has not been investigated in detail, perhaps because the drug is no longer widely used, but low drug concentrations (5 μmol/L) facilitate hERG currents while higher drug concentrations block hERG currents leading to an uncertain effect on cardiac depolarization (Ulens et al., 1999).

Whatever the role of hERG channel activation, norpropoxyphene disrupts the orderly sequence of myocardial depolarization, conduction is delayed, and the QT interval is

dispersed (Hantson et al., 1995; Karunakara et al., 2003). Myocardial contractility is also decreased, causing cardiac output and blood pressure both to drop. Neither treatment with β-adrenergic agents nor pacing has proven very effective (Whitcomb et al., 1989; Wu et al., 1997b; Ulens et al., 1999).

It is believed that when propoxyphene is coadministered with ethanol, first-pass hepatic transformation is decreased and higher blood concentrations of propoxyphene result (Oguma and Levy, 1981). The importance of an ethanol–propoxyphene interaction is difficult to assess, but ethanol is a frequent finding in cases of propoxyphene-related deaths. In the case series reported from Sweden, where propoxyphene was detected in 7.5% of forensic autopsies ($n = 1782$), ethanol was simultaneously detected in less than one-quarter of all cases (Jonasson et al., 1998). Clearly, co-ingestion of ethanol is not required to cause fatalities (Koski et al., 2003) but it may well facilitate them.

5.11.12.3 Tissue Distribution

Propoxyphene is highly lipid soluble, and large amounts are sequestered in fat tissue. Propoxyphene-related fatalities were frequent during the mid-1970s and concentrations at autopsy have been reported in hundreds of cases (Worm, 1971; Adjutantis et al., 1974; Finkle et al., 1976; McBay, 1976; Christensen, 1977; Finkle et al., 1981; Caplan et al., 1985; Jonasson et al., 1998). In the past, it was believed that serious toxicity was associated with levels greater than 1 mg/L, and that fatalities were associated with levels of over 2 mg/L. But, as with all opiates, tremendous overlap exists—fatalities have occurred at much lower concentrations, and higher values have been observed as incidental findings.

Quantitative postmortem measurements are, in general, unreliable, and probably none more than propoxyphene. Measured concentrations depend entirely on the area of the body from which the blood samples are drawn. This variability was dramatically illustrated by a study completed in the early 1990s (Yonemitsu and Pounder, 1992). Multiple blood and tissue samples were obtained from four decedents who had died of propoxyphene poisoning. A second and third set of samples was obtained after 24 and 48 h had elapsed. In every case, the lowest blood concentrations were observed in peripheral blood samples. When the levels in the peripheral blood measured 3.5 mg/L, the concentration in the aorta was 1.9 g/L, nearly 55 times higher! When blood was drawn from the pulmonary artery (PA), the propoxyphene concentration increased twofold at 24 h and threefold at 48 h.

Given the enormous variations that have been demonstrated in cadaver blood, drawing any conclusions from quantitative propoxyphene levels is unwise and unjustified. The same caveat applies to measurements made with muscle and other tissues. Analysis of these tissues is certainly a valid way to demonstrate the presence of propoxyphene, but results cannot be assumed to reflect plasma values at the time of death (Langford et al., 1998). Since death is more likely to be the result of metabolite cardiotoxicity than propoxyphene-induced respiratory depression, it hardly makes sense to measure propoxyphene without, at the same time, measuring concentrations of norpropoxyphene, but even that approach is unlikely to prove useful because norpropoxyphene is a more polar molecule than propoxyphene and, therefore, would be expected to have a lower volume of distribution (it has never been measured) and different pattern of postmortem redistribution.

For forensic purposes it may be more useful to look at the individual's electrocardiogram. Truly toxic propoxyphene concentrations will produce distinctive EKG changes (Whitcomb et al., 1989) including QRS prolongation, bundle branch block, and torsades de pointes (Adler et al., 2011). Studies with human volunteers have shown up to a

30 ms prolongation of the QT interval (FDA, 2010b). In extreme cases, asystole can occur (Madsen et al., 1984).

The antemortem demonstration of these abnormalities, particularly QRS prolongation (Afshari et al., 2005), is likely to be more probative than postmortem blood concentration measurements. As with all opiates, the cause of death should never be determined by reference to blood and tissue concentrations reported from earlier postmortem studies or by comparison with "therapeutic" concentrations reported in the living.

5.11.12.4 Excretion and Detectability

Propoxyphene is not on the National Institute on Drug Abuse (NIDA) prohibited list, so no effort would be made to detect it on limited workplace screening tests, nor would there be any financial incentive to develop the antibodies necessary for ELISA testing. However, propoxyphene is included in widely used 10-drug screening panels (e.g., Online KIMS assay [Roche] and enzyme multiplied immunoassay technique II assays) (Lu and Taylor, 2006). Propoxyphene has a half-life of 22 h, so it will remain detectable in the urine for at least 4 days. In the past, the changes of putrefaction were known to cause false-positive EMIT screening tests for propoxyphene, though there have been no recent studies of the subject (Sloop et al., 1995). In any case, the issue is easily resolved with confirmatory testing.

5.11.12.5 Maternal/Fetal Considerations

Propoxyphene is excreted in mothers' milk, but not in quantities likely to produce any effect on their infants. The excretion of propoxyphene and norpropoxyphene in breast milk was studied in six healthy nursing mothers. Breast milk concentrations generally followed plasma levels, with approximately the same ratio of norpropoxyphene to propoxyphene (2.6) observed both in plasma and milk. The ratio of drug in the milk and plasma was 0.417 for propoxyphene and 0.382 for norpropoxyphene. Both parent drug and metabolites are cleared from the milk at the rate of 4 mL/h, with a mean half-life of 3.68 h for propoxyphene and 5.49 h for norpropoxyphene (Kunka et al., 1984). Nursing infants are unlikely to ingest amounts that will cause any detrimental effects during short-term treatment (Bar-Oz et al., 2003). The possibility of norpropoxyphene toxicity occurring after long-term exposure cannot be ruled out (Spigset and Hagg, 2000), although actual examples of such toxicity have never been reported.

5.11.12.6 Histologic Abnormalities and Autopsy Findings

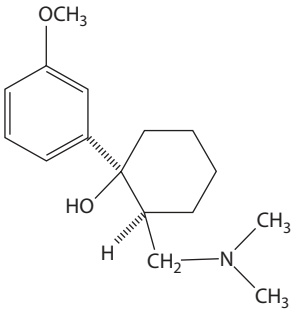
With the exception of cases of suicidal overdose, propoxyphene-related deaths are most likely to occur in chronic drug abusers, and the usual stigmata of injection drug abuse are to be expected. In some countries, titanium dioxide (TiO_2) was used as an excipient along with talc. When abusers crushed these tablets and injected them, titanium deposits became visible in many organs as chalk streaks, which are actually composed of granule-laden macrophages. The titanium appears a green-tan color with routine hematoxylin and eosin stains. These granules appear pink under polarized light (de Lima et al., 2004). Other case reports have described an acute generalized exanthematous pustulosis (AGEP), but it is rarely seen. During the 1990s there were reports of liver disease characterized by centrilobular cholestasis, portal tract inflammation, and bile duct abnormalities (Bassendine et al., 1986; Rosenberg et al., 1993); however, it has been 20 years since additional cases have been reported.

5.11.13 Tramadol (Table 5.28)

5.11.13.1 General Considerations

Tramadol is a centrally acting analgesic with only 10% of morphine's potency when administered parenterally. Structurally it is related to both codeine and morphine. Like codeine, tramadol itself is not an active opioid (Meyer and Maurer, 2011). For it to become active, it must first be converted to *O*-desmethyl-tramadol. The conversion is performed by CYP2D6. The *N*-demethylated form is catalyzed by CYP2B6 and CYP3A4. Tramadol has two enantiomers, but unlike methadone, each enantiomer has analgesic activity, even though different mechanisms are involved. *l*-Tramadol and the metabolite *l*-*O*-desmethyltramadol (M1) are agonists of the μ-opioid receptor, and they also inhibit 5-HT reuptake, but *d*-tramadol only inhibits norepinephrine reuptake. As a consequence, the *d* form enhances the inhibitory effects on pain transmission in the spinal cord. Because CYP2B6 is highly polymorphic, the analgesic effect may be unpredictable. Studies have shown that both pain relief and plasma tramadol concentrations correlated with the CYP2D6 isoform (Stamer et al., 2007). These distinctions are of more than academic interest. In one case report, genotyping CYP2D6 in a young man with cardiac arrest revealed the patient to be heterozygous for a duplicated wild-type allele, predictive of a CYP2D6 UM phenotype. Because tramadol had inhibited norepinephrine reuptake, and because the man was an ultrametabolizer CYP2D6 phenotype, excessive blood nor epinephrine levels led to stunning of the myocardium (Elkalioubie et al., 2011).

Table 5.28 Physiochemical Properties and Pharmacokinetics of Tramadol

Chemical names	2-(Dimethylaminomethyl)-1-(3-methoxyphenyl)-cyclohexan-1-ol
Physiochemical properties, structure, and form	Available as HCl salt MW: 263.38 CAS: 27203-92-5 p*K*a: 8.3, 9.4 V_d: 3 L/kg Protein binding: 20% 
Synonyms and brand names	Anadol, Dolol, Dromadol, Ralivia, Tramal, Tramacet, Ultram, Upziva, Zamadol, Zydol
Pharmacokinetic parameters	Bioavailability: 87%–95% C_{max}: 11 ng/mL after 100 mg PO T_{max}: 1 h $T_{(1/2)\beta}$ (half-life): 8.0 ± 1.3 h
Metabolism	*O*-demethylation by P450 (CYP)2D6, *N*-demethylation by CYP2B6 and CYP3A4
Excretion	30% excreted as unchanged drug
Postmortem artifacts	Increases occur postmortem; at least twofold expected
Interactions	CYP3A4 inducers, CYP3A4 inhibitors
Key papers	Grond and Sablotzki (2004), Curry et al. (2007), and García-Quetglas et al. (2007)

5.11.13.2 Metabolism

The major metabolites present in plasma are *O*-desmethyltramadol and *N*-desmethyltramadol, and to a minor extent *N*,*N*-didesmethyltramadol, *N*,*N*,*O*-tridesmethyltramadol (M4), and *N*,*O*-desmethyltramadol. All of these metabolites are potent μ-agonists. The *O*-desmethylated metabolite is formed by cytochrome CYP2D6, but CYP2B6 and CYP3A4 form *N*-desmethyltramadol. The role played by CYP2D6 is very important, partly because 10% of U.S. Caucasians are deficient and partly because 2D6 is highly polymorphic. Individuals may be ultrametabolizers, normal, intermediate, or PMs, and metabolizer status, more than any other single factor, determines how a particular individual will respond to the drug. For reasons that remain unclear, when PMs are given tramadol, they have higher epinephrine plasma concentrations than normal metabolizers. It is thought by some that, in addition to binding to the μ-receptor, tramadol has ability to prevent the reuptake of both norepinephrine and serotonin and that both actions contribute to its significant analgesic properties (Barkin, 2008).

Tramadol is rapidly distributed in the body and is approximately 20% protein bound. It is mainly metabolized by *O*- and *N*-demethylation and then by conjugation reactions to form glucuronides and sulfates that are all mainly excreted by the kidneys. The mean elimination half-life is about 6 h.

5.11.13.3 Clinical Issues

Pharmacokinetic–pharmacodynamic characterization of tramadol is difficult because of differences between tramadol concentrations in plasma and concentrations at the site of action, not to mention pharmacodynamic interactions between the two enantiomers of tramadol and their active metabolites. Tramadol provides postoperative pain relief comparable with that of meperidine. The analgesic efficacy of tramadol can further be improved by combination with nonopioid analgesics. Tramadol is one of the more common causes of serotonin toxicity when it is used simultaneously with other serotonin-active drugs, for example, SSRIs, MAOIs, and MDMA. It should be avoided when patients are also on other serotonin-active drugs (Pilgrim et al., 2011).

Tramadol is thought to be particularly useful in patients with poor cardiopulmonary function, especially after surgery of the thorax or upper abdomen. Tramadol is reported to be an effective, well-tolerated agent, useful in the treatment of pain secondary to trauma, renal or biliary colic, labor, and neuropathic pain. Tramadol appears to produce less constipation and dependence than equianalgesic doses of strong opioids (Grond and Sablotzki, 2004).

Tramadol must be used with caution in patients taking vitamin K antagonists as there is evidence that concurrent use of tramadol with oral vitamin K antagonists creates a risk of excessive anticoagulation (Elkalioubie et al., 2011; Pottegard et al., 2012). In spite of a relatively good clinical profile, tramadol use is associated with two different significant side effects: seizures and the serotonin syndrome. These two adverse reactions may develop during tramadol monotherapy but appear much more likely to emerge during misuse/overdose as well as when tramadol is taken with other drugs, particularly reuptake blockers of any type but especially the newer classes of antidepressants (Gardner et al., 2000; Ripple et al., 2000; Sansone and Sansone, 2009). There are also case reports that ingestion of large doses may lead to rhabdomyolysis (Yousef Khan et al., 2010).

There are reports of tramadol diversion to the illicit markets (Cicero et al., 2005a,b; Inciardi et al., 2006), particularly in the Middle East. Tramadol is neurotoxic and can cause generalized seizures, usually within the first 24 h of use (Jovanovic-Cupic et al., 2006).

Studies in human users are lacking, but when rats were exposed to tramadol for up to 10 days (doses of 20–80 mg/kg), they exhibited raised liver enzymes. Light microscopy revealed severe centrilobular congestion and focal necrosis in the liver, as well as vacuolization in tubular cell groups (Atici et al., 2004). The results of other animal studies suggest that tramadol exerts a seizurogenic effect on mice via an H_1 receptor activation-linked pathway, possibly through an opioid receptor-dependent release of histamine from the mast cells (Rehni et al., 2010). Human epidemiologic studies confirm that tramadol, when used in conjunction with phenacetin, is more hepatotoxic than combinations of phenacetin with either codeine or propoxyphene (Tavassoli et al., 2009).

5.11.13.4 Postmortem Considerations

Very few tramadol deaths have been reported or studied. There are only nine case reports in the literature, and all of those have occurred in individuals taking multiple other drugs. Peripheral blood concentrations of tramadol in these polydrug users have ranged from 1.6 to 15 ng/mL. Whether or not the drug produces any recognizable lesion is not known. One report describes a case of pure tramadol poisoning (De Decker et al., 2008). A 28-year-old hospitalized man, taking only tramadol, was found apneic. He was asystolic and acidotic when the paramedics arrived to resuscitate him. Routine toxicology screening of blood and urine, performed on samples obtained during resuscitation, was negative. Other tests disclosed liver failure. He died after 2 days. Autopsy disclosed pulmonary edema with alveolar hemorrhage, diffusely hemorrhagic gastric mucosa, "shock" liver, and acute tubular necrosis. Toxicologic analysis of serum and gastric lavage specimens, obtained on admission at the ICU, revealed a tramadol concentration of 8 and 400 mg/L, respectively. Analysis of postmortem blood (site of origin not specified) disclosed tramadol and the metabolite *N*-desmethyltramadol in the blood, liver, and kidney; tramadol concentrations were 5.2 mg/L, 6.5 μg/g tissue, and 4.5 μg/g tissue, respectively.

Two case reports of fatal intoxication due solely to tramadol have been published. In one case, concentrations of tramadol and its metabolites were 7.7 mg/L (tramadol), 1.33 mg/L (*O*-demethylated), and 0.6 mg/L (*N*-demethylated). In the second case the total blood tramadol concentration was 48.34 mg/L (the highest ever to be described in the literature), of which 2.43 mg/L was the *O*-desmethylated form and 10.09 mg/L the *N*-demethylated form (De Backer et al., 2010).

Tramadol screening tests can cause false-positive tests for PCP, especially in those dying of tramadol overdose (Hull et al., 2006). It may be that some reported PCP deaths are actually tramadol deaths, and the results should always be confirmed.

5.12 Medical Consequences of Opiate Abuse

Patterns of drug abuse vary from place to place, from time to time, and from drug to drug. Opiate users experience excess morbidity and mortality and die younger than the general population. In 2010, there were 38,329 drug overdose deaths in the United States; most (22,134; 57.7%) involved pharmaceuticals; 9429 (24.6%) involved only unspecified drugs. Of the pharmaceutical-related overdose deaths, 16,451 (74.3%) were unintentional, 3780 (17.1%) were suicides, and 1868 (8.4%) were of undetermined intent. Opioids (16,651; 75.2%), benzodiazepines (6497; 29.4%), antidepressants (3889; 17.6%), and antiepileptic and antiparkinsonism drugs (1717; 7.8%) were the pharmaceuticals (alone or in combination

Table 5.29 Most Common Anatomic Diagnoses in Heroin Abusers (n = 154)

Diagnosis	Percent (%)
1. Pulmonary edema	46
2. Track marks	44
3. Birefringent crystals in lung or liver	27
4. Pneumonia	18
5. Hepatic steatosis	16
6. Severe coronary artery disease	12
7. Hepatitis	12
8. Myocardial fibrosis	11
9. Extensive aortic atherosclerosis	9
10. Cerebral edema	9

Source: Office of the San Francisco Medical Examiner, San Francisco, CA, unpublished data.

with other drugs) most commonly involved in pharmaceutical overdose deaths. Among overdose deaths involving opioid analgesics, the pharmaceuticals most often also involved in these deaths were benzodiazepines (5017; 30.1%), antidepressants (2239; 13.4%), antiepileptic and antiparkinsonism drugs (1125; 6.8%), and antipsychotics and neuroleptics (783; 4.7%) (Jones et al., 2013). Skin infection with methicillin-resistant *Staphylococcus aureus* (MRSA) remains the principal disorder that brings addicts to inner-city hospitals, though the frequency of this complication seems to have stabilized after rising precipitously at the turn of the century, when the proportion of skin infections caused by MRSA increased from 29% in 2001–2002 to 64% in 2003–2004 (Buckingham et al., 2004). No clinical or historical features reliably predict MRSA etiology, but many of these cases are presumed to be secondary to injection drug use. In a study of 1200 soft tissue infections (both heroin-related and otherwise) reported from the United States Midwest, Gram-positive aerobes plus anaerobes represented approximately 80% of the pathogens, with the anaerobic rates being underestimated (Zimmerman et al., 2009).

The 10 most frequent anatomic diagnoses encountered by the San Francisco Medical Examiner in 1999 are listed in Table 5.29.

5.12.1 Dermatologic Sequelae

Skin lesions are associated with all types of intravenous drug abuse, but they are more common among opiate abusers. The difference has to do with the properties of the drugs themselves. Stimulants and hallucinogens do not cause histamine release, so their use is not associated with pruritus or excoriations. Cutaneous complications in injecting users are often related to the adulterants and excipients injected along with the drugs (Figure 5.35). Occasionally the consequences can be dire. In Europe, more so than in America, spore-forming bacteria are often present in the drugs injected by abusers. In the United Kingdom, in the year 2000, *Clostridium novyi* caused 63 cases of severe illness; 7 additional cases were reported in 2001 (Murray-Lillibridge et al., 2006). Nearly 100 years after World War I, wound botulism has reemerged in England. The first cases occurred in 2000 (6 cases) with 51 further cases through March of 2004. Infections with tetanus, *Clostridium histolyticum*, *Clostridium sordellii*, and *Bacillus cereus* have also been reported (Brett et al., 2004).

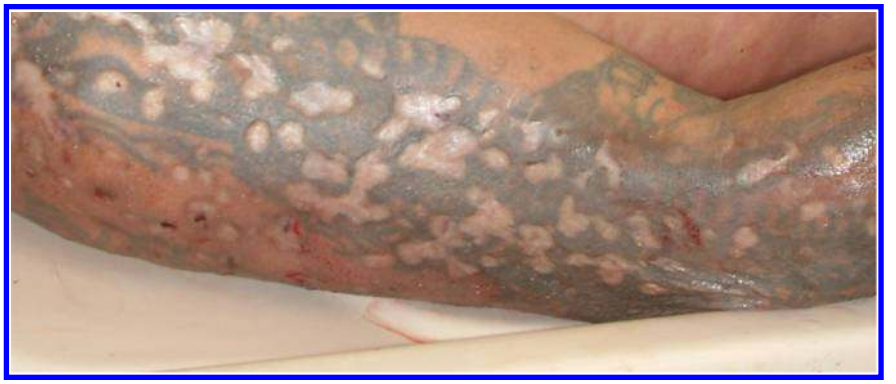


Figure 5.35 *Skin popping*. When an addict has thrombosed all accessible veins, they often resort to injecting under the skin. This may introduce infectious agents and form an indolent ulcer.

Clusters of cases of wound botulism have also been reported from Germany (Galldiks et al., 2007). Additional cases were reported from Norway in 2013 (MacDonald et al., 2013). A review of UK cases reported from 1990 to 2009 strongly suggests that while cases of tetanus, *C. novyi* infection, and anthrax most often occur in clusters, botulism tends to occur as sporadic cases (Hope et al., 2012).

Over the last 15 years, there has been increased recognition of a toxic shock-like syndrome associated specifically with *C. sordellii*. This complication is seen almost exclusively in individuals skin-popping black tar heroin and in women undergoing childbirth or other gynecologic procedures including medically induced abortion. Like their cousins, illness is secondary to *Clostridium*-produced endotoxins (Stevens et al., 2012).

The U.S. state of California is in the midst of an ongoing *Clostridium* outbreak, and many of the reported cases are actually recurrences. From 1993 to 2006, 17 IVDUs had recurrent wound botulism, 14 with 1 recurrence and 3 with 2 recurrences. Of 25 laboratory-confirmed episodes, 22 were confirmed through serum testing and 3 through wound testing. Patients were 32–61 years old and 94% were male. All were heroin injectors and 88% specified they had been using black tar heroin (N.B., despite certain resemblances, Iranian black tar heroin has a different composition than Mexican black tar heroin and is not available in California); 76% reported subcutaneous injection (Yuan et al., 2011a).

In early 2010 an outbreak of anthrax occurred among subcutaneous and intravenous heroin injectors living in the United Kingdom and Europe. As Afghanistan is now the major supplier of heroin worldwide, the outbreak no doubt originated in tainted Afghan heroin. When anthrax occurs, cutaneous disease accounts for over 95% of cases. At least 47 cases were reported in the 2010 outbreak, with 13 deaths having occurred through the end of 2010. Strangely, many of these individuals lacked the usual external signs of anthrax (raised itchy bumps resembling insect bites that develop into vesicles, then painless ulcers, with a characteristic black necrotic [dying] area in the center) (Knox et al., 2011). Although systemic anthrax infection is rare, it should be considered when faced with severe cutaneous infection in IVDUs. This case shows that patients with life-threatening bacteremia may manifest no signs of hemodynamic compromise or give any indication of how sick the patient actually is (Powell et al., 2011).

Traditionally, three types of anthrax syndromes were recognized: cutaneous, gastrointestinal, or inhalational. A fourth syndrome characterized mainly by severe soft tissue

infection and seen only in injecting drug users is now emerging in the United States. The estimated mortalities of cutaneous, gastrointestinal, inhalational, and injectional anthrax are 1%, 25%–60%, 46%, and 33%, respectively. As discussed previously, the nonspecific nature of the early symptoms makes initial identification of anthrax cases difficult. Clues to anthrax infection include history of exposure to herbivore animal products, heroin use, or clustering of patients with similar respiratory symptoms simulating a bioterrorist event. Diagnosis can be confirmed with a simple Gram stain, but for epidemiologic purposes, PCR may prove useful (Sweeney et al., 2011).

5.12.1.1 Fresh Needle Punctures

Finding recent injection sites is usually a simple matter but sophisticated abusers often take great pains to conceal evidence of injection, and these lesions may be hard to find. The presence of dried blood on the surface of the skin surrounding a puncture is confirmatory evidence that death occurred almost immediately following injection (Hirsch, 1972). The antecubital fossa is the preferred site for self-injection, but punctures may be found at the wrist, under a watchband, or between the toes. The path of the needle may be confirmed by making a skin incision immediately adjacent to the suspected site. This will reveal the presence of small subcutaneous hemorrhages that occur after venipuncture (Hirsch, 1972). Alternatively, a single longitudinal incision can be made on the flexor surface of the arm from mid-biceps to distal forearm and the subcutaneous tissues exposed by either blunt or sharp dissection. Subcutaneous hemorrhage may not be evident in every case, but chemical analysis of tissue around the needle track often yields evidence of the drug injected.

Demonstration of drug in the skin taken from a suspected injection site does not, by itself, prove that the drug was injected at that site. If the decedent survives for even a few minutes, the circulation will have distributed drug throughout the body, including the skin! The only way to prove that drug was introduced into the body at a particular site is to sample skin from both sides of the body; the concentration on the side in question should be significantly higher than on the other.

The purity of street heroin is now so high (well over 60%) that heroin *snorting* is a common practice. In cases where no track marks are evident, but narcotic overdose is strongly suspected, the nasal cavity should be examined and then swabbed with saline for toxicology testing. Of course, the same considerations apply to swabbing the nasal mucosa (or mucosa at any other site). The detection of drug on the mucosa does not prove that it was applied there, only that the drug was circulating throughout the body and that some minutes elapsed between the drug being taken and the time of death.

5.12.1.2 Atrophic Scarring

Subcutaneous injection is a fairly common practice, especially among novice users or chronic users with difficult venous access. The flexor aspect of the arm is the preferred site for injection, followed by the anterior thigh. Absorption of heroin is good by this route, but the deposition of excipients in the subcutaneous tissue eventually leads to the development of oval or irregularly shaped lesions measuring 1–3 cm. Lesions are slightly depressed and often hyperpigmented. Most lesions are found at the sites of healed abscesses, but they may occur without abscess formation. Alternatively, they may become confluent.

This type of lesion has been recognized for more than half a century but the dermatopathology remains poorly characterized and the etiology unclear. Early workers suggested that the lesions were a direct result of the effect of heroin on the skin (Light and Torrance, 1929),

but adulterants or infectious agents are more likely to be the cause, though some evidence indicates that the pH of the solution injected rather than the drug itself may be what determines whether tissue injury occurs (Pollard, 1973; Thomas et al., 1995). Microscopic examination of healed atrophic lesions usually reveals subcutaneous fibrosis. Foreign body granulomas may or may not be present, but birefringent material, such as talc or starch crystals, is likely to be seen with the aid of nothing more complex than a polarizing filter (Hirsch, 1972).

5.12.1.3 Abscesses and Ulceration

Abscesses are common in heroin abusers who inject subcutaneously. The practice is known as *skin popping* (Webb and Thadepalli, 1979; Orangio et al., 1984). Lesions occur primarily on the extensor surfaces and lateral aspects of the arms and hands but can be seen almost anywhere on the body. Injection into the subclavian area (Espiritu and Medina, 1980; Tsao et al., 2002) and the femoral triangle may cause life-threatening infections (Pace et al., 1984; Schondorf et al., 2000; Rhodes et al., 2007; Zador, 2007; Muller et al., 2008; Higgs et al., 2009), as can injection into the intercostal vessels (Gyrtrup, 1989). One case report described avascular necrosis of the femoral head after repeated injection into the femoral triangle (Gramenz et al., 2010), while others have reported limb ischemia—presumably from particle embolization and necrosis as a result of this practice (Betz et al., 2011). The ulcers have a punched-out appearance, with indurated borders surrounding a central core of granulation tissue. Nothing distinguishes the appearance of injection site abscesses from any other sort of soft tissue abscess (Webb and Thadepalli, 1979).

5.12.1.4 Track Marks

Track marks (Figure 5.36) were first described in 1929. They were observed in a heroin addict who had contracted malaria following intravenous injections (Biggam, 1929). Lesions were said to resemble railroad tracks because they were linear, indurated, and hyperpigmented. What the lesions actually look like, and how rapidly they form, depends on the substances being injected. The excipients found in nonpharmaceutical cocaine and methamphetamine are usually water soluble, so *track* marks are a less common finding

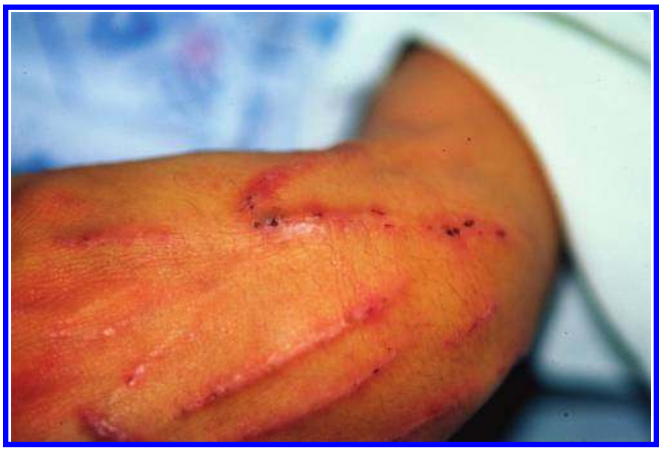


Figure 5.36 *Track* marks are the result of repeated injection with heroin that is contaminated with substances that irritate the veins. Microscopic examination will show birefringent crystals of the diluent and localized inflammation.

in this group of abusers than in opiate users, where they may be prominent (Wetli et al., 1972). Paregoric, which is also injected by desperate heroin users, causes an intense sclerotic reaction. When paregoric injecting was popular in the 1960s, addicts quickly ran out of peripheral veins and were forced to inject themselves in the neck and groin (Lerner and Oerther, 1966). Heroin, even in its adulterated form, is less toxic to veins than paregoric, but prolonged use will eventually cause thickening and sclerosis of the subcutaneous veins, if only because impure heroin is being injected. On the other hand, subcutaneous injection of heroin (unlike morphine) is relatively painless, allowing the doctors of some countries to give hospice care using syringe drivers (small infusion pumps) connected to subcutaneously placed needles.

The skin overlying the sclerotic veins becomes hyperpigmented, probably as a result of the underlying chronic inflammatory process (Vollum, 1970), but the degree of hyperpigmentation depends largely on the individual's native coloration, not necessarily on how long the addict has been injecting himself. Discoloration of the surrounding skin can also be the result of inadvertent tattooing. Addicts may try to sterilize their needles with a match flame, causing small amounts of soot to be deposited on the outside of the needle. The soot is then carried into the skin at the time of injection. Injection drug users have traditionally tried to conceal these marks by tattooing or even by burning themselves in the hopes of scarring the entire area (Wetli, 1984; Martinez and Wetli, 1989; Sperry, 1991, 1992).

The histology of sclerotic veins is variable (Schoster and Lewis, 1968). Only fibrous thickening of the vein wall may be evident, suggesting a low-grade, chronic inflammatory process. In other instances thrombophlebitis, sterile or septic, may occur. The results are difficult to predict and Halpern (1972) even commented that on occasion the veins repeatedly used by addicts "show less evidence of closure by thrombosis than the veins of patients subjected to repeated punctures by physicians for medical purposes."

5.12.1.5 Tattoos

Tattooing derives its name from the Tahitian word *tatau*, which means "the results of tapping," the way in which Tahitian tattoos were applied. The practice dates back to antiquity. Tattoos have been found on Egyptian mummies from the 11th Dynasty, making the practice at least 4000 years old (Sperry, 1991). In prison, tattoos are applied by using the "melted-toothbrush" technique. Any pointed object, such as a bedspring or matchbook staple, can be used as a needle. The end of a plastic toothbrush is then melted in a flame and the smoky residue collected. The residue is mixed with soap and water to form ink (Sperry, 1991). A great deal of significance was once attributed to the design and location of these tattoos. Symbols on the thumb webbing were said to indicate criminal specialties. Within various prisons and gangs, tattoos of tear drops signify the number of murders the individual has committed. The results of more recent studies suggest that hand-web tattoos probably have significance only in the prison where they are applied (Martinez and Wetli, 1989). In some specific subpopulations, such as the Marielitos, tattoos may represent religious symbols or themes, but these interpretations cannot be generalized to other subgroups, nor does the presence of tattoos seem to correlate with any particular personality traits or beliefs (Koch et al., 2004). This type of skin ornamentation is thought to explain the extraordinarily high incidence of hepatitis C among addicts (greater than 90% in most urban areas) (Hellard et al., 2007).

The meaning of tattoos has become more obscure in the modern era. Tattoos and body piercings are becoming increasingly popular, particularly in adolescents and young adults

who have nothing to do with criminal enterprises. The main reasons for obtaining body art seem to be the need for individual expression and peer support. Techniques for tattooing are advancing, along with the development of newer inks that may be less reactive. When patients become tired of their body art, or develop complications from the art itself, removal needs to be considered, and newer laser modalities may provide tattoo removal options with less scarring potential. Alternatively, criminals may resort to these same laser procedures in order to help assume new identities (Desai and Smith, 2011).

Infrared imaging techniques are now used to visualize faint tattoos, and animal studies have even demonstrated that, with the assistance of infrared visualization, it may be possible to reassemble skin fragments to reconstitute original tattoos from mutilated skin parts (Starkie et al., 2011). Occasionally these body markings may have medical significance (over and above the transmission of HIV and hepatitis). Tattoos are now known to be associated with dermatofibrosarcoma protuberans, an uncommon skin tumor associated with a number of different medical conditions. Sites of local trauma (the more common cause for this tumor) and tattoos may undergo malignant transformation and should be examined with some frequency (Reddy et al., 2011).

5.12.1.6 Puffy Hands Syndrome

Lymphedema sometimes occurs in chronic opiate injectors. The condition was first described in the 1960s as a complication of heroin abuse (Neviaser et al., 1972), but it is also seen with buprenorphine and pentazocine abuse (Andresz et al., 2006). This abnormality is generally thought to be an indicator of long-term abuse, and usually does not appear until 3–5 years after the initiation of drug use (Simonnet et al., 2004). It is difficult to say with certainty, because the incidence of this disease is not recorded and the mechanism is not really understood (Simonnet et al., 2004), but puffy hands appear to be more likely to suggest the use of buprenorphine or other synthetic narcotics than heroin (Andresz et al., 2006). Drugs like buprenorphine are very insoluble and injection of ground tablets into the hand is thought to destroy the lymphatic system. The same considerations apply to pentazocine, though abusers of this drug are rarely seen today. The hands become smooth and slightly edematous with obliteration of the normal anatomic landmarks, but pitting edema is absent. In contrast to the changes seen in the hands of myxedematous patients, the skin in addicts with "puffy" hands is thin and smooth. The skin on the volar aspect of the forearm is also normal, even though evidence of repeated injections will be seen in both antecubital fossae (Prasad et al., 2005).

5.12.1.7 Necrotizing Fasciitis

Necrotizing fasciitis was first described over 120 years ago. The term describes a severe infection of the superficial fascia and subcutaneous tissue (Figure 5.37). Initially, the infection does not involve the overlying skin (Wojno and Spitz, 1989). In the absence of drug abuse, necrotizing fasciitis usually occurs in diabetics or in patients with severe atherosclerosis, where the infectious process is initiated by surgery or even by minor trauma to ischemic tissue.

Reports and studies suggesting a link between necrotizing fasciitis and the use of nonsteroidal anti-inflammatory drugs (NSAIDs) continue to appear (Zerr and Rubens, 1999; Chikkamuniyappa, 2004; Souyri et al., 2008). Whatever the inciting factor, when these cases occur, virulent strains of exotoxin-producing *Streptococcus*, almost always group A organisms such as *Streptococcus pyogenes*, are usually present.

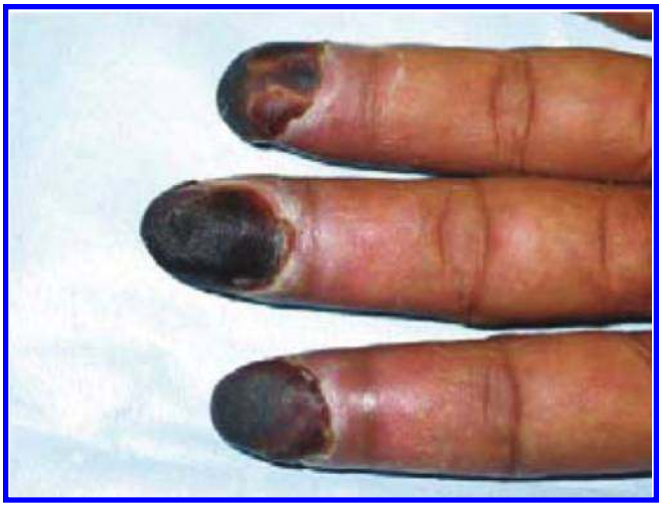


Figure 5.37 Necrosis of finger tips in buprenorphine abuser with acute brachial artery necrosis at the injection site. (From Chong, E. et al., *Singapore Med. J.*, 50(1), 34, 2009.)

Another cause for this condition is the process of drug injection itself. In one recent study, it was discovered that nearly half of the cases of necrotizing fasciitis seen by the medical examiner could be related to the injection of black tar heroin (Mexican origin). Of those infections contracted from heroin, nearly half were due to clostridial species, and nearly one-third of those were *C. sordellii*, an obscure species of clostridia recently linked to the occurrence of spontaneous abortion (Zane and Berg, 2006). Clostridia were found in only a few of the cases not associated with intravenous drug abuse. Roughly half studies were found to be polymicrobial infections (Dunbar and Harruff, 2006).

The pathophysiology of this disorder is only now becoming apparent. The invading streptococcus must possess pyrogenic endotoxin B (a protease secreted by group A streptococci, shown to degrade a wide range of host group A streptococcal proteins in vitro). Recent evidence shows that streptococcal endotoxin B is critical for the pathogenesis of severe invasive disease caused by the streptococcus. In experimental models, when the gene for endotoxin B is turned off, the resulting bacteria have a significantly diminished ability to cause necrotizing fasciitis (Carroll and Musser, 2011). Although there is very little doubt about these observations, there is evidence that the spectrum of causative agents is changing (Tsitsilonis et al., 2013). Even *Serratia marcescens* has been implicated. When *Serratia* is the responsible agent, tissue destruction is rapid and death can occur in less than 72 h, in spite of intensive treatment (Rehman et al., 2012).

Once the infection of the fascia is established, necrosis spreads rapidly through fascia and subcutaneous tissues. The overlying skin looks normal until very late in the course of the disease, and the underlying muscle is usually not involved (Tehrani and Ledingham, 1977). Hematogenous seeding may occur with spread to organs throughout the body. Even purulent myocarditis has been reported as a complication. The fact that the overlying skin looks normal may delay the diagnosis and lead to a fatal outcome (Wojno and Spitz, 1989).

At one time it was thought that only Gram-positive aerobes were the causative agents, but in more recent studies the etiology has been seen to be polymicrobial. In a review of 182 patients with documented necrotizing soft tissue infections, wound cultures grew an average of 4.4 different microbes. Infection was due to a single pathogen in only

28 instances (15%). Nearly half the patients had combined aerobic and anaerobic growth. The most common organisms, listed in declining order, were *Bacteroides* species, aerobic *Streptococcus*, *Staphylococcus*, *Enterococcus*, *E. coli*, and other Gram-negative rods (Elliott et al., 2000). Evidence suggests that *Klebsiella* is increasingly responsible for cases in Asia but infections with this agent remain rare in the United States (Persichino et al., 2012).

Whether this same hierarchy applies today is not known, but one interesting new observation is that when Gram-negative organisms were predominant in wound isolates, Gram-positive organisms were predominant blood isolates (Awsakulsutthi, 2010). All in all, this is a very virulent disease. A 2011 study of 321 patients found that patients with hypotension, heart disease, liver disease, presence of *Vibrio* spp. in wound cultures, presence of fungus in wound cultures, and presence of Streptococcus group A, *Aeromonas* spp., or *Vibrio* spp. in blood cultures all had a significantly higher risk of in-hospital mortality. Mortality was especially high in those infected with *Vibrio* spp. and was similarly elevated in those with preexisting conditions like hypotension, heart disease, and liver disease (Chen et al., 2011).

5.12.1.8 Histamine-Related Urticaria

Skin excoriations are common, but it is not always clear if they are the result of narcotic-induced pruritus or psychological disorder (Young and Rosenberg, 1971). Drugs may cause urticaria by different mechanisms, but the mechanism responsible for opiate-induced histamine release is somewhat different than other types of urticaria. The most well-known mechanism leading to histamine release is the allergic reaction mediated by immunoglobulin (Ig)E antibodies, which induce acute generalized urticaria. Allergic reactions to beta-lactams are the most common cause of adverse drug reaction mediated by IgE antibodies (Mathelier-Fusade, 2006).

Histamine release in narcotic abusers is not a true IgE-mediated allergic response (Hermens et al., 1985). Opiates act directly on mast cells to produce histamine release. The process is thought to be G-protein mediated (Barke and Hough, 1993). How much histamine will be released depends on the type and amount of opiate administered. For example, there is some evidence that equipotent doses of heroin cause less pruritus than morphine (Haemmig and Tschacher, 2001). In one series, more than 20% of the patients receiving postoperative opiates developed urticaria (Withington et al., 1993). In some instances, the amount of histamine liberated can be large enough to cause hypotension, in addition to erythema and tachycardia. Not all narcotics cause histamine release. Elevations in plasma histamine occur after dosing with intravenous morphine, meperidine, and diacetylmorphine (heroin) but not after treatment with fentanyl or sufentanil (Flacke et al., 1987).

5.12.1.9 Acute Generalized Exanthematous Pustulosis

A serious drug-induced eruption, AGEP, usually accompanied by fever and neutrophilia with elevated total white blood cell count, has been reported occasionally in heroin abusers (Lee et al., 1995; Kardaun, 2011). Many small follicular pustules can be seen superimposed on areas of bright erythema. This disorder has been reported in association with the use of many different drugs, including morphine, though most of the time the offending agent is an antimicrobial or acetaminophen. This disorder overwhelmingly involves women. Some studies implicate T cells, while others suggest the induction of an antigen–antibody complex by the culprit drug or infection, with subsequent activation of the complement system followed by neutrophil chemotaxis (Lee et al., 1995; Tamir et al., 2006).

In severe drug-induced eruptions, bullous lesions can be associated with immune-complex–mediated vasculitis and/or with T-lymphocyte–mediated keratinocyte apoptosis. In a recent study of 32 patients with T-lymphocyte–mediated drug-induced eruptions, ranging from groups of patients with toxic epidermal necrolysis/Stevens–Johnson syndrome, to cases of drug rash with eosinophilia and systemic symptoms, to cases of AGEP and drug-induced maculopapular exanthema, cellular apoptosis was prominent not just in the skin but in many other organs of the body (Verneuil et al., 2011). This suggests that AGEP is, indeed a systemic disease, though why it should follow heroin injection remains a mystery. It may well be that the reaction was not to heroin itself, but to some adulterant.

5.12.1.10 Fungal Lesions

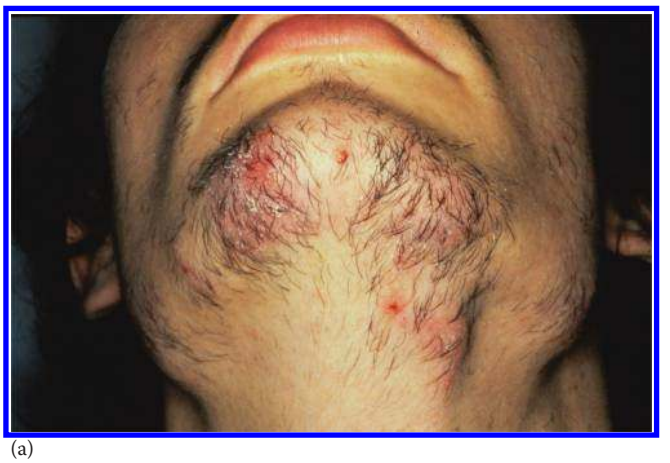
Oral candidiasis is the most common opportunistic infection seen in HIV patients. When the HIV pandemic first began, the prevalence of oral thrush in AIDS patients was 40%–90%, but with the introduction of HAART, the pattern of HIV-related oral disease has changed and in industrialized countries the prevalence of this disorder is estimated to range between 10% and 50% of those infected (Hodgson et al., 2006). No similar study has been published since, so the true incidence of this complication today is not known. The prevalence of esophageal involvement is much lower, but fully 75% of those who develop oral candidiasis will at some time progress to esophageal involvement (Flores-Figueroa et al., 2011). As a rule, *Candida albicans* infections in AIDS patients are limited to the mucosa.

Disseminated disease does not occur unless the infected individual is also a heroin user or has some other similar risk factor (steroid therapy, indwelling catheter, severe granulocytopenia) that would diminish the immune response (Vinceneux et al., 1981).

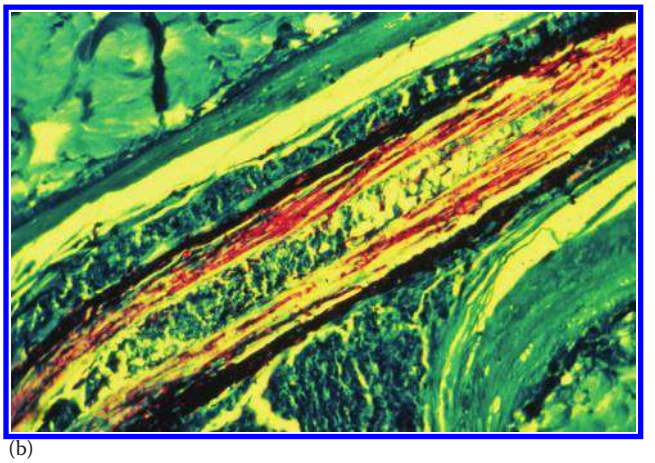
Candida-related febrile septicemia with cutaneous involvement is a disorder confined to heroin addicts. The syndrome was first described in 1981 when a cluster of cases occurred in Paris (Vinceneux et al., 1981). Subsequently, hundreds of additional cases were reported across Europe and Australia, but only sporadic cases have been reported in the United States (Collignon and Sorrell, 1983; Martinez-Vazquez et al., 1998). Epidemiologists eventually linked the outbreak to the use of poorly soluble heroin that had been exported from Iran ("brown" heroin). In order to dissolve the heroin so that it could be injected, users added lemon juice or some other acidifying agent (Mellinger et al., 1982). It was found that even bottled lemon juice could become contaminated with the *C. albicans* found on an addict's skin (Berger et al., 1988). It was found that most of the lemon juice had been contaminated with a single strain of *Candida*, serotype A, biotype 153/7 (Shankland and Richardson, 1989). Subcutaneous lesions are seen in 75%–100% of the cases, ocular involvement in approximately 60%, and osteoarticular involvement in 20%–50% (Vinceneux et al., 1981).

In a typical case, symptoms occur within 2–24 h after the last heroin injection. Chills, fever, headache, and profuse diaphoresis quickly follow one after another. Within 1–3 days, patients develop disseminated folliculitis and scalp nodules. Any hair-bearing area may be involved, but the scalp is the most common site (Dupont and Drouhet, 1985). Painful cutaneous nodules, usually measuring less than 1 cm, erupt quite suddenly. As many as 100 of these nodules may be present, and it is said that the scalp of such an individual feels like "a sack of marbles." Figure 5.38a and b illustrates the facial appearance of a typical patient. Fungal filaments are visible within a hair.

Smaller pustules may be seen adjacent to the nodules. The pustules strongly resemble lesions produced by staphylococcal or streptococcal infection, but microscopic examination



(a)



(b)

Figure 5.38 (a) Facial appearance of a heroin addict with *Candida* septicemia. (b) Yeast filaments in the hair of the same individual.

discloses yeast and filaments of *C. albicans*. Biopsy of the follicular nodules is more likely to be diagnostic than blood cultures. Gomori methenamine–silver staining will reveal bifurcated filaments of *C. albicans* admixed with an intense, mixed inflammatory infiltrate (Dupont and Drouhet, 1985). Since the initial reports were first published, others have appeared describing the same syndrome after injection of methadone-containing syrup diluted with orange juice (Scheidegger and Frei, 1989; Moller et al., 1997), after injection of buprenorphine tablets diluted in lemon juice, and even after intravenous injection of methamphetamine and cocaine (Upton et al., 1998).

For reasons that are not clear, this disorder is rare, at least within the United States. It would seem to be a difficult diagnosis to miss.

5.12.1.11 Miscellaneous Cutaneous Abnormalities

Other skin disorders are occasionally seen, but none with sufficient frequency to be of any diagnostic value. After self-injecting with opiates, abusers may fall asleep with cigarettes in their mouths, resulting in burns of the anterior chest when the head falls forward (Sapira, 1968). First described more than 50 years ago, this lesion is still sometimes

encountered. Sometimes the burns occur in a circular pattern. Other lesions reflect usage patterns that were unique to a specific time and place and are mainly of interest as historical curiosities. In the late 1800s, when opium smoking was still popular, the presence of cauliflower ears (swelling of the auricles) was considered almost pathognomonic for opium use. They were the result of lying for long periods on opium beds with hard wooden pillows (Owens and Humphries, 1988).

5.12.2 Cardiovascular Disorders

5.12.2.1 Introduction

The hearts of heroin abusers are more susceptible to endocarditis and to the various complications associated with HIV infection. A study published in 2012 analyzed 2036 cases of HIV and compared prospectively the incidence of medical disorders against those occurring in 35,718 controls. Persons with the following disease categories had a high risk of HIV diagnosis during the subsequent 5-year period: sexually transmitted infections and viral hepatitis (adjusted odds ratio [aOR] = 12.3, 95% CI: 9.60–15.7), hematologic diseases (aOR = 4.28, 3.13–5.85), lower respiratory tract infections (aOR = 3.98, 3.14–5.04), CNS infections (aOR = 3.44, 1.74–6.80), skin infections (aOR = 3.05, 2.47–3.75), other infections (aOR = 4.64, 3.89–5.54), and substance abuse (aOR = 2.60, 2.06–3.29) (Søgaard et al., 2014).

Several specific diseases were associated with aORs greater than 20 including syphilis, hepatitis A, non-A viral hepatitis, herpes zoster, *Candida* infection, endocarditis, thrombocytopenia, and opioid abuse. Causation is becoming increasingly difficult to determine because most opiate abusers abuse other drugs at the same time.

In Siegel and Halpern's classic 1966 papers on the *Diagnosis of death from intravenous narcotism* and *Deaths from narcotism in New York City*, heart disease was not even discussed (Helpern and Rho, 1966; Siegel and Halpern, 1966). Wetli et al. noted no significant cardiac abnormalities in their study published in 1972, in which 100 consecutive autopsies of cocaine abusers were reviewed (Wetli et al., 1972). Now that most drug abusers are polydrug abusers, any disease encountered could be the consequence of the drugs being taken with heroin, not the heroin itself. This is especially true in the case of combined opiate–stimulant abusers (Karch, 2005), as stimulant abuse causes myocardial remodeling. It is also true in heroin abusers who may be injecting aminorex (levamisole, an adulterant to cocaine and heroin, is metabolized to aminorex) (Karch et al., 2011).

Making sense of the older studies and even some of the newer ones is difficult. The phrase *narcotic addict* has never been applied consistently. Early workers used the term to apply to any sort of intravenous drug abuse, even though the effects of sympathomimetic (cocaine, methamphetamine) drugs are manifestly different from those of opiates. In early studies, chemical confirmation of the diagnosis of drug abuse was impossible except by observing associated clinical signs. Such inferences can be very useful at the bedside, and sometimes even in court, but the only reason these early observations ever made their way into the peer-reviewed medical literature was the inability, at the time, to accurately detect drugs in postmortem tissue.

Worse, almost all of the older studies on drug abusers were uncontrolled. This led to some very strange conclusions, which should have been suspect even at the time. In 1989 Dressler and Roberts reported finding lesions in the hearts of every abuser they examined, but their study lacked controls. Nearly all of the 168 cases they studied had been referred to a tertiary center, only because the original prosecutors suspected that cardiopulmonary

abnormalities were present (Dressler et al., 1990). The abnormalities reported by Dressler and Roberts are utterly at odds with the experience of most medical examiners. Unfortunately, very few controlled studies have compared the cardiopulmonary pathology in opiate-related deaths with age-matched controls, but in general, the hearts of the opiate addicts, unless suffering from endocarditis, or myocardial fibrosis (either a consequence of lung disease or interstitial fibrosis), or lung-related disease, appear to differ in no significant way from those of the controls (Seltenhammer et al., 2013).

Another problem is the nonspecific nature of the histologic alterations. Cardiac fibrosis may be the result of ischemia, infarction, cardiomyopathy (which would include toxic cardiomyopathy), and myocarditis but, more than likely, is simply another manifestation of myocardial remodeling, whatever the cause (stimulant abuse, old infarction, hypertension). No matter the initiating cause, the process is mediated by fibromyocytes differentiating into fibroblasts and differentiating satellite cells that merge with existing fibroblasts (Giampietri et al., 2010; Park et al., 2010). Fortunately, the process can be halted, and possibly even reversed, by medical interventions such as treatment with angiotensin-converting enzyme inhibitors (Giampietri et al., 2010; Hellawell and Margulies, 2010).

There are still other reasons why results of the older studies are problematic. At the time it was simply not possible to measure what effects the drugs were exerting at the molecular level. For example, it is now obvious that users of some opiates, particularly those taking very large doses of synthetic racemic methadone, are at risk for QT interval prolongation and a potentially lethal arrhythmia known as torsades de pointes (Almehmi et al., 2004; Andrews et al., 2009; Karch, 2011). Certain polymorphs for CYP2D6 cannot metabolize the *S* form of methadone, and it is the *S* form that binds to the hERG potassium channel, thereby causing the QT prolongation and dispersion leading to torsades de pointes (Eap et al., 2007). But a decade ago no forensic pathologist had ever heard of QT dispersion or hERG potassium channels, nor was it any clearer to early researchers that heroin/morphine is, in fact, cardioprotective (Peart and Gross, 2004). In fact, the pendulum has now swung in the opposite direction. Laboratory limits of detection are far lower now than they were in the past and it is now possible to detect drugs, even though they are present in concentrations well below those associated with any physiologic change.

The relative scarcity of heart disease (excluding endocarditis in IVDUs) may be due to the cardioprotective effects of heroin/morphine. The mechanism involves a process known as *preconditioning*. If the heart is exposed to sublethal myocardial ischemia, it is actually protected from later, severe ischemic insults for periods as long as 72 h. The protected period can be divided into two phases, acute and chronic. During the first 24 h of myocardial protection is conferred by the increased production of inducible nitric oxide. During the second, longer phase, protection is the result of increased cyclooxygenase-2 production. Treatment with other opioids produces exactly the same results and the same degree of cardioprotection as ischemic preconditioning (Peart et al., 2011).

The frequency with which any particular cardiac lesion is observed is also a function of the pattern of drug abuse within the population being studied. When Rajs and Falconer (1979) reviewed the cardiac pathology in a group of 25 IVDUs, they found contraction band necrosis, fibrosis, and inflammatory cells in the myocardium, but amphetamine, not opiate, abuse was common in the population being studied, and the changes observed by Rajs and Falconer are consistent with that fact. In some areas, especially parts of Europe, the injection of pills meant for oral use is still a very common practice (and anecdotal evidence suggests that popularity is increasing in the United States again as well). Where this

practice is popular, granulomatous lung disease and pulmonary hypertension are common, and the spectrum of cardiac lesions seen at autopsy is likely to reflect that fact (Crouch and Churg, 1983). Fibrosis resulting from pulmonary talcosis seems ever more common (Marchiori et al., 2010), and it appears that even cocaine *snorting* can lead to the same outcome (Mancano et al., 2008). As drug abuse is now a universal phenomenon, the picture has become far less clear, and it has become harder and harder to generalize about what pathologic changes are to be expected. The observation that levamisole is now being used to adulterate some heroin (Schneider and Meys, 2011) complicates the picture even further. Chronic exposure to levamisole can induce pulmonary hypertension that may affect the heart secondarily.

The frequency of incidental cardiac lesions in addicts dying of trauma has never been tabulated. Because of the HIV pandemic, heart disease in intravenous opiate users has come to be almost synonymous with HIV infection, though the picture of involvement has changed as treatment has improved. HIV/AIDS is more prevalent in people of working age, whereas heart disease and malignant neoplasms occur more frequently in people over 65 years of age, whether or not they are drug abusers, and no matter whether they are HIV infected. Since 1995, largely as a result of treatment advances, there has been a rapid reduction in the effect of HIV/AIDS on life expectancy, at least in the U.S. population. This is especially true for black males of working age (Sudano et al., 2006), but it would be a mistake to exclude HIV from any differential diagnosis.

5.12.2.2 HIV-Associated Cardiovascular Pathology

The role of HIV in cardiovascular disease is still evolving. The most recently published survey analyzed the deaths of 305 patients (roughly 10%) of 2943 South African adults on retroviral therapy. Acute sepsis (20%) was the most common cause of death followed by tuberculosis (18%) and *Mycobacterium avium* complex bacteremia (14%) (Karstaedt, 2012). However, as described later, any number of bizarre complications may be encountered.

In a separate study designed mainly to assess the effects of HAART, Hellberg et al. found that there had been no change in the incidence of heart disease from 1995 to 2008 (Helleberg et al., 2012). The clinical introduction of HAART has dramatically reduced mortality and morbidity in the HIV-positive population, which, ironically, has led to an increase in cardiovascular disease among this group of patients. The increase has come about chiefly as a result of age-related complications like cardiovascular diseases, causing increased morbidity and mortality (Table 5.30).

The relative contributions of HIV infection versus potential adverse effects of HAART on coronary heart disease risk remain unclear, but recent reports implicate both HIV infection per se and HAART therapy-induced metabolic derangements as being proatherogenic. HIV-infected individuals have an increased risk of coronary heart disease (CHD), possibly as a consequence of the viral infection itself, and quite possibly the use of HAART, or other

Table 5.30 Cardiac Findings in AIDS Patients at Autopsy, in Probable Order of Frequency

1. Pericardial effusion
2. Myocardial disease (cardiomyopathy, myocarditis, lymphoma, drug toxicity)
3. Pulmonary hypertension
4. Cardiac tumors
5. Endocardial disease

poorly characterized immune factors. Nonetheless, clinical presentations of CHD in HIV-infected patients tend to be different from those of the general population because patients present at much earlier ages (mean age 50 years) (Ho and Hsue, 2009).

5.12.2.3 HIV-Related Vascular Disease

It is estimated that by 2015 more than 50% of HIV-positive patients will be older than 50 years. Since age is a major unmodifiable cardiovascular risk factor, the risk for cardiovascular disease in this population will significantly and progressively increase. A large part of the risk for cardiovascular events appears to be a result of lipid abnormalities associated with HAART therapy. Lipid abnormalities may be related to viral infection, HAART, or both. HAART-treated patients have atherogenic lipid profiles comprised of low HDL cholesterol levels, hypertriglyceridemia, and increased levels of small LDL particles. In addition to the risks posed by hyperlipidemia, there is the added risk of inflammatory vascular disease (Triant, 2012) that is thought to be even worse than the disease produced by abacavir. Controlled trials have now shown that patients infected with HIV, when compared to noninfected controls with similar cardiac risk factors, have evidence of increased arterial inflammation, associated with circulating markers of monocyte and macrophage activation (Subramanian et al., 2012). Other studies have indicated the presence of platelet and endothelial dysfunction (Gresele et al., 2012).

The prevalence of lipid disorders in the HIV infected is very high (estimated at 20%–80%). All of these individuals appear to be subject to dyslipidemias, lipodystrophy syndromes, endothelial dysfunction, and associated metabolic events such as insulin resistance (Troll, 2011). These disorders include both pericarditis and myocarditis.

Coronary artery lesions in the HIV infected have features intermediate between the lesions observed in common coronary atherosclerosis and the type of disease associated with chronic rejection of cardiac transplants. Thickening of the media and increased production of elastic fibers have both been demonstrated (Tabib et al., 2000). The etiology of these changes remains obscure but increasingly appears to be a complication of treatment with protease inhibitors (Meng et al., 2002a). There is also emerging evidence that this group of drugs may even promote ventricular remodeling and left ventricular hypertrophy (Meng et al., 2002b; Mermis et al., 2011).

5.12.2.4 HIV Disorders of the Myocardium and Pericardium

Pericarditis may lead to pericardial effusion but rarely to tamponade (Moreno et al., 1997; Chen et al., 1999). Cardiomyopathy in the HIV-infected individual is often clinically silent, with asymptomatic left ventricular systolic dysfunction. Endocarditis is mainly seen in the HIV-infected individuals who also happen to be intravenous drug abusers. Pulmonary hypertension, potentially leading to right heart failure, is another recognized HIV complication (Porter et al., 2013). The incidence of cardiomyopathy and pericarditis in the HIV infected has been reduced by HAART. However, premature coronary atherosclerosis is now a growing problem.

Treatment with ARVs can also have serious metabolic consequences, not all that different from those seen in the noninfected suffering from metabolic syndrome. The prolonged use of protease inhibitors can cause lipodystrophy, a clinical syndrome of peripheral fat wasting, central adiposity, dyslipidemia, and insulin resistance. Underlying these symptoms may be all the other elements of metabolic syndrome, including hyperlipidemia, hyperglycemia, and hypertension, which is why these individuals are good candidates for

lipid-lowering therapies. Metabolic syndrome only complicates the issue of HIV treatment because it is very likely that the patient will be treated with lipid-lowering drugs that interact with the P450 system, leading to inactivation of some ARV agents (Sudano et al., 2006).

The incidence of pericardial effusion seems to be decreasing. In a 2009 echocardiographic study of asymptomatic patients, fewer than 12% were found to have effusions (Aggarwal et al., 2009). Effusions large enough to cause tamponade are extremely uncommon. In developing countries the effusion is more likely to be the result of tuberculosis, but in the industrialized world, it is more likely to be a consequence of myocarditis (Moreno et al., 1997; Nemunaitis et al., 2009). Underlying Kaposi's sarcoma-associated lymphoma has, since its discovery in 1994, along with the sarcoma-associated herpesvirus (KSHV), been associated with lymphoproliferative disorders, particularly in patients infected with HIV. The disorders most strongly linked to KSHV are multicentric Castleman's disease (MCD), primary effusion lymphoma, and diffuse large B-cell lymphomas. Figure 5.39 shows a myocardial biopsy.

Since their discovery in 1994, an array of lymphoproliferative manifestations of KSHV infection have been recognized, mostly in HIV-infected patients. The best characterized diseases are MCD consisting of diffuse large B-cell lymphomas accompanied by primary lymphoma effusion (Nador et al., 1996).

Since the introduction of HAART, there has been a 30% reduction in the prevalence of HIV-associated cardiomyopathy, possibly related to a reduction of opportunistic infections and myocarditis. As previously indicated, some HAART regimens, especially those including protease inhibitors, can cause metabolic syndrome (HIV lipodystrophy syndrome). This syndrome is linked to a high frequency of cardiovascular events that could culminate in ischemic cardiomyopathy. Whatever the fundamental etiology, the most common outcome is clinically silent left ventricular systolic dysfunction, a finding that cannot be proven of disproven at autopsy.

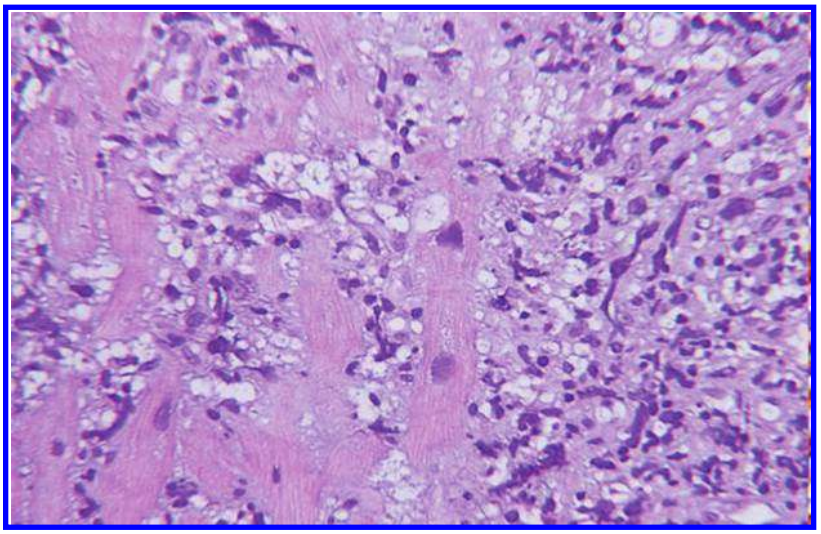


Figure 5.39 Primary cardiac B-cell lymphoma with atrioventricular block and paroxysmal ventricular tachycardia from HIV patient. (From Chen, K.W. et al., *J. Cardiothorac. Surg.*, 7, 70, 2012. With permission.)

An association between cardiomyopathy and severe mitochondrial damage in an HIV-infected patient, treated with nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), was described in 2002 (Frerichs et al., 2002). The mitochondrial toxicity results from pyrimidine NRTIs used to treat HIV/AIDS. In the heart, this can deplete mitochondrial DNA and cause cardiac dysfunction, including left ventricular hypertrophy (Kohler et al., 2009). How often this problem occurs is not known.

Myocarditis occurs, but it is often as a consequence of the treatment (HAART), not the disease itself. Before HAART became available, myocarditis was evident at autopsy in 40%–50% of those with AIDS. The incidence is now considerably lower. Histologic findings in HIV-infected patients with myocarditis do not appear any different than myocarditis in the general population. Lymphocytes, along with smaller numbers of macrophages, may be focal or more widely distributed through the heart. If there is any apparent difference between the HIV infected and the general population, it is simply that the infiltrate will be more widely distributed. Primary involvement of the conduction system also appears more frequently (Barbaro, 2005). Histologic and immunohistochemical studies rarely detect the presence of viruses in the myocardium, though their DNA may be identified by resequencing. Among patients with myocarditis, coinfection with coxsackievirus B3 will be evident in nearly a third, Epstein–Barr virus in 8% and cytomegalovirus in 4% (Klatt, 2003).

The most common opportunistic infectious agent causing myocarditis in AIDS is *Toxoplasma gondii*. In the early 1990s the incidence was on the order of 12%, though it has fallen considerably since then. Microscopically, the myocardium shows cellular infiltrates, polymorphonuclear leukocytes, macrophages, and lymphocytes. *T. gondii* infection may not always evoke intense inflammation but usually is associated with muscle fiber necrosis. The response generally falls into one of three patterns: acute diffuse myocarditis, focal myocarditis, or the presence of organisms without significant inflammation or necrosis. The extracellular cysts, or pseudocysts, are always located within the myocardial fibers (Klatt, 2003; Barbaro, 2005).

Primary pulmonary hypertension is seen in between 0.5% and 12% of patients with HIV infection, much higher than the general population where the yearly incidence is only approximately one to two per million people (Sudano et al., 2006). While it is certainly true that repeated opportunistic pulmonary infections can cause right ventricular dysfunction and cor pulmonale, there does not appear to be a connection between opportunistic infection and pulmonary hypertension in these patients (Mesa et al., 1998), although this assumption too has been called into question (Frustaci et al., 2014).

The histopathology of HIV-associated pulmonary hypertension is similar to that of primary pulmonary hypertension. The most common alteration observed is HIV-associated plexogenic pulmonary arteriopathy. Thrombotic pulmonary arteriopathy and pulmonary veno-occlusive disease also occur but are much less common than the arteriopathy. Chronic release of cytotoxic cytokines (e.g., endothelin-1 [ET-1], interleukin-6, interleukin-1β, and TNF-α) leads to progressive tissue damage, regardless of HAART (Barbaro, 2004). Pulmonary arterial hypertension (PAH) represents one of the most severe complications of HIV infection and it may occur at any stage of the disease. Its occurrence does not seem to be related to the degree of immune deficiency. Many of the symptoms in HIV-PAH result from right ventricular dysfunction (Cicalini et al., 2011). If the HIV-infected patient is an intravenous drug abuser, then the possibility also exists that he has been using heroin adulterated with levamisole. The combination can also cause pulmonary hypertension (Karch et al., 2012).

Myxoma, fibroelastoma, sarcoma, and lymphoma all occur in the hearts of the HIV-infected individuals, and the incidence of lymphoma seems to be increasing (Carbone et al., 2005; Zhang et al., 2010; Andrews et al., 2011). Kaposi's sarcoma generally occurs in the setting of widespread mucocutaneous disease (Stotka et al., 1989). It is very rare for the heart to be the only organ infected, and the lesions are likely to be asymptomatic. The pericardium, epicardium, and myocardium can all be involved and there may also be a pericardial effusion. Primary cardiac lymphomas, especially B-cell lymphomas of the right atrium, have a very poor prognosis.

5.12.2.5 Endocarditis

After HIV infection, infectious endocarditis (IE) is the only other cardiovascular disease where there is overwhelming evidence that the incidence is clearly higher among intravenous drug abusers than in the general population (Table 5.30), although the numbers appear to be changing. In developed countries IE is now affecting older patients and patients with no previously known valve disease. The occurrence of prosthetic IE (prosthetic valve endocarditis [PVE]) and endocarditis in patients with implanted devices such as pacemakers and internal defibrillators complicates the picture (Figure 5.40). At the same time, the number of cases involving *S. aureus* is increasing, and so is the number of cases that can be related to health care-associated procedures, especially in diabetics or patients on chronic hemodialysis. These changes in the general population probably explain the increasing numbers of cases that have been reported (Tornos et al., 2011).

Overall, *S. aureus* is the most common organism causing IE (>70% of all cases) (Table 5.31) and its incidence has been steadily increasing in recent decades (Baddour et al., 2005; Fowler et al., 2005; Tleyjeh et al., 2007). It is felt that the increase has largely to do with the increasing use of intravenous catheters and devices (Baddour et al., 2005). *S. aureus* IE tends to involve the mitral valve more often than the aortic, and the mitral valve is the one most commonly involved when IE occurs in IVDUs (Fowler et al., 2005).

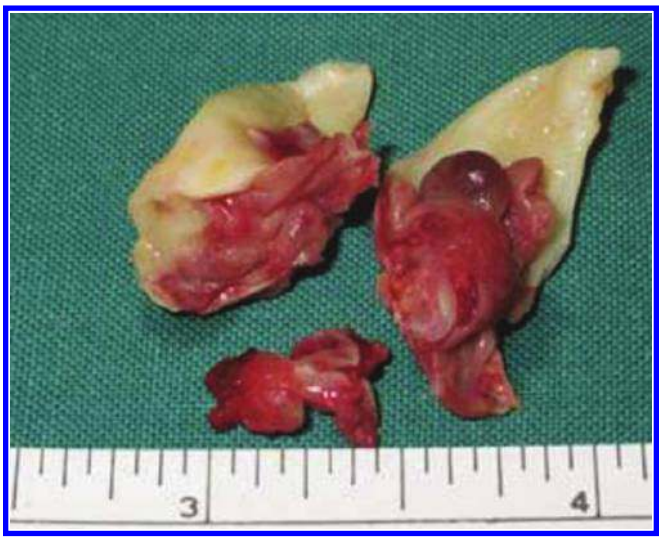


Figure 5.40 Removed tricuspid value with vegetation detached. (From Chong, E. et al., *Singapore Med J*, 50, 34, 2009.)

Table 5.31 Pathogens Reported in Addicts with Infectious Endocarditis

1. *S. aureus* (>50%)
2. Coagulase-negative staphylococci (6%)
3. Streptococci (30%) mainly *viridans* streptococci, but also *S. mitis*, *S. mutans*, *S. salivarius*, *S. sanguis,* and the *S. intermedius* group (*S. intermedius*, *S. anginosus*, and *S. constellatus*)
4. Enterococci (10%)
5. Gram-negative bacilli (5%); includes *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella*

Among IVDUs, the tricuspid valve is still more commonly involved than the pulmonic valve but the reason why is not understood. In the general population, the left heart is involved more than 90% of the time, but IVDUs are much more likely to have right-sided involvement. They also typically tend to be younger and without other underlying disease, such as diabetes. They typically present with symptoms of septic pulmonary emboli, including pleuritic chest pain, dyspnea, and hemoptysis. Pulmonary emboli are common.

Autopsy studies indicate that most (>80%) vegetations occur on previously normal valves (Dressler and Roberts, 1989). The results of echocardiographic studies suggest that the valves of intravenous heroin users, even those with no clinical evidence of endocarditis, are abnormal: small areas of thickening on both the mitral and tricuspid valves are often present (Pons-Llado et al., 1992). Similar changes have been observed in intravenous cocaine users, where nearly half of the patients studied showed, at a minimum, valve thickening (Willoughby et al., 1993). The findings suggest that some type of endothelial trauma must occur to allow deposition of the microscopic thrombi, which, in turn, constitute the first stage of infection. Some studies have found that those with antiphospholipid syndrome, which results in valvular thickening, may be more at risk (Tincani et al., 2005; Blank et al., 2005).

While most vegetations occur on normal valves, it is important to remember that they may also occur on prosthetic valves and that they are not always due to heroin injection. Figure 5.41 was taken from a drug abuser who injected buprenorphine into his axilla.

The origin of the infectious agent in intravenous users is a matter of dispute. Addicts seldom practice sterile techniques: the needles they use may be contaminated, and the injected material is likely to be unsterile. The injection site, especially if the groin is chosen (Roszler et al., 1989), may be colonized with pathogenic organisms. Thus, a number of possible sources for infection exist. With the exception of *Candida* infection (Dupont and Drouhet, 1985), studies have failed to link the heroin itself, or the paraphernalia used, to any particular infectious organism (Tuazon et al., 1974; Risser et al., 2007). More often than not, the infectious organism is derived either from the addict's normal surface flora (Hubbell et al., 1981) or from a preexisting infection such as cellulitis or suppurative thrombophlebitis.

Platelet deposition, no matter what the cause, damages valvular epithelium, exposing the matrix of subendothelial connective tissue below, and allowing the further deposition of fibrin and platelet thrombi. Vegetations are friable, white or tan, and most likely to be found along the line of valve closure. Bacterial vegetations tend to arise on the atrial aspect of the atrioventricular valves and on the ventricular surfaces of the aortic and pulmonary valves. With time, they may proliferate and involve the opposite side of the valve or spread to the chordae tendineae or onto the parietal pericardium. The lesions ulcerate, and the ulcerations seen in acute endocarditis tend to be larger and deeper than those associated with subacute disease (Silber, 1987). To some degree, the size, color, and appearance of the vegetations depend on the type of infectious agent responsible. Fungal lesions tend to be

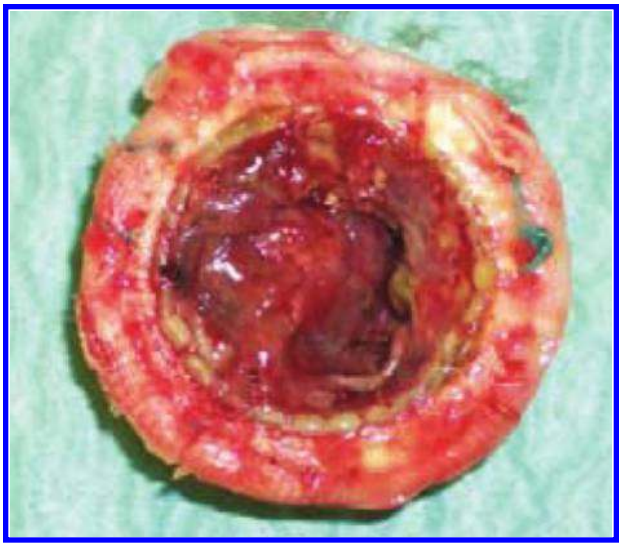


Figure 5.41 Infected prosthetic tricuspid valve ring with abscess. (Reproduced from Chong, E. et al., *Singapore Med J*, 50, 34, 2009.)

larger and bulkier than bacterial vegetations and are more likely to cause valvular insufficiency and embolization. Streptococcal vegetations grow more slowly than staphylococcal vegetations, but they may eventually be much larger (Ciliberto et al., 1999; Ellis et al., 2001).

Vegetations much smaller than those seen with bacterial or fungal infection are seen at autopsy in approximately 2% of severely cachectic patients. The lesions are sterile, and the process of their formation is generally referred to as nonbacterial thrombotic endocarditis (NBTE; also marantic endocarditis) (Angrist and Oka, 1963; Eftychiou et al., 2005). This is a rare disorder that tends to be associated with hypercoagulable states or advanced malignancy such as adenocarcinomas (Mazokopakis et al., 2010). It also occurs in association with autoimmune disorders, but here too, diagnosis during life is rare unless the verrucae embolize and cause a thromboembolic event (Reisner et al., 2000). NBTE associated with systemic embolism usually occurs as a complication of advanced or terminal malignancies, and it appears to be associated more with some malignancies than others (Eftychiou et al., 2005).

The verrucae of NBTE are composed of bacteria-free, amorphous material. Depending on how much fibrin has been deposited, the color of the vegetations can range from white to tan or gray. On microscopic examination, the lesions of marantic endocarditis are easily distinguished from those of IE; masses of fibrin, platelets, and polymorphonuclear leukocytes can be seen surrounding colonies of bacteria located directly on the surface of the valve. Necrotic areas of valve become surrounded with a mixed cellular infiltrate that often includes giant cells. In older lesions, capillary proliferation occurs, along with the formation of granulation tissue (Saphir et al., 1950). Fibrous tissue eventually proliferates over the vegetations, and the necrotic material becomes organized and endothelialized. Healed lesions are often calcified.

Until recently, polymicrobial infection was a distinctly rare entity. In one retrospective study of nearly 1000 patients seen from 1951 to 1966, only one case was found (Lerner and

Weinstein, 1966). In more recent reports, the incidence has crept from 8% (Crane et al., 1986) to as high as 15% (Saydain et al., 2010) and even above 20% in individuals with hospital-acquired disease from central venous catheters (Chrissoheris et al., 2009). As many as seven or eight different organisms may be involved at one time, and because many of these organisms are quite fastidious, all may not be diagnosed by routine laboratory methods (Mah and Shafran, 1990; Adler et al., 1991; Tran et al., 2007). In recent years infection with *Neisseria sicca/subflave* (*N. sicca*) appears to have increased, though the reason is not apparent (Mehrzad et al., 2013).

Right-sided cardiac involvement results in symptoms that are more pulmonary than cardiac in nature. Dislodged vegetations frequently embolize to the lung, producing multiple segmental infiltrates, especially in the lower lobes (Chan et al., 1989). Tricuspid vegetations can, on occasion, grow quite large and may even interfere with valve function. Papillary rupture, on the other hand, produces relatively few symptoms on the right because of the low intracavity pressure (Conway, 1969). Aneurysm of the sinus of Valsalva may result when infection dissects into the valve ring. This process is most often seen in cases of staphylococcal infection. Staphylococcal infections may also extend outward from the ring, resulting in ring abscess, and the infection may also spread to involve the interventricular septum (Conde et al., 1975). Lethal arrhythmia can result.

Extension of a valvular infection outward may result in purulent pericarditis or even cardiac rupture. In fact, it is said that purulent pericarditis occurs in nearly 20% of all cases of endocarditis, even without the rupture of any large abscess (Silber, 1987). Smaller abscesses may be scattered throughout the myocardium, and even though abscess formation is more common in cases of acute endocarditis, it may be seen in subacute cases as well. Abscesses may be subendocardial or subpericardial but are most likely to be found in the left ventricle (Arnett and Roberts, 1976). A spectrum of other myocardial alterations short of frank abscess formation can also be seen. In acute cases there may be cloudy swelling of the myocytes, hemorrhage, or even tiny areas of infarction. Small infarcts occur in subacute cases where microemboli obstruct distal branches of the coronary arteries (Saphir et al., 1950).

The peripheral sequelae of valve infection have changed little since Osler described them in the Goulstonian Lectures in 1885 (Ostler, 1886). The most frequent complications associated with endocarditis in addicts are the same as those in the general population who suffer with endocarditis. Many of the extracardiac manifestations are the result of arterial embolization of friable vegetations. Mycotic aneurysm results in septic emboli, most of which occur at the bifurcation of medium-sized arteries. This process is especially common in the brain but can also occur elsewhere. In the kidneys, septic emboli can cause infarction, especially when *Staphylococcus* is the etiology. Glomerulonephritis is seen in more than half of the patients and is the result of immune complex deposition (Bell, 1932). In addition to the classic focal embolic changes seen in the kidneys of patients with endocarditis, diffuse proliferative glomerulonephritis may also be seen. In these latter cases, there is strong evidence for an immune-related etiology. It may well be that other peripheral lesions, such as Roth's spots and even Osler's nodes, are immune in nature (Bayer and Theofilopoulos, 1990).

If there is any suspicion that a decedent was suffering from IE, aseptic techniques should be used at autopsy to ensure the collection of uncontaminated material. The major

vessels should be clamped before removing the heart from the body. An area on the surface of the heart adjacent to the affected valve (e.g., entrance through the posterior right atrial wall would give access to the tricuspid valve) should then be seared and the center of the area incised with a sterile scalpel, allowing direct access to the valve, which can be sampled and cultured. If such an approach is not followed, the samples obtained may well be contaminated. In addition to routine Gram stains, slides should also be stained for fungi (Gomori stain) and for acid-fast organisms.

In the HIV-infected individual, a whole spectrum of agents can cause endocarditis. These range from multiresistant *Candida* to *Histoplasma* to *Torulopsis* to *Bartonella* (Jinno et al., 2010; Kumar et al., 2011; Solomons, 2011) but the most common remains *S. aureus*.

5.12.2.6 Myocardial Fibrosis

Interstitial fibrosis is also a frequent finding in the hearts of drug abusers (Figures 5.42 through 5.44). Certain patterns of fibrosis play a role in the generation of malignant rhythm disorders and sudden cardiac death (Strain et al., 1983; John et al., 2004). Microfocal fibrosis is most typically seen in stimulant abusers (Rajs and Falconer, 1979), where it results partially from healing contraction band necrosis secondary to catecholamine excess (Szakacs et al., 1959), partly from cytokine-induced damage from differentiating fibromyocytes (a type of interstitial cell that responds to injury) (Vracko et al., 1989), and partly from myocardial remodeling caused by cocaine activating calmodulin kinase II (Henning and Cuevas, 2006). Whether it is also caused directly by heroin and the chronic abuse of other opiates is still not known. Fibrosis is also seen in roughly 2% of young adults with sudden

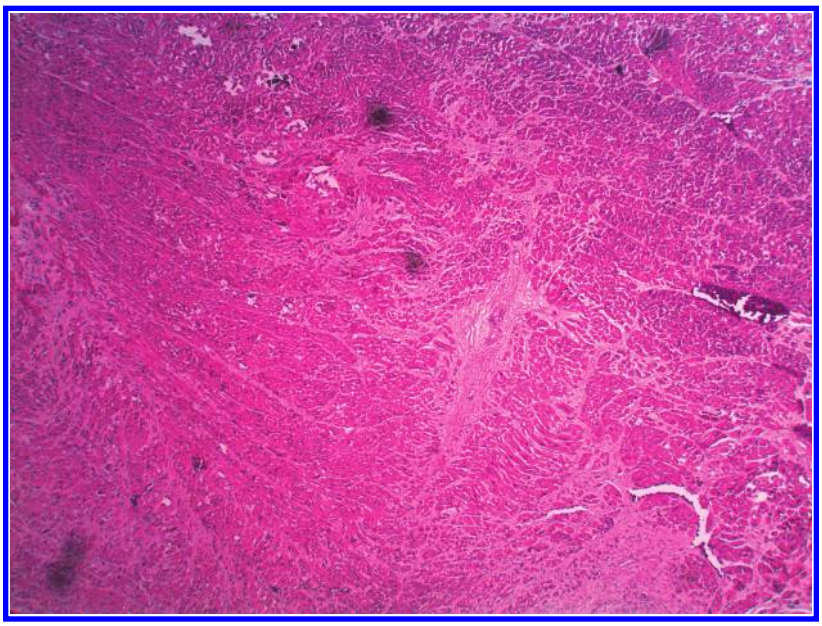


Figure 5.42 Like cocaine abusers, heroin users are subject to myocardial fibrosis that can, on occasion, be extreme. Note the interlacing fingers of dense fibrosis and the fatty infiltrate that is thought to be directly produced by heroin. Alternatively, the presence of fibrosis may simply reflect the fact that most heroin abusers are polydrug users who also take cocaine and similar stimulants. (Micrograph by Author.)

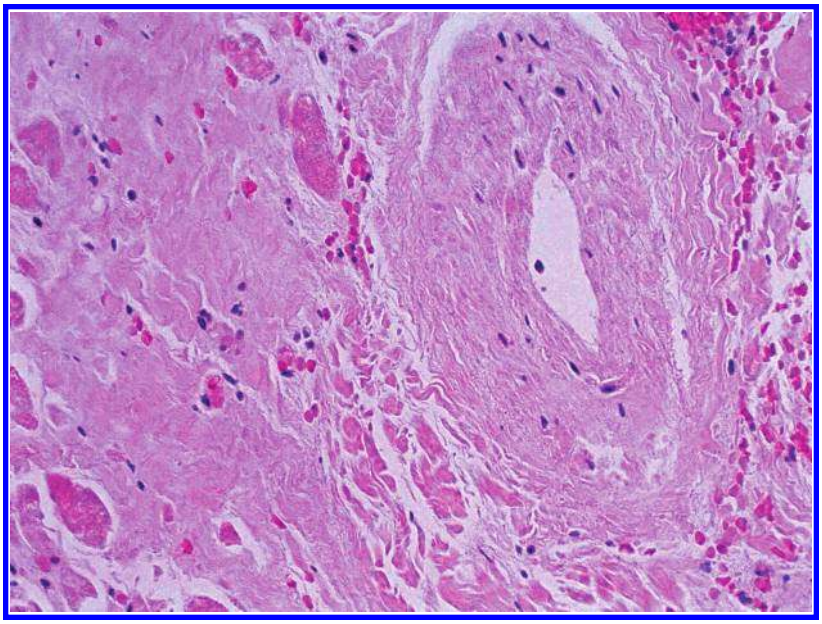


Figure 5.43 Chronic opiate abusers manifest most of the features of *myocardial remodeling*. Note the endothelial proliferation reducing the lumen of a small intramyocardial artery. Note also the presence of transforming fibroblasts and perivascular fibrosis—fibrosis prevents the normal occurrence of *flow-mediated dilation*. (Micrograph by Author.)

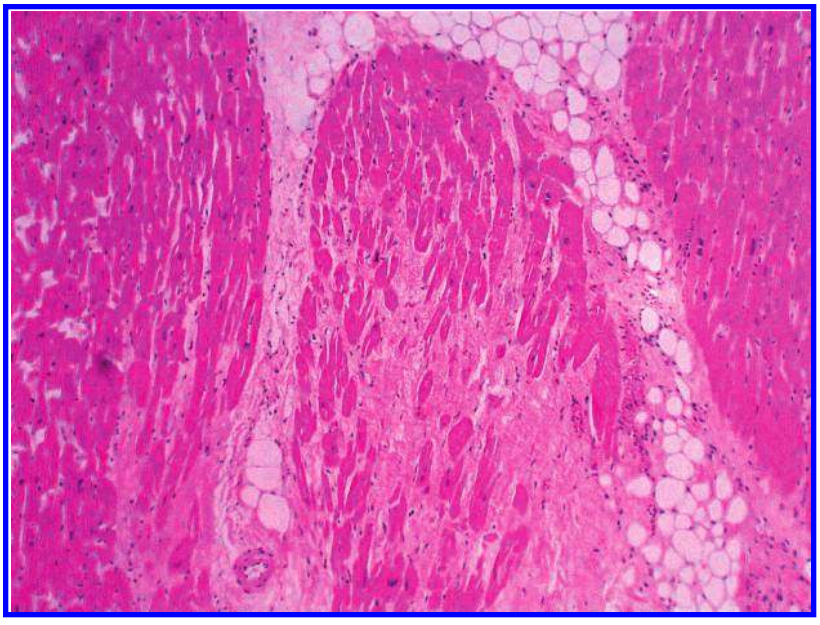


Figure 5.44 Most of the features commonly seen in opiate abusers are obvious in this illustration, including fibrofatty replacement and interstitial fibrosis that is extensive. The decedent was probably also a stimulant abuser as myocyte hypertrophy is obvious from the presence of the enlarged myocytes and abnormal cell nuclei. (Micrograph by Author.)

death and no other obvious risk factor (Lecomte et al., 1993). Fibrosis is a well-known complication of Kawasaki disease (Yu et al., 2014) and hypertrophic cardiomyopathy (Chan et al., 2014), but more often than not, drug abusers combine narcotic and stimulant drugs, so it may well be that the occurrence of myocardial fibrosis in opiate abusers is just the result of stimulant cardiotoxicity.

Endocarditis can be the outcome of many disorders, not just healed myocarditis; uncontrolled hypertension with ventricular remodeling is perhaps more likely to cause this change than simple healing of myocarditis. The mechanism in non-catecholamine fibrosis involves increased expression of the proteinases (urokinase-type plasminogen activator and matrix metalloproteinases) (Heymans et al., 2006). The mechanisms responsible for other forms of interstitial myocardial fibrosis are not so clear, but seem to involve increased production of tissue growth factor-β (John et al., 2004).

Larger zones of fibrosis are likely to represent healed areas of prior ischemic infarction. Large zones of fibrosis could also be related to emboli that may cause infarction in some of the smaller coronary artery branches (Silber, 1987), but this is relatively uncommon. The detection of fibrosis is not simply an incidental finding; it may very well be the cause of death. Viable muscle tissue trapped within scar tissue can give rise to reentrant circuits, leading to fatal arrhythmias (Nakahara et al., 2010). When identified in life, these areas are increasingly treated with ablation.

5.12.2.7 Myocardial Hypertrophy

Right-sided cardiac enlargement in intravenous drug abusers who have lung disease is to be expected; the injection of contaminated heroin and crushed pills will diminish the size of the pulmonary bed. However, the finding of hypertrophic myocardium in a pure heroin abuser is most likely to reflect untreated hypertension, concurrent cocaine use, or previously undiagnosed idiopathic hypertrophic cardiomyopathy. As more and more heroin users also abuse cocaine, and some methamphetamine, the latter explanation becomes more likely. Whereas cocaine activates calmodulin kinase and therefore causes myocyte enlargement, hypertrophy in methamphetamine abusers is more likely to be a consequence of chronic catecholamine excess and/or HIV disease. It is clear that methamphetamine activates the *d*-calcium (high-voltage-activated) channel of neurons, and there is also evidence that methamphetamine inhibits rat myocardial function, but whether it does the same things to *d*-calcium channels in humans is not clear (Kanyshkova et al., 2014).

5.12.2.8 Coronary Artery Disease

No mention of coronary artery disease is made in any published autopsy series of heroin abusers (Halpern and Rho, 1966; Siegel and Halpern, 1966; Louria et al., 1967; Froede and Stahl, 1971; Wetli et al., 1972). Whether the incidence of coronary artery disease in heroin addicts is any different from that in age-matched controls is not known. It is known that intravenous heroin abusers have abnormal, atherogenic lipid profiles (Maccari et al., 1991; Sztajzel et al., 1994), just as it is known that heroin abusers die at a younger age than the rest of the population (Figure 5.45). Interestingly, there is also evidence that long-term opiate use may prevent against coronary artery disease. When the degree of coronary artery disease was measured in 98 MMT patients and 97 controls, the MMT patients were found to have significantly less coronary artery disease (Marmor et al., 2004).

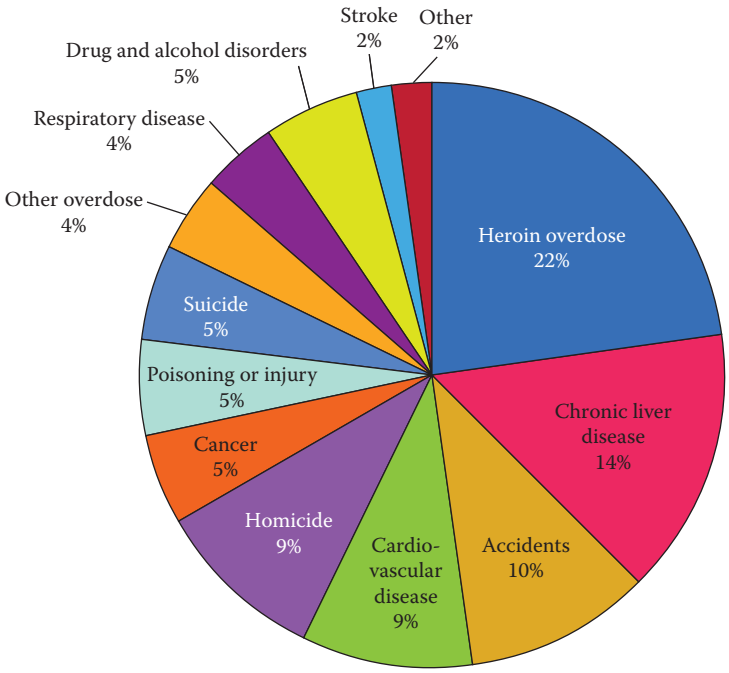


Figure 5.45 The life span and cause of death in heroin users, from 1962 to 1967. (From National Institute on Drug Abuse, Research Report Series, Heroin, available online at http://www.drugabuse.gov/publications/research-reports/heroin/scope-heroin-use-in-united-states, last accessed August 28, 2014, 2014.)

Morphine is one of several opiates that protect the heart against ischemia and reperfusion injury. Opiates do this by μ-receptor activation (Gross, 2003). When given intravenously, prior to ischemia or reperfusion, morphine (Schultz et al., 1996), fentanyl (Lessa and Tibirica, 2006), and remifentanil (Zhang et al., 2004) reduce myocardial infarct size and preserve myocardial function. This protective effect is even exerted when the opiate is given intrathecally, a sort of remote preconditioning (Wong et al., 2010), but the ultimate mechanism is not known. The feeling among many, however, is that a link between the endogenous release of opioids and the production of heart shock proteins mediates cardioprotection (Patel et al., 2002). This situation is analogous to the situation seen in chronic methamphetamine abusers who are subject to accelerated atherosclerosis but, because methamphetamine induces heat shock protein, suffer relatively few infarcts (Karch et al., 1999).

There are case reports describing coronary spasm and acute myocardial infarction in heroin abusers, where acute myocardial infarction occurred after the injection of heroin, but there is no way to tell whether the episodes were due to the heroin, an adulterant, concurrent myocarditis, or some direct effect of heroin on coronary arteries (Yu et al., 2004). However, it is known that nearly all heroin users smoke cigarettes, if not marijuana as well.

5.12.3 Pulmonary Complications

5.12.3.1 Pulmonary Edema

The first person to describe narcotic-related pulmonary edema was a physician named Lee who was in practice in New York City during the 1850s, when he first encountered a case of narcotic overdose. Lee noted the simultaneous occurrence of cerebral edema and

pulmonary congestion in a man dying from a laudanum (morphine) overdose (Ostler, 1886). One hundred and fifty years later, the mechanism of narcotic-induced pulmonary edema remains an enigma. It is generally presumed that pulmonary edema in heroin abusers is in some way related to respiratory depression and respiratory failure, although there are those who still adhere to the notion that it is the result of an allergic or anaphylactic reaction (Edston and van Hage-Hamsten, 1997; Dettmeyer et al., 2000; Perskvist et al., 2007) or histamine release (Brooks, 1999). All opiates decrease the responsiveness of brain stem respiratory centers to increased concentrations of PCO_2, and if enough narcotic is given, the respiratory drive disappears. In practice, postmortem examination will reveal pulmonary congestion of varying degrees, but not always florid pulmonary edema.

The edema fluid associated with narcotic overdose is rich in protein (Figure 5.46a through c). Agonal respiratory efforts will cause the fluid to froth, much like beaten egg white. In extreme cases, congealed froth is seen in the mouth and nares. In one large autopsy series, the average weights of the right and left lungs were 830 and 790 g, respectively (Levine and Grimes, 1973). In the case series reported by Siegel and Halpern in 1966, the average total was slightly lower (1400 g) (Siegel and Halpern, 1966). Fluid accumulation occurs in a lobular distribution, with areas of congestion and edema alternating with other areas of air trapping and acute emphysematous change. The posterior lower lobes are most severely affected, especially if gastric aspiration has also occurred. Histologic examination may reveal a spectrum of changes. In less severe cases, the only abnormality found will be widening of the interstitial spaces, especially around the bronchi and extra-alveolar vessels (Pietra, 1991). In more extreme cases, the alveolar spaces become flooded with protein-rich fluid.

If there is enough time for hypoxic heart failure to occur, blood vessels in the nose and pharynx rupture, giving a pink tinge to the edema fluid. After 24 h, hyaline membranes will be visible in the alveoli. They are composed of necrotic alveolar cell debris, mixed with

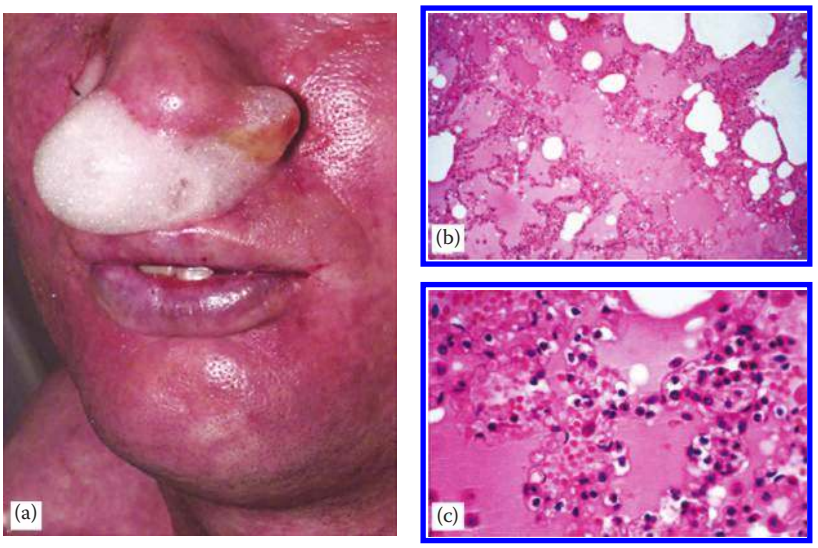


Figure 5.46 (a–c) Pulmonary edema; the pink staining material that fills the alveoli is highly proteinaceous and typical of narcotics overdose. Because of the high protein content of the edema fluid, it will form froth that may exude from the upper airway and even out of the mouth of the cadaver. (Reprinted from *Curr. Diagn. Pathol.*, 10, J.F. Tomashefski Jr., J.A. Felo, The pulmonary pathology of illicit drug and substance abuse, 413–426, Copyright 2004, with permission from Elsevier.)

the protein-rich edema fluid deposited on the alveolar walls. This phase is followed by a recovery phase. During this final phase, the cut surface of the lung will be firm and brownish, suggesting the diagnosis of pneumonia. Type II alveolar cells and fibroblasts proliferate, and the fibrinous exudate in the alveoli is replaced by granulation tissue (Kringsholm and Christoffersen, 1987). The microscopic appearance of the lung tissue cannot be overemphasized, as the mere presence of pulmonary edema is a nonspecific finding. Increased lung weight could equally well be attributed to the presence of fluid administered during resuscitation (Ornato et al., 1985). It could also be a consequence of mechanical ventilation that worsens lung edema and inflammation through the syndrome of ventilator-induced lung injury (Jurek et al., 2014).

Studies of heroin users published in the older literature frequently describe thickening of the alveolar septa, fibrosis, and as hypercellularity, with hemosiderin-laden macrophages (see Figure 5.60) often found to be present in the alveolar walls and even in the lamina of the alveoli and respiratory passages (Rajs et al., 1984). More recent studies point to the frequent finding of hyperplastic pulmonary perivascular lymphatic tissue. At one time, it was thought that the presence of hemosiderin-containing macrophages was nearly diagnostic for chronic heroin use, but because so many heroin abusers also smoke "crack" cocaine, such conclusions are no longer warranted since they look almost identical.

Sputum from "crack" smokers is usually turbid, gray, or even black, and considerably darker than sputum seen in heavy tobacco smokers dwelling in the same urban environment. "Crack" smokers tend to have carbonaceous sputum and, not infrequently, emphysematous changes in their lungs (Klinger et al., 1992). Carbon-laden macrophages can also be found in the pleural fluid of "crack" smokers who develop malignancy or HIV-related pulmonary disease (Singh et al., 1995), and small intrapulmonary hemorrhages are also common in this subgroup. The pattern is obvious in microscopic sections, even before the sections are placed under the microscope; it is highly reminiscent of the pattern seen in "coal miner's lung" or, for that matter, the changes seen in "crack lung." When the pattern of injury produced by cocaine smoking is superimposed on the pattern of injury produced by intravenous heroin abuse, the resultant picture is difficult to predict (Forrester et al., 1990; Bailey et al., 1994; Gallouj et al., 1999; Milroy and Parrai, 2011).

One of the more puzzling features of this syndrome is why some individuals with opiate-induced respiratory failure should develop florid pulmonary edema and others do not. In a retrospective review of 1278 cases of heroin overdose treated in a large urban emergency room over a 53-month period, only 27 patients met the criteria for the diagnosis of noncardiogenic pulmonary edema (Sporer and Dorn, 2001). It is not impossible that some of our basic observations are, if not mistaken, at least incomplete. Polish researchers studied 41 heroin-related deaths. Every one tested negative for α_1-antitrypsin, but each displayed alveoli and alveolar ductules that were enormously expanded by fluid, each containing numerous air bubbles of variable size, some of which were quite large. Perhaps not surprisingly, given the patient population, analysis of the fluid showed that it contained multiple hemosiderin-loaded macrophages (Krus et al., 2010).

Nearly 50 years ago it was suggested that heroin had direct toxic effects on pulmonary capillaries or even the heart, leading to hypoxia-induced heart failure (Menon, 1965). A role for altered capillary permeability is suggested by the fact that the protein content of the edema fluid is almost twice that of serum (Katz et al., 1972), but immunochemical studies of IgE, collagen IV, and laminin have failed to disclose any abnormalities in the capillary membranes (Dettmeyer et al., 2000). Other theories that have been proposed include

acute allergic reactions to heroin and the presence of contaminants in the heroin, causing histamine release, a centrally mediated effect, or locally induced cardiovascular disruption (Katz et al., 1972). This last possibility seems unlikely, given that whenever hemodynamic measurements have been made in these patients, the only abnormality detected is moderately elevated PA pressure.

The picture has recently been complicated with the recognition of takotsubo cardiomyopathy, also called transient left ventricular apical ballooning or ampulla cardiomyopathy. Pulmonary edema occurring simultaneously with the cardiac changes was first described a decade ago (Daly and Dixon, 2009). In this instance, the edema would appear to be cardiogenic, especially because this disorder is seen particularly in women of postmenopausal age. Takotsubo derives its name from a type of left ventricular dysfunction that manifests a distinctive shape on the end-systolic left ventriculogram, reminiscent of a *takotsubo*, the type of octopus trap used in Japan.

Takotsubo is characterized by a peculiar, yet characteristic, combination of transient regional systolic dysfunction, mainly of the left ventricular apex and midventricle, with hyperkinesis of the basal left ventricular segments. Despite the ventricular dysfunction, there is absence of obstructive atherosclerotic coronary disease. There is much conjecture but little evidence to explain the etiology of the syndrome. Several possible mechanisms have been suggested to account for these changes, including multivessel epicardial spasm, microvascular coronary dysfunction, catecholamine-induced injury, and neurohumoral-related myocardial stunning (Talawarm and Mahajan, 2007). Emotional stress including sudden accidents, natural disasters, death, or the funeral of a family member, in addition to other triggering factors, such as cerebrovascular accidents, epileptic attacks, and noncardiac surgery, might play a key role in the development of this reversible cardiomyopathy, but the precise etiologic mechanisms remain unidentified. Patients with takotsubo cardiomyopathy have been shown to possess higher catecholamine levels than other patients with acute myocardial infarction of the same severity (Wittstein et al., 2005). Most of the theories involve elevated catecholamines, and the most plausible explanation, at least for the moment, is myocardial stunning induced by catecholamine excess.

Alveoli normally stain negatively for laminin and type IV collagen, but in disease states, when membrane destruction is present, both compounds leach out. When stained with IgE antibodies, lung specimens from opiate overdose victims do not appear significantly different from controls, which is just the opposite of what would be expected if the edema was due to anaphylaxis. For the present, the most plausible explanation seems to be the one that was first proposed more than 30 years ago: respiratory depression leads to hypoxia, which in turn causes increased capillary permeability allowing fluid extravasation into the alveoli.

5.12.3.2 Needle and Mercury Emboli

In the remote past, drug abusers occasionally injected themselves with mercury in hopes of improving athletic and sexual prowess and, to some extent, this practice still occurs (Kobidze et al., 2014). Chest x-rays show small metallic opacities in the distribution of the pulmonary vascular bed, often with a small pool of mercury in the apex of the right ventricle (Cassar-Pullicino et al., 1985; Clague et al., 1989). Horrifying though the picture may be, the mercury will disappear over the course of time (chelation therapy is sometimes used to speed the process), and the injection is usually asymptomatic. The diagnosis is easily made with routine chest films. If a large amount is injected, however, pulmonary embolus may result.

Cases where needles have embolized to the lung have been reported, especially in drug users who resort to central venous sites for injections (Lewis and Henry, 1985; Angelos et al., 1986; Thorne and Collins, 1998; Shapiro et al., 2007). These emboli cause few deaths and are best managed conservatively. Patients usually have subcutaneous needle densities visible on radiographs at the site of venous self-injection.

5.12.3.3 Foreign Body Granulomas

Foreign particle embolization is frequent in intravenous drug abusers, but clinical symptoms are not. Granuloma formation (Figures 5.47 and 5.48) is an inconsistent but relatively frequent finding at autopsy (Halpern and Rho, 1966; Sapira, 1968; Gottlieb and Boylen, 1974; Glassroth et al., 1987; Wolff and O'Donnell, 2004). Granulomas form when drug users repeatedly inject themselves with aqueous suspensions of pharmaceutical preparations designed for oral administration. Heroin has been available since the turn of the century, and morphine for nearly 200 years, but pulmonary granulomatosis occurring in the lungs of drug users was only first described in 1950 (Spain, 1950). The time lapse suggests that the injection of oral medications is a relatively recent innovation.

Some granulomas are caused by cotton fibers (Table 5.32). The cotton is introduced when addicts load their syringes by drawing up the liquid through a cotton ball; small fibers of cotton are drawn up at the same time. Most granulomas, however, are due to magnesium trisilicate (talc), because talc is widely used in the pharmaceutical industry as a filler. Photographs of talc particles in an addict's lung are shown in Figure 5.49. The amount of active ingredient in most pills can be quite small, so talc is added to create a pill of manageable proportions. When injected, talc particles become trapped in the pulmonary arterioles and capillaries, producing acute focal inflammation and thrombosis. The reported incidence of talc-containing granulomas ranges from 15% to 90% in some series (Hopkins, 1972).

The tissue reaction to cotton closely resembles the reaction to talc. In regions where the injection of crushed pills is common, the frequency of foreign body granulomas is increased (Tomashefski and Hirsch, 1980; Kringsholm and Christoffersen, 1987).

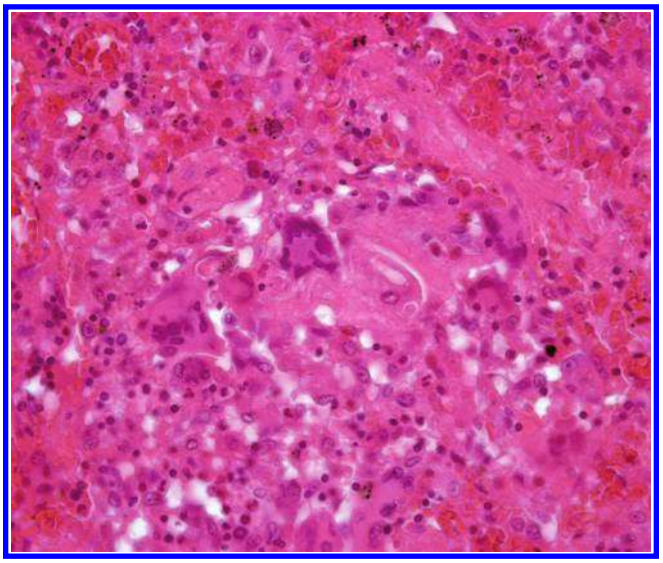


Figure 5.47 Foreign body granuloma; adulterants mixed with heroin lodge in smaller pulmonary vessels leading to granuloma formation. (Photograph courtesy of Vittorio Fineschi.)

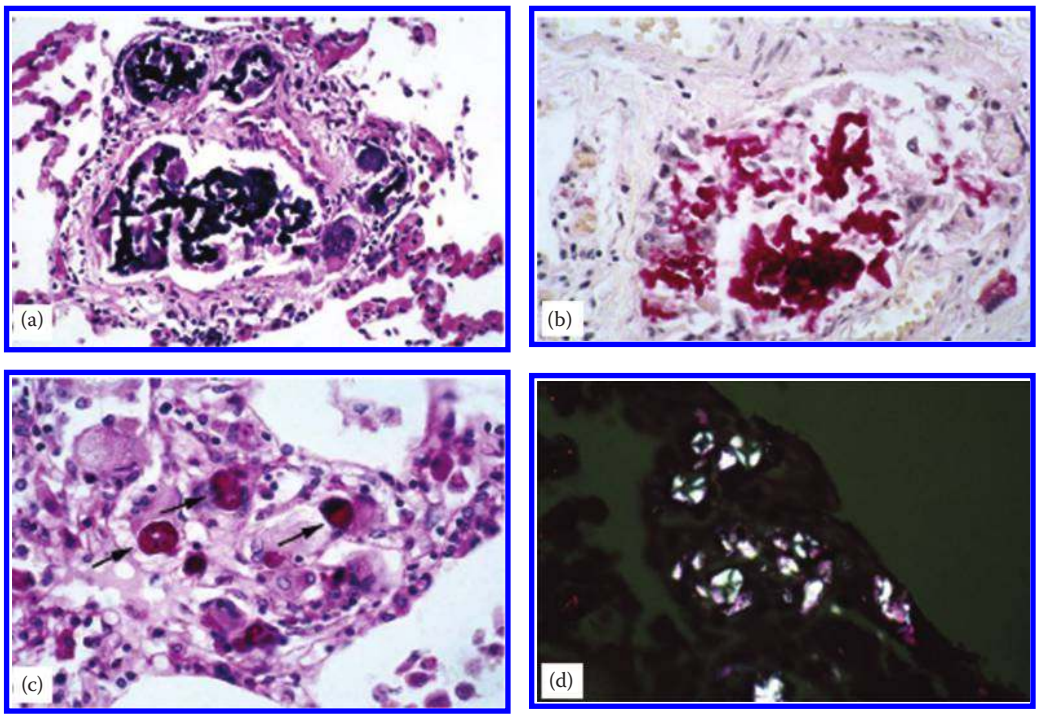


Figure 5.48 Birefringent crystals are especially easy to detect in the liver, kidney, and lungs. A mixture of different tablet filler crystals is represented here. (a) Intravascular and perivascular deposits of crospovidone with foreign body giant cell reaction (H&E stain). (b) Intense staining of crospovidone with mucicarmine. (c) Small interstitial foreign body granuloma with cornstarch particles (arrows) (PAS stain). (d) Central Maltese cross birefringence of cornstarch. Note also small spicules of microcrystalline cellulose (polarized light). (From Tomashefski, J.F. Jr. and Hirsch, C.S., *Hum. Pathol.*, 11(2), 133, 1980. With permission.)

Table 5.32 Characteristics of Birefringent Materials Found in the Lungs of Intravenous Drug Users

Substance	Shape	Size (μm)	PAS Staining
Talc	Needle-shaped	5–15	Negative
Potato starch	Maltese cross, eccentric center	20–200	Positive
Maize starch	Maltese cross, concentric	10–30	Positive
Microcrystalline cellulose	Elongated rod	25–200	Positive
Cotton fibers	Irregular	Variable	Negative

Source: Adapted from Kringsholm, B. and Christoffersen, P., *Forensic Sci. Int.*, 34(4), 245, 1987.
Note: Talc and cellulose are frequently seen in conjunction with granulomatous reactions, but other agents are not.

Fungal spores are an extremely rare cause of granulomatous disease. The soil saprophyte *Scopulariopsis brumptii* has been found to be the cause of hypersensitivity pneumonitis in at least one addict (Grieble et al., 1975), and analysis of confiscated heroin samples has always shown the presence of many different fungal varieties. Nonetheless, following the epidemic of clostridia poisoning in Scottish heroin abusers, an analysis of the then available street heroin disclosed the presence of multiple bacteria, but no fungal spores (Jones et al., 2002; McLauchlin et al., 2002).

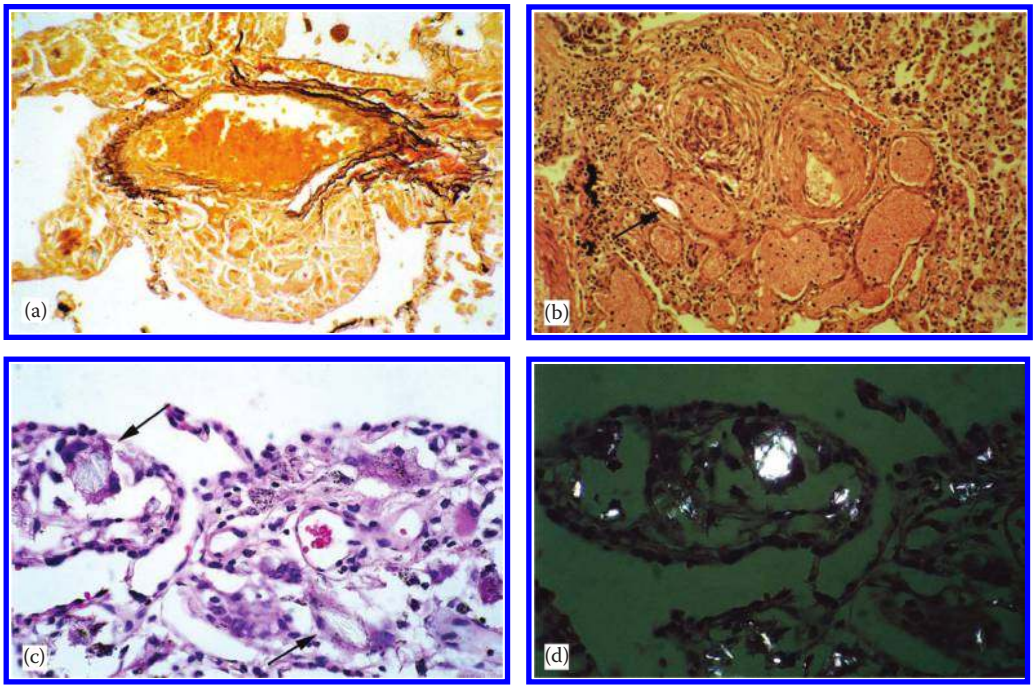


Figure 5.49 (a–d) Talc-induced emphysema, usually the result of injecting pills meant for injection. (a) Diffuse, finely dilated airspaces (barium sulfate impregnation, scale = 0.5 cm). (b) Airspace dilation with alveolar septal destruction and perivascular talc granulomas (partially polarized light). (c) Perivascular granulomas with stacked, gray-green spicules (arrows) in foreign body giant cells. (d) Brightly birefringent aggregates of talc particles (polarized light). Examination for birefringence is simple, takes only a few seconds, and often may be diagnostic. (From Tomashefski, J.F. Jr. and Hirsch, C.S., *Hum Pathol,* 11, 133, 1980.)

It makes no difference whether the offending agent is talc, cotton, cornstarch, or cellulose; the clinical course and pathologic findings will be much the same (Figure 5.49). Trapped particles cause microthrombosis and granuloma formation. Some of the trapped material may migrate into the perivascular space, where more granulomas form. If the process is ongoing, a reduction in the size of the pulmonary bed occurs, and pulmonary hypertension can result. Associated anatomic changes include medial hypertrophy and eccentric/concentric intimal fibrosis. The tissue diagnosis can be confusing, because organizing and recanalizing thrombi seen in IVDUs can appear very much like the plexiform lesions of primary pulmonary hypertension. The two conditions can be distinguished by the fact that plexiform lesions are typically seen only at the branching points of stenosed small arteries (Pietra et al., 1989).

Microcrystalline cellulose, a depolymerized form of cellulose, is also used as a filler and binder in the manufacture of oral medications. Cellulose crystals measure anywhere from 20 to 90 μm across, making them a good deal larger than talc or cornstarch crystals. The larger size of these crystals explains granuloma formation in the larger elastic pulmonary arteries and even the right ventricle. Cellulose granulomas can be identified by their distinctive Maltese cross pattern (visible with a polarizing microscope) and by the fact that they stain as carbohydrates (Tomashefski et al., 1980). The presence of foreign bodies in the pulmonary interstitium is consistent with a longstanding process. The presence of foreign material only in the media of vessels is consistent with more recent use.

The distressing feature about talc granulomas is that they can recur years after the last episode of intravenous drug abuse, as is demonstrated by a case where a patient became symptomatic three decades after the last likely exposure. Talc-induced lung disease should be considered in any patient with multiple scattered pulmonary lesions and a history of intravenous drug use. Confirmation of the disease by biopsy is essential, but unfortunately there are few successful proven treatments, even if the diagnosis is made correctly (Krimsky and Dhand, 2008).

5.12.3.4 Injuries of the Great Vessels

The adulterants and excipients mixed with illicit heroin can provoke an inflammatory reaction; peripheral veins become sclerotic, and abusers must use central veins for access. The two most popular sites are the vessels of the groin and neck (the "groin hit" and the "pocket shot") (Roszler et al., 1989; Maliphant and Scott, 2005; Higgs et al., 2009).

The neck vessels are especially difficult for abusers to inject, and for a fee, other addicts will do the injecting for them. The results are predictable. Pneumothorax is a frequent occurrence, as is a spectrum of disorders ranging from hemothorax to laceration of one of the great vessels (Espiritu and Medina, 1980). Pyohemothorax and pseudoaneurysm (Johnson et al., 1984; Navarro et al., 1984; Zorc et al., 1988) are also seen, though less frequently. Vocal cord paralysis secondary to repeat neck injection has also been described (Hillstrom et al., 1990). None of these complications is reported with any great frequency, quite possibly because the purity of street heroin has increased and more people either "snort" or "chase the dragon" (Gupta et al., 2009), but injury to the great vessels seems to be increasingly common in addicts, especially when heroin is injected with cocaine. An epidemiologic study of heroin/cocaine users, undertaken in London in 2006, found that individuals who simultaneously injected heroin/crack saw nothing particularly dangerous about the process, and some actually preferred neck injection, partly because of cocaine's local anesthetic effect (Rhodes et al., 2007).

5.12.3.5 Aspiration Pneumonia

Depressed cough reflex, decreased level of consciousness, and a general tendency to retain secretions are all associated with narcotic abuse and all favor aspiration (Cherubin et al., 1972). If aspirated stomach contents are of very low pH, acute chemical pneumonitis may also occur. If there is much particulate matter present, then acute airway obstruction is possible. Pneumonitis is usually a result of infection with Gram-negative and anaerobic organisms (Warnock et al., 1972). Aspiration pneumonia in narcotics abusers is no different from aspiration pneumonia in alcoholics or in patients debilitated by any other chronic disease, but it seems to occur much more often than had previously been thought. In one recent series, more than 20% of the drug users admitted to the ICU had aspiration pneumonia (Grigorakos et al., 2010), and in a series of more than 800 autopsies published in 2005, approximately 12% of the decedents were found to have pneumonia (Passarino et al., 2005).

5.12.3.6 Community-Acquired Pneumonia

The infection rate among IVDUs is higher than that of the general public and is a major cause of morbidity and hospitalization in this population. While numerous exotic infections are possible, bacterial infections such as tuberculosis, pneumonia and viral infections such as HIV-1 and hepatitis dominate. An important but previously overlooked problem is

that patients receiving medically indicated chronic opioid treatment may, for any number of reasons, go into withdrawal. Data on bacterial virulence in the context of opioid withdrawal suggest increased bacterial load and shortened survival times for those in withdrawal. Morphine diminishes innate and adaptive immunity simply by its interaction with the μ-opioid receptor and the signal transduction activated in the process (Roy et al., 2011).

Even before the HIV epidemic, intravenous drug abusers were at increased risk for pneumonia (Cherubin et al., 1972; Harris and Garret, 1972; Moustoukas et al., 1983; Scheidegger and Zimmerli, 1996). If opiate users had normal immune function, which they do not, the injection of unsterilized material through contaminated syringes would still cause a transient septicemia that may or may not become established. HIV-positive IVDUs are much more prone to develop community-acquired pneumonia and tuberculosis than are their HIV-negative counterparts, and when they do develop pneumonia, their clinical course is said to be more severe (Scheidegger and Zimmerli, 1996). Among HIV-infected patients, including those without AIDS, the increased rate of infection can be striking. In one study, the annual attack rate for *Streptococcus pneumoniae* was only 0.7–2.6/1000 in the general population, compared to 21/1000 in asymptomatic HIV-infected intravenous abusers (Selwyn et al., 1988).

HIV-infected heroin abusers are more likely to develop empyema, which may be the first evidence of infection (Hernandez Borge et al., 1998). The rate of opportunistic lung infections among HIV-positive intravenous drug abusers is similar in frequency to that of other HIV-positive subgroups (Niedt and Schinella, 1985; Ambros et al., 1987). Eosinophilic pneumonia occurs (Talebzadeh et al., 1990; Brander and Tukiainen, 1993; Tsapas et al., 2008), but whether opportunistic infections are more or less frequent than in cocaine users and "crack" smokers is hard to say. Diffuse pulmonary infiltrates composed of eosinophilic bronchoalveolar fluid have been described, and appear to be the result of an IgE-mediated hypersensitivity reaction (Tsapas et al., 2008), but no new reports of this syndrome have appeared for nearly a decade.

Bronchiolitis obliterans has been described in heroin abusers. One case involved a patient who presented with rapidly deteriorating pulmonary function after injecting heroin. When no etiology could be determined, open-lung biopsy was performed. It revealed patchy, temporally homogeneous, interstitial pneumonia with lymphocytic infiltration and intraluminal fibroblastic proliferation, all consistent with organizing pneumonia. Scattered nonnecrotizing granulomas with rare giant cells were also present and they were seen to contain polarizable foreign bodies (Bishay et al., 2008). Just how frequently this syndrome occurs is not known, but it may be that many possible cases are not diagnosed for lack of proper tissue sampling.

5.12.3.7 Fungal Pneumonia

Pulmonary fungal infections occur even in HIV-negative IVDUs (Rosenbaum et al., 1974; Mellinger et al., 1982; Collignon and Sorrell, 1983). Street heroin is often contaminated with fungal species, and precipitins to *Aspergillus*, *Saccharopolyspora rectivirgula*, and *Thermoactinomyces vulgaris* can, if sought, be found in the blood of many intravenous drug abusers (Smith et al., 1975). The preponderance of evidence suggests that most of the fungi found in illicit drug samples is there largely because of airborne contamination, introduced when the users prepared their drugs for injection. The presence of specific fungi cannot be used to identify the origin of a sample, although some types of heroin seem to contain more contaminants than others. Whatever the cause, *Candida* pneumonia

is a rare occurrence, and when it does occur it is more likely to be a consequence of nosocomial hospital infection, particularly in patients who are already ventilator dependent (Albert et al., 2014).

Analysis of several outbreaks of fungal pneumonia among addicts suggested that the cause of infection was contaminated paraphernalia, including preserved lemon juice used to prepare the heroin injection (Clemons et al., 1991). Some types of heroin (Mexican brown) are poorly soluble in water and can only be dissolved after they have been acidified. The two most popular agents for acidifying are lemon juice and vinegar. *Candida* species are present as contaminants of the lemon rind.

Candida-infected patients most often present with lobar pneumonia. In a high percentage of cases, peripheral nodules, with or without cavitations, may be seen. Lung abscess and empyema may also develop (Mellinger et al., 1982). Hilar and mediastinal adenopathy can be a prominent finding that resolves over the course of weeks or months. Pleural effusions are seen in about 20% of cases, and pleural thickening may also result (Lazzarin et al., 1985). DNA analysis has shown that all strains of *Candida* appear to be equally infective, and no particular genotype is linked with disease in the addict population (McCullough et al., 1998).

Disseminated *Candida* infections have a rapid onset, with symptoms appearing only a few hours after injecting. The infection may be manifest as a self-limiting lobar pneumonia or as a generalized infection with endocarditis, chorioretinitis, and hepatitis, with or without soft tissue abscesses. Occasionally the septicemia is manifested only as an isolated endophthalmitis (Dupont and Drouhet, 1985; Shankland and Richardson, 1989). Repeated showers of emboli cause mycotic aneurysms of the pulmonary arteries but these are usually asymptomatic and are only incidentally found at autopsy. Histologic diagnosis can sometimes be made by examination of scalp biopsy specimens, which will show infiltration of the hair follicles with chronic inflammatory cells and *C. albicans*.

Most cases of *Candida* pneumonia are secondary to hematologic dissemination of *Candida* from some distant site, usually the gastrointestinal tract (the general population) or the skin (IVDUs). The diagnosis of pulmonary candidiasis is difficult because there is no specific clinical or radiologic presentation. In addition, the presence of *Candida* in sputum or other respiratory specimens usually just represents contamination. A definitive diagnosis of *Candida* pneumonia requires histopathologic proof of inflammatory lung invasion. Children can also be affected by pulmonary allergic reactions caused by *Candida* species (Pasqualotto, 2009).

5.12.3.8 Tuberculosis

As was confirmed by the 2010 NSDUH survey of nearly 30,000 drug abusers, a definite connection exists not only between IVDUs and HIV infection but also tuberculosis, asthma, and sexually transmitted diseases (Han et al., 2010; SAMHSA, 2011). The incidence of tuberculosis in both HIV-positive and HIV-negative opiate abusers continues to increase, and the disease seems to be much worse in patients who also use heroin. A retrospective case-controlled study of pulmonary tuberculosis in heroin-abusing patients was performed in China. Patients with tuberculosis and heroin addiction suffered from much worse tuberculosis than those in the non-heroin-addicted group. More lesions were found in their chest x-rays, higher sputum tuberculosis-positive rates were recorded, and the results of medical treatment were not as good (Fang et al., 2006). The connection between heroin and tuberculosis derives from the diminished immunity triggered by opiate abuse

(see Section 5.12.3.7) but also has to do with lifestyle. In a study of heroin users with tuberculosis who were treated in Barcelona, a history of prior imprisonment was found to be a much better predictor for infection than either concurrent HIV infection or number of years of heroin use (Manzanera et al., 2000). Pulmonary melioidosis (due to *Burkholderia pseudomallei*), which can resemble tuberculosis on x-ray, also occurs rarely in narcotic addicts (Cooper et al., 2000).

5.12.3.9 Septic Pulmonary Emboli

Septic pulmonary emboli, occasionally associated with pneumothorax, occur not infrequently in intravenous abusers. Recurrent emboli of infected material may arise from infected bone or soft tissue at the injection site, leading to septic thrombophlebitis or even endocarditis. In the past, the most probable source for these emboli was vegetations on the tricuspid valve, and recurrent septic pulmonary emboli should always raise the possibility of tricuspid vegetations (Reiner et al., 1976). However, Asian addicts have developed a new way of administering parenteral heroin, and this new pattern of injection could also be the source of emboli and infection.

Heroin injectors in Southeast Asia, particularly Vietnam, use a novel injection process known as *cay ma*, a Vietnamese idiom meaning "injection sac." The "sac" is formed by repeatedly inserting a hypodermic needle into the same area on the skin surface. The area chosen always overlies a large vein. Repeated injection leads to sclerosing of the injection site, in turn leading to the increased formation of fibrocytes and fibroblasts, leading to the production of type I collagen, giving the "sac" an elastic quality. An addict wanting to inject drugs simply inserts the needle through the sac directly into the vein, decreasing the chances for extravasation and increasing bioavailability (Clatts et al., 2007). Although never evaluated in a controlled study, it seems likely that the use of this method would be particularly likely to lead to infection.

5.12.3.10 Emphysema

Emphysematous changes are occasionally seen in the subset of intravenous abusers who inject medications meant for oral use. The process may involve both the upper (Pare et al., 1989) and lower lobes (Smeenk et al., 1990). Rarely, disease is panacinar (Groth et al., 1972) but typically the upper lobes show the most damage. Intravenous drug abusers with emphysema are in their late 30s, which distinguishes them from those with emphysema due to smoking or α_1-antitrypsin deficiency; victims of the latter tend to be much older. Emphysematous changes have always been more common in stimulant abusers than in individuals taking opiates (Stern et al., 1994), but now that simultaneous abuse of stimulants and narcotics is common practice, it may be impossible to determine the etiology of the changes.

5.12.3.11 Cotton Fever

Hardly any new cases have been reported for more than a quarter century, but the disease still occurs (Torka and Gil, 2013). *Cotton fever* is a benign syndrome occasionally seen in intravenous narcotic abusers. They acquire this disorder when they filter their "fix" through a wad of cotton. Unfortunately, the cotton often contains a limited amount of a preformed endotoxin (Shragg, 1978; Ferguson et al., 1993). Cotton plants are heavily colonized with Gram-negative bacteria, especially *Enterobacter agglomerans* (Rylander and Lundholm, 1978). Endotoxin released by *E. agglomerans* may activate pulmonary

macrophages and neutrophils. Activation of those cells promotes the release of other chemicals, causing fever and leukocytosis. The same symptoms occur in cotton workers who inhale the endotoxin, which floats freely in the air of cotton mills. There is no effective way to immediately differentiate patients who have injected themselves with limited amounts of preformed endotoxin and those who have actually inoculated themselves with *E. agglomerans* or other bacterial agents. Because the latter group is at risk for sepsis or endocarditis, prudence dictates that patients presenting with *cotton fever* should have blood cultures drawn and then be treated, at least initially, with empiric antibiotic therapy.

5.12.3.12 Idiopathic Pulmonary Hypertension

Sporadic cases of idiopathic pulmonary hypertension (IPH) have been reported over the years (Overland et al., 1980; Restrepo et al., 2007), though in the past this disorder was also considered a consequence of chronic granuloma formation due to drug excipients. Now that levamisole has emerged as the preferred adulterant for cocaine, and is increasingly popular among heroin dealers, it may be that heroin injectors are at risk for IPH. The explanation has to do with the fact that levamisole is converted into aminorex, and aminorex is notorious for causing pulmonary hypertension (Bertol et al., 2011). Figures 5.50 and 5.51a through f illustrate the changes apparent in a chronic heroin abuser. Levamisole contamination of cocaine came to notice in early 2005, but the adulteration of heroin with levamisole seems to be a phenomenon of recent onset. The U.S. DEA estimates that over 70% of street cocaine is levamisole contaminated, though the amount of levamisole present is variable. Heroin is becoming more widely levamisole contaminated, roughly in the same portions as cocaine (Casale et al., 2008).

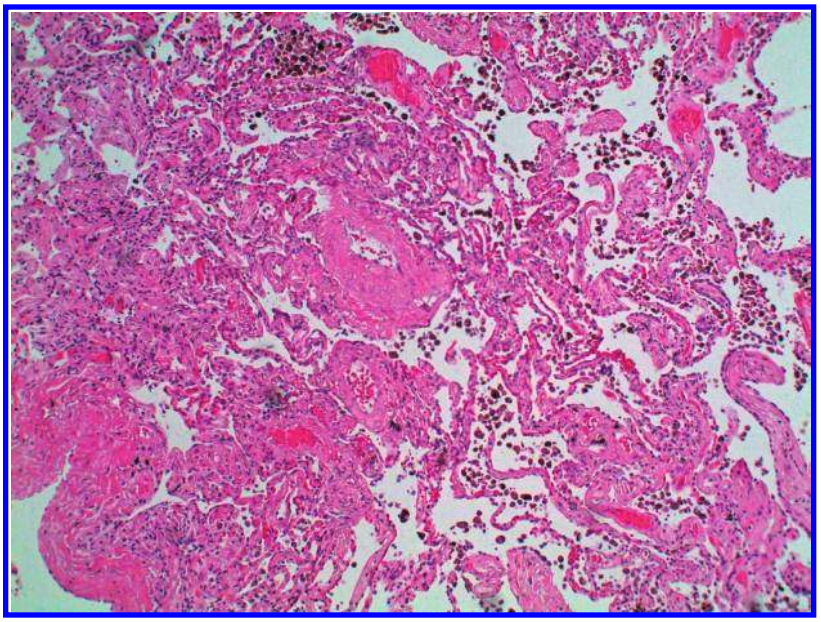


Figure 5.50 Lung from a chronic polydrug abuser. Note the hemosiderin-containing macrophages. Humans convert levamisole to aminorex, a serotonin agonist. Chronic use can lead to pulmonary hypertension. (From Angelos, M.G. et al., *J. Emerg. Med.*, 4(5), 391, 1986.)

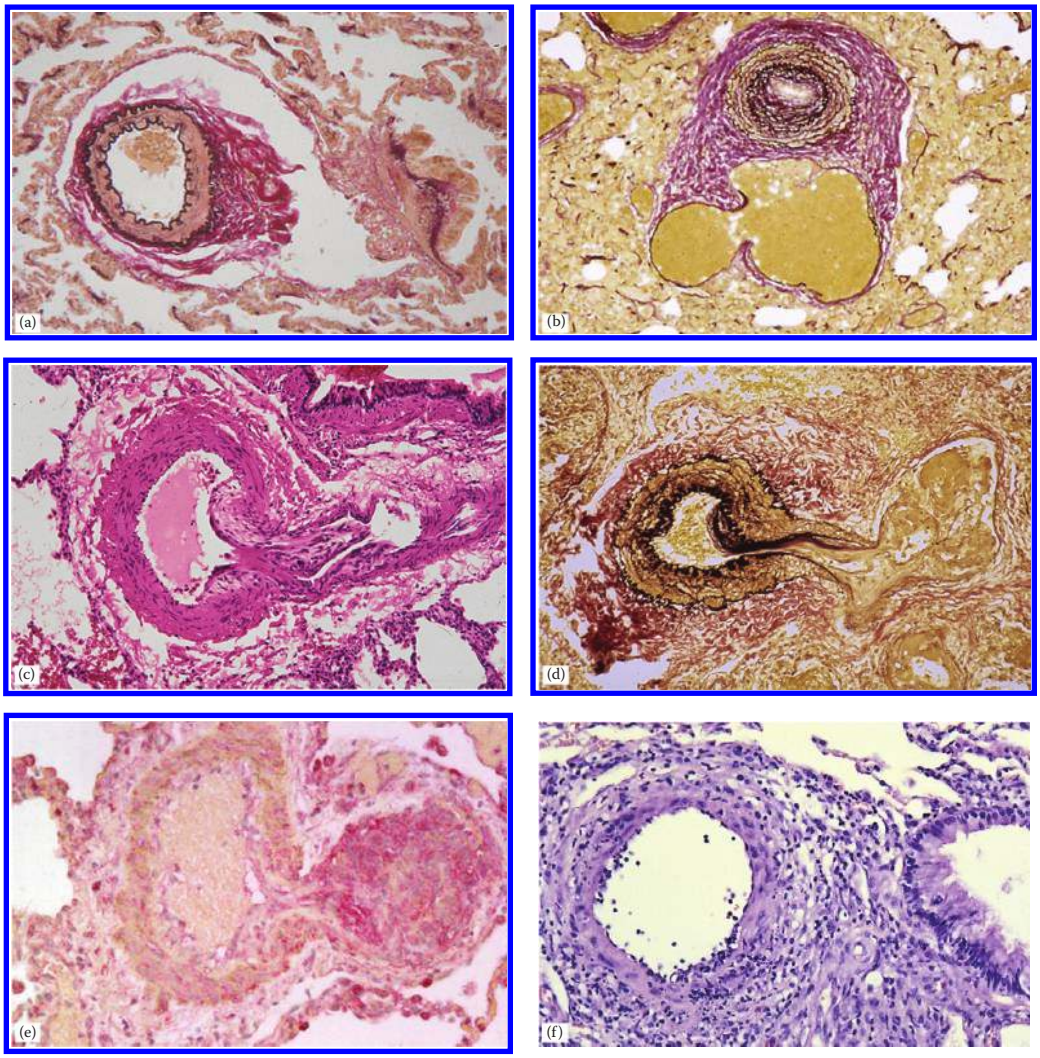


Figure 5.51 (a–f) Plexogenic pulmonary arteriopathy (WHO group 1.1–1.3). (a) Medial hypertrophy and some adventitial thickening of muscular pulmonary artery. In the early (and presumably reversible) phase of the disease process, these nondiagnostic alterations are the only findings. (b) Advanced plexogenic pulmonary arteriopathy; a dilatation lesion, presumably representing greatly widened and very thin-walled supernumerary arterial vessels, lies next to parent artery showing marked medial hypertrophy. (c, d) Plexiform lesion. Axial muscular pulmonary artery (left) giving rise to supernumerary artery showing intimal fibrosis at the orifice and proximal part, with associated increased cellularity, irregular shape of lumen, some mural thrombus formation (d), marked widening of lumen of most distal part of the lesion (right). (e) Vascular endothelial growth factor immunostain highlighting plexiform lesion (red). (f) Focal pulmonary arteritis in a case of advanced plexogenic pulmonary arteriopathy. (a, b, d, f) Hematoxylin–eosin (HE) stain; (c) elastic van Gieson (EvG) stain; (e) immunostain (alkaline phosphatase). (Reproduced from *Curr. Diagn. Pathol.*, 12(6), Mooi, W.J. and Grünberg, K., Histopathology of primary hypertensive diseases, 429–440, 2006, with permission from Elsevier.)

5.12.4 Gastrointestinal Disorders

5.12.4.1 Introduction

Liver disease is common in intravenous heroin abusers (Edland, 1972; Passarino et al., 2005) (Table 5.33). Infiltration of the portal triads, though nondiagnostic, is easily demonstrated in most chronic abusers, as are hepatic steatosis and limited portal fibrosis. In many instances these infiltrates are, in fact, evidence of previously undiagnosed hepatitis. Although the incidence and prevalence of hepatitis C seems to be declining (Des Jarlais et al., 2005), hepatitis C remains endemic among drug users (Hagan et al., 2005). Most IVDUs with hepatitis C remain asymptomatic, but in a small percentage the infection leads to cirrhosis and, even less often, to hepatocellular carcinoma. In fact, HCV-related end-stage liver disease represents the primary indication for liver transplantation in Western countries, but this procedure is associated with poor posttransplantation outcomes and a rapid progression of HCV-related disease (Angelico, 2011).

5.12.4.2 Bowel Disorders

Opiates that bind the μ-receptor decrease gut motility, leading to constipation or even obstipation. The diagnosis of narcotism often can be made just from the appearance of the colon at autopsy; it will be distended with hard feces. The other bowel disease associated with opiate abuse is the "body packer" syndrome. Suarez et al. first noted this disorder in a cocaine courier in 1977 (Suarez et al., 1977). Most "body packing" cases have involved cocaine rather than heroin (Greenberg et al., 2000). The incidence of heroin body packing seems to be increasing, but the increase is not worldwide, and whether it occurs depends on the logistics of the smuggling operation. For example, heroin body packing is rare in the United Kingdom because the drug is usually imported in vehicles driven into that country from the Balkan states, having first been processed in Turkey after being grown in Afghanistan (Booker et al., 2009). Smugglers, known as "mules," ingest anywhere from 20 to 100 rubberized packets containing multiple-gram quantities of drug. At first, the packets were made from condoms,

Table 5.33 Frequency of Hepatic Lesions in 150 Randomly Selected Drug Addicts

Lesion	Incidence (%)
Steatosis	70
Portal fibrosis	47
Portal phlogosis	93
Piecemeal necrosis	46
Lymphoid follicles	40
Plasma cells	34
Acidophil bodies	23
Viral antigens	16
Bile duct proliferation	6
Bridging necrosis	5
Granulomas	2
Birefringent material	<1
Mallory's hyaline	Absent

Source: Adapted from Paties, C. et al., *Forensic Sci. Int.*, 35(1), 11, 1987.
Note: Patients had a mean age of 23.3 years and were predominantly male (86%).

balloons, or the fingers cut from surgical gloves. Today more care is devoted to the packaging process than previously, not only because the packets occasionally rupture and kill the courier but also because the earlier versions of these packets were too easy to see on x-ray (Krishnan and Brown, 1999). More than 40 years have elapsed since the first reports of this syndrome, but methods for detection still remain imperfect.

In the most recently published study, packets were detected in 28 of 32 known body packers, implying that more than 15% of these drug smugglers do go undetected (Gsell et al., 2010). Complicating research efforts at detecting these smugglers is that there are relatively few heroin mules to be studied. A recent meta-analysis failed to identify a single large case series where the mules were carrying heroin; all of those detained were carrying cocaine (Booker et al., 2009). Smugglers often manage to avoid detection by plain film x-ray by minimizing the contrast difference between the packets and the surrounding feces. To this end, sophisticated smugglers may drink mineral oil that helps to eliminate x-ray contrast differences. Urine testing, at least in the case of cocaine smugglers, is often positive even if none of the packets rupture; the rubber wrapping acts as a semipermeable membrane through which small amounts of the contents of the packet gradually diffuse and enter the bloodstream (Gherardi et al., 1988). With the introduction of abdominopelvic MRI, the problem has more or less disappeared, save for the fact that not many airports have facilities for MRI screening, which is the most reliable method of diagnosing body packing (Bulacki and Ozbalir, 2013). In the past, managing physicians often resorted to surgical intervention. That trend has been reversed: today fewer than one in three mules requires surgery and the morbidity rate seems to have decreased substantially. Laparotomy is not undertaken unless there are immediate signs of intoxication or if the patient begins to deteriorate clinically.

5.12.4.3 Liver Disease

The first paper suggesting direct, heroin-related hepatotoxicity was published in 1935 (Edland, 1972), and a recent epidemiologic study of Australian addicts found that liver disease had become the most common cause of mortality among the aging opioid dependent (Anon., 2014). An autopsy study compared the findings in 40 samples of liver tissue taken from intravenous heroin abusers and 10 controls: 2 sorts of changes were apparent. All of the heroin users had obvious degenerative vesicular and fatty changes, along with chronic hepatitis, cirrhosis, occasional amyloidosis, dysplastic changes, and cellular dropout. All of the changes correlated (some more, some less) with the duration of heroin abuse. There was also a change in the number of Kupffer and endothelial cells. The most frequently encountered abnormality was vesicular degeneration, which appeared to be the only direct effect exerted by heroin. The remaining morphologic changes could all be accounted for by viral infections, and the severity of these changes also correlated with the duration of narcotic abuse (Granel et al., 2006; Ilic et al., 2010a).

Ultrastructural studies of the same group of decedents were performed. As indicated earlier, the livers of the heroin addicts all displayed degenerative vesicular and fatty change, along with the expected findings of chronic active and persistent hepatitis, cirrhosis, and reduced hepatocyte glycogen content. Hypertrophy of the Kupffer cells was again evident. Hyperplasia and hypertrophy of the smooth endoplasmic reticulum, resulting from increased enzymatic activity, were evident as were pathologic changes in the sinusoids and microcirculation (Ilic et al., 2010b).

More often than not, the principal finding in the liver in death from acute narcotic overdose is enlargement and congestion (the liver often weighing over 2000 g). Other abdominal

organs are also likely to be congested because of acute cardiac decompensation. It is important to bear in mind that not all opiates exert the same effects. There is some evidence, both clinical and experimental, that buprenorphine is directly hepatotoxic. Buprenorphine is mainly metabolized in the liver by the cytochrome CYP3A4 system, with only 10% excreted by the kidneys. This particular cytochrome is highly polymorphic, leading to great interindividual variation in the way buprenorphine is metabolized. If too much of this drug accumulates, whether because of overdose or PM status, mitochondrial damage may occur (Berson et al., 2001; Zuin et al., 2009).

Whereas buprenorphine metabolites do not seem to have any significant effect on mitochondria, clinically relevant doses of buprenorphine alter the cell membrane potential and inhibit beta-oxidation, at the same time uncoupling and inhibiting respiration, at least in rat liver mitochondria. These combined actions lead to a reduction in the manufacture of ATP and appear to be CYP3A4 mediated, although it is still not known which polymorphism is responsible. It is not known if similar changes occur in humans. Only a handful of possible cases of buprenorphine-associated hepatotoxicity have been reported (Berson et al., 2001).

5.12.4.4 Porta Hepatis Adenopathy

Enlargement of lymph nodes located in direct proximity to the liver is a common and nearly diagnostic indicator for chronic intravenous heroin abuse. The exact incidence of this lesion has never been tabulated, but some have placed it at over 75% (Edland, 1972; Kringsholm and Christoffersen, 1987). The porta hepatis, subpyloric and peripancreatic lymph nodes, the cystic node at the neck of the gallbladder, and other nodes located along the common duct may all be involved. Not infrequently, the gastroduodenal and pancreatoduodenal nodes will also be enlarged. These nodes are gray, firm, and sharply demarcated. The degree of enlargement may be striking. Nodes measuring as much as 2 cm across are not uncommon. Microscopic examination of these nodes shows only a nonspecific pattern of reticuloendothelial hyperplasia.

There are at least three possible explanations for this type of adenopathy, all unproven. Node enlargement could be a reaction to the injection of particulate material. In one series, birefringent material was found in 39% of nodes from confirmed addicts (Kringsholm and Christoffersen, 1987). Another possible explanation is recurrent infection. Long before the existence of HCV was even recognized, histologic changes consistent with nonspecific reactive hepatitis were observed in more than half the known drug users coming to autopsy (Paties et al., 1987). Deep abdominal lymphadenopathy can also be seen in HIV infection, though usually only in individuals with overt AIDS and secondary malignancy (Subramanyam et al., 1985). Finally, there is the possibility that morphine itself might exert some direct effect on lymph nodes, causing them to enlarge, though there is little evidence to support that notion. Morphine is easily detectable in nodes draining the portal areas, and in most cases the concentration of morphine is greater in the nodes than it is in the blood. Lymph node morphine concentrations measuring anywhere from 300 to over 8000 ng/mL have been recorded (Nakamura and Choi, 1983).

5.12.4.5 Inflammatory Disease

Inflammation of the portal tracts is a nearly constant autopsy finding in long-term intravenous drug abusers. The incidence was over 92% in one of the first studies to examine the question (Paties et al., 1987). The pattern of inflammation seen in addicts is commonly referred to as *triaditis*, referring to a predominantly lymphocytic infiltrate frequently admixed with plasma cells, located within the portal triads (Figure 5.52). On occasion,

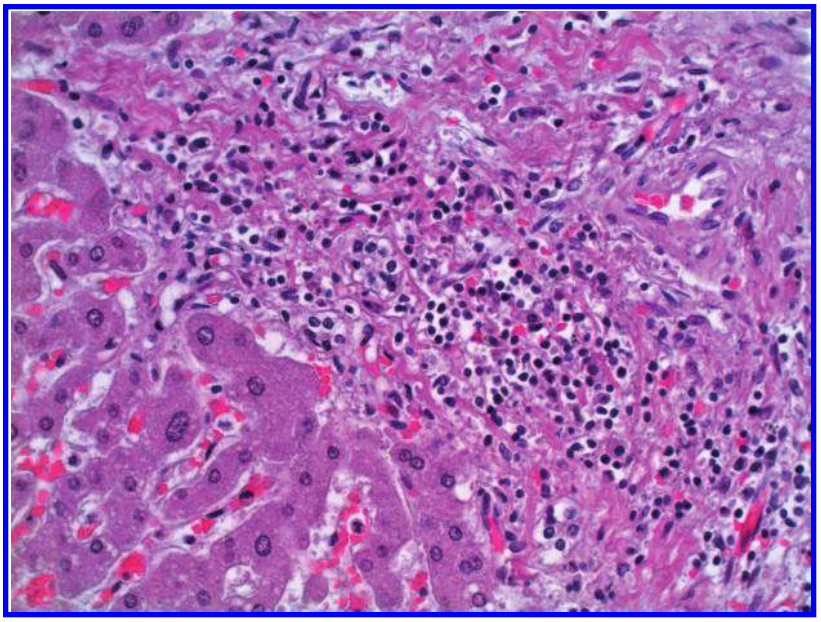


Figure 5.52 Liver *triaditis* and microsteatosis in the liver of a chronic heroin abuser. The collection of lymphocytes in the portal triad probably represents hepatitis C. The fat droplets are fine and widely dispersed. Often the degree of steatosis is much more pronounced (see Figure 5.53) and best described as macrosteatosis. Steatosis is a nonspecific finding caused by many different drugs and diseases, including hepatitis. (From the collection of the author.)

neutrophils may also be present, but these infiltrates are usually devoid of eosinophils (Kaplan, 1963; Siegel and Halpern, 1966; Paties et al., 1987). Recent advances in cell biology suggest that apoptosis may be an important part of the inflammatory process in drug abusers and could partly explain the occurrence of triaditis (though most cases almost certainly represent viral infection). Cell death by apoptosis is a prominent feature in a variety of liver diseases. It is likely that apoptosis is the initial cellular response to liver and biliary injury, thereby initiating harmful cellular and cytokine cascades. Together, these cascades may account for some of the hitherto unexplained necrosis and fibrosis seen in the livers of chronic abusers (Kilicarslan et al., 2009).

Lobular inflammation is almost as common as *triaditis* (85%) but necrosis is less common (46%) and, when it occurs, it tends to be widely scattered. The changes in addicts should be distinguishable from those seen in alcoholics, as there is no centrilobular necrosis, no Mallory's hyaline, and only rarely are neutrophils present. True bridging necrosis is also uncommon. Infiltrates in areas of necrosis are composed mainly of monocytes. Steatosis, which was once believed uncommon, can be found over 70% of the time. Fatty accumulations may be microvesicular, macrovesicular, or mixed, but these distinctions are of little diagnostic value. Given the reality of polydrug abuse, multiple abnormalities may be detected on the same slide. There is, for example, some evidence that methamphetamine is associated with steatosis (Karch et al., 1999; Kahraman et al., 2006). Until very recently, the relationship between fatty infiltration of the liver and cardiovascular disease was obscure. It is now an area of intense interest.

Conditions ranging from simple steatosis to progressive nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD) are extremely prevalent.

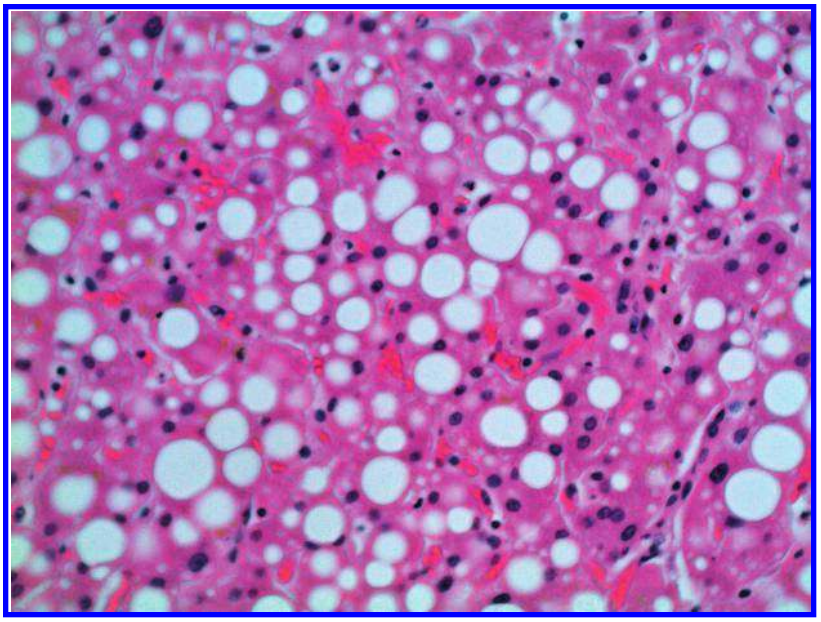


Figure 5.53 Macrosteatosis. As opposed to the fine dispersed droplets evident in Figure 5.52, large fat droplets occupy much of the hepatocyte. This form of fatty infiltrate is more common in the obese and, in life, easily diagnosed with MRI scanning. Some of the changes observed may be secondary to the direct action of heroin. (From the collection of the author.)

The prevalence of NAFLD depends on adiposity, age, gender, and ethnicity. The natural history of patients afflicted with NAFLD critically depends on histologic changes in the liver and these, unfortunately, are not always sought. The issue that has only recently been appreciated is that cardiovascular mortality is increased in NAFLD, particularly in middle-aged adults. The question that remains unresolved is whether all types of NAFLD, including those associated with drug abuse, carry an equal risk, either because of hyperinsulinism, with prothrombotic potential, or metabolic syndrome or because of some combination of all three (Maurantonio et al., 2011).

Hepatic foreign body granulomas are uncommon because most injected contaminants are trapped in the pulmonary vascular bed and never enter the systemic circulation. Whether or not birefringent material will be found in the liver or within hepatic nodes depends, in large part, on the population being studied. If the population of addicts consists of crushed pill injectors, then the probability of finding birefringent material is greater. Foreign bodies can become widely disseminated if a septal defect and shunt are present, and users with widespread systemic granulomas have been reported occasionally (Riddick, 1987).

5.12.4.6 Hepatitis

In 1987 Paties et al. reviewed the autopsies of 150 addicts: changes consistent with chronic active hepatitis were found in 24%, while changes consistent with acute hepatitis were diagnosed in 12%. Most of these individuals had immunohistochemical evidence of one or more viral antigens. In acute cases, scattered foci of parenchymal cell loss with acidophilic necrosis and swelling, along with proliferating reticuloendothelial cells and mononuclear infiltrates, were seen. During the last 10 years, the prevalence of intravenous drug abusers infected with either hepatitis B virus (HBV) and HCV, as well as HIV, has increased but

the changes being reported are regional and, to some degree, unpredictable. In a study that examined health conditions among an aging cohort of 108 male narcotics addicts in California, half of the sample had abnormal liver function, 94.2% tested positive for hepatitis C, 85.6% for hepatitis B, 3.8% for syphilis, and 27.3% for tuberculosis (Hser et al., 2004). When seroprevalence was measured in Italian prison inmates, 12.5% of inmates were HIV positive, 8.1% HBV positive, and 31.1% HCV positive; 25 subjects were found to be positive for both HIV and HCV (Sabbatani et al., 2004). In Germany, blood screening of chronic intravenous drug abusers showed even lower rates for HBV and HCV. Accurate diagnosis is vital as it is likely to affect both treatment and outcome.

5.12.4.6.1 Hepatitis A Virus Ancient Chinese, Greek, and Roman physicians recognized hepatitis A infection, but the first documented report was not published until the eighteenth century. Today the prevalence of hepatitis A virus (HAV) is much lower than that of HBV or HCV, largely because the introduction of a hepatitis A vaccine in 1995 led to a drop in the number of reported cases of hepatitis A in all age groups. During 1999–2006, the overall seroprevalence of anti-HAV was 34.9% (95% confidence interval [CI] 33.1, 36.7). During 1999–2006, U.S.-born children living in vaccinating states (33.8%, 95% CI 26.2, 42.2) had a higher seroprevalence than children in nonvaccinating states (11.0%, 95% CI 9.4, 12.8; $p < 0.001$). Seroprevalence among children increased from 8.0% (95% CI 6.3, 10.1) during 1988–1994 to 20.2% (95% CI 16.0, 24.8) during 1999–2006 ($p < 0.001$). For U.S.-born children aged 6–19 years, the strongest factor associated with seroprevalence was residence in vaccinating states. Among U.S.-born adults aged >19 years, the overall age-adjusted seroprevalence of anti-HAV was 29.9% (95% CI 28.3, 31.5) during 1999–2006, which was not significantly different from the seroprevalence during 1988–1994 (32.2%, 95% CI 30.1, 34.4) (Klevens et al., 2011).

Transmission is primarily fecal–oral, although there have been rare instances of transmission through blood products, including contaminated and reused needles.

The various viruses that cause hepatitis belong to the Picornaviridae family and carry a single strand of RNA. There are seven genotypes. IgM and IgA antibodies appear in the serum early in the disease, at about the time of symptom onset. The diagnosis of hepatitis A infection is confirmed by the finding of IgM anti-HAV antibodies, routinely performed using an ELISA test. Treatment is supportive. Intramuscular anti-A gamma globulin is used for passive immune prophylaxis, and there is an efficient vaccine for active immune prophylaxis. Ten to twenty percent of symptomatic patients experience a prolonged or relapsing course of illness and, occasionally, chronic infection has been reported. Fulminant infection, with fatal outcome, occurs in less than 1% of those infected (Pereira and Goncalves, 2003).

5.12.4.6.2 Hepatitis B Virus A small, circular, partially double-stranded DNA virus, hepatitis B is a member of the Hepadnaviridae family. Its incidence in the United States has been declining. The current rate of newly reported cases is less than 2000 cases/100,000/year. The highest rates were observed among persons aged 15–44 years; the lowest rates were among persons aged <15 years. In 2008, rates were highest for persons aged 25–44 years (2.6 cases/100,000 population); the lowest rates were among children <15 years (0.02 cases/100,000 population).

Traditionally, the rate of symptomatic hepatitis B infection has been higher among males than females. During 1990–2008, the male-to-female ratio remained stable (1.5–1.8). In 2008, the rate for males was approximately 1.8 times higher than for females. In 2008, incidence among males was 1.7 cases per 100,000 population, compared with 1 case per 100,000 population among females.

Historically, acute, symptomatic hepatitis B rates have differed by race; the highest rates occurred among non-Hispanic blacks and Asian/Pacific Islanders (APIs). In 2008, the rate of acute, symptomatic hepatitis B was highest for non-Hispanic blacks (2.2 cases/100,000 population). In 2008 the downward rate trend among APIs (0.7 cases/100,000 population) was similar to that for Hispanics (0.8 cases/100,000 population) and non-Hispanic whites (0.9 cases/100,000 population) (CDC, 2010).

Estimates suggest that more than 350 million people worldwide (Wright, 2006) are infected with hepatitis B, and over 1 million die annually of HBV-related chronic liver disease. Many, perhaps most, of those infected eventually become noninfective. However, if a prolonged immunologic response persists, it may lead to cirrhosis, liver failure, or hepatocellular carcinoma in up to 40% of patients. In endemic areas, where carrier rates are over 5%, most become infected in early childhood or even at birth.

In the United States, the main routes for transmission are high-risk sexual activity and intravenous drug abuse. In San Francisco, nearly one-third of IVDUs under the age of 30 report sharing needles, and nearly two-thirds report having had more than two sexual partners in the previous month; only 10% reported having received vaccinations for hepatitis B infection (Seal et al., 2000). Asians living in San Francisco also have a high rate of infection and there have been intensive public health efforts to improve the situation. As of this date, there have been no striking breakthroughs (Sarkar et al., 2012).

Approximately 2.1% of patients with chronic HBV develop cirrhosis each year. It had been thought that the annual incidence of hepatocellular carcinoma was only 0.1% in asymptomatic patients, rising to 1% in patients with chronic HBV, and increasing still further to 3%–10%, when cirrhosis is present. However, recent genetic studies have shown that the role of HBV in adenocarcinoma may be much higher. In a 2011 study of tumor and adjacent normal liver tissues obtained from 33 cryptogenic HCC patients and 28 HCC patients with identifiable causes (13 with chronic hepatitis B, 6 with chronic hepatitis C, and 9 cases that were alcohol related), viral DNA for hepatitis was identified in 24 (73%) of the patients with adenocarcinoma of undetermined cause and half of the patients whose tumors were alcohol related (Wong et al., 2011).

Many otherwise healthy HBV-infected patients first come to medical attention only after they have become infected with a different virus, such as hepatitis C, or even A. The combined infection places them at greatly increased risk of fulminant hepatic failure. For reasons that are not clear, HCV superinfection may lead to negative HBV tests (HBsAg positivity) (Chu, 2000).

There are no histopathologic differences between patients with HBV and HCV—not in the severity of inflammatory activity, degree of architectural damage, or appearance of the bile ducts (Thorne et al., 1982). Nor is it possible to distinguish, at least not with any certainty, patients with a hepatotropic virus from those with nonalcoholic hepatic steatosis. More than a third of the viral cases will have steatosis, and 80% have some evidence of necrosis (Goldstein et al., 1995). Some recent reports have suggested that high viral load is associated with poorer patient outcomes, for example, more rapid progression to cirrhosis and a higher incidence of hepatocellular carcinoma (Gish and Locarnini, 2006).

5.12.4.6.3 Hepatitis C Virus The WHO estimates that 170 million individuals worldwide are infected with HCV, but the incidence varies widely from location to location. According to the U.S. CDC, an estimated 1.8% of the U.S. population is positive for HCV antibodies and at least 2.7 million people have active disease. HCV accounts for 20% of all

cases of acute hepatitis, an estimated 30,000 new acute infections, and 8000–10,000 deaths each year in the United States and is the most common cause for liver transplantation.

The infective status of an individual can be determined by serologic testing. Subjects with detectable HCV core antigen (HCVcAg) or measurable HCV RNA are presumed to be viremic, and therefore carriers. Subjects negative for both HCVcAg and HCV RNA (i.e., viremia negative) but with a past history of hepatitis are considered as having had a prior HCV infection from which they have recovered and, therefore, are no longer carriers. In one large, recent epidemiologic study, 1125 seropositive individuals were studied over 10 years. Among the anti-HCV-positive subjects included in the analysis, 758 (67.4%) were HCV carriers, and 367 were noncarriers. A total of 231 deaths occurred in these subjects over a mean follow-up of 8.2 years: 176 deaths in the HCV carrier group and 55 in the noncarrier group. Clearly, the overall mortality rate was higher in HCV carriers than in noncarriers. Among HCV carriers, a higher level of HCVcAg (≥100 pg/mL) and persistently elevated alanine aminotransferase levels were important predictors of liver-related mortality (Uto et al., 2009).

One explanation for the increased mortality rate associated with this infection is its role in oncogenesis. Many infectious agents, especially viruses, account for several of the most common malignancies. Some estimate that as many as 20% of all cancers may arise in this fashion. Lymphomas are frequently found in association with viral infections, such as Epstein–Barr virus, HIV, human herpes virus 8, and HCV (De Falco et al., 2011) and infection with the bacterium *Helicobacter pylori*. Other recent studies have shown that genetic polymorphism may play a role in susceptibility to HVC infection (Tulio et al., 2011).

HCV is caused by a single-stranded RNA virus. In 60%–80% of patients, infection with the virus leads to chronic hepatitis. A strong multispecific T-lymphocyte reaction against HCV proteins is associated with viral clearance. Both CD4+ and CD8+ lymphocyte functions are required to clear the virus. In chronic infection, genetic and environmental factors determine the progression of inflammation and fibrosis in individual patients. Of the individual factors that can alter outcome, age, gender, race, and alcohol use seem to be the most important. The development of hepatocellular carcinoma is mainly restricted to patients who already have cirrhosis (Neuschwander-Tetri, 2000).

The early stages of HCV infection produce no unique histologic features, and the picture may even resemble that of unrelated disorders, such as NASH. Besides fatty change, a mixed cellular inflammatory infiltrate may be seen extending across the lobule, with evidence of hepatocyte injury and fibrosis (Neuschwander-Tetri, 2000) Similar alterations were first noted in intravenous heroin abusers more than 25 years ago and recognized in stimulant abusers a decade later, both presumably the result of the same virus whose existence was not then suspected. Depending upon the stage of the disease, biopsies of HCV-infected patients usually provide a varied picture. The disease may take an acute course, in which case the morphologic features will be those of classical acute hepatitis, usually without bridging necrosis. In particular, features of HCV infection include prominent portal inflammation with lymphoid follicles, hepatic bile duct lesions, and the lobular changes of eosinophilic hepatocytes, eosinophilic bodies, and steatosis (see Section 5.11.4.10). See Figure 5.54a and b (Dienes et al., 1999).

Occasional prominent sinusoidal reactions are seen. They may have an inflammatory pattern simulating the pattern seen in infectious mononucleosis. Cholestasis, if present, is usually mild, but cholestatic hepatitis can occur. It is rare to encounter submassive or massive hepatocyte necrosis. Within portal areas, lymphoid infiltrates are nearly always present with lymphoid aggregates or follicles; germinal centers are present in 50%–78% of cases. The picture becomes even more confusing when there is HIV co-infection (Bach

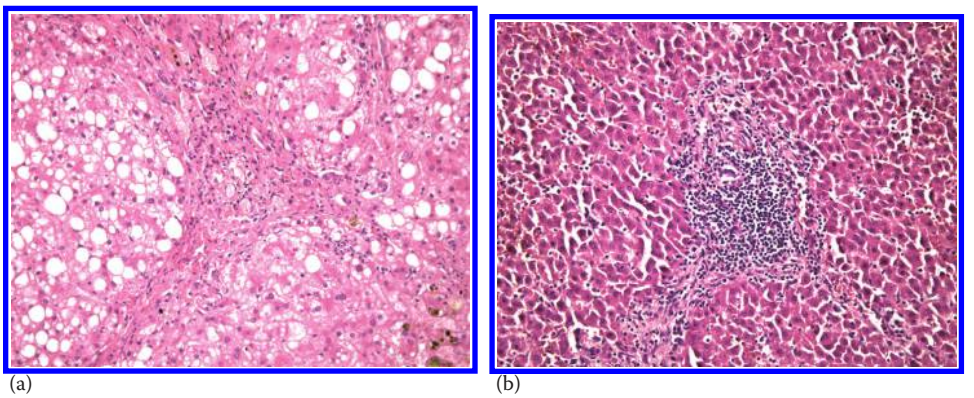


Figure 5.54 (a, b) *Triaditis*. Infiltration of the portal triads is a common finding in both stimulant and opiate abusers. For many years the etiology of this finding was unclear, but now it is generally accepted that it is hepatitis related. Virtually all heroin users in California are hepatitis C infected. The rate is not much lower in the rest of the country or in Europe. (Photograph courtesy of Vittorio Finneschi, Sienna.)

et al., 1992; Scheuer et al., 1992). Often these lymphoid follicles are located close to bile ducts that manifest various degrees of damage. Still, it may be hard or even impossible to distinguish these features from those of chronic hepatitis.

5.12.4.7 Steatosis

Steatosis (fatty liver) is a frequent finding in the livers of chronic drug abusers. It is usually attributed to co-ingestion of alcohol and possibly to the abuse of stimulant drugs. In alcoholics, steatosis may be the only finding in otherwise unexplained cases of sudden death (Chejfec, 2001). Sudden death in patients with steatosis due to chronic alcoholism is the result of an abnormality in the cardiac conduction system that is manifested as a prolonged QT interval. The triggering event in these cases of sudden death is not known, but hypoglycemia, hypophosphatemia, and hypomagnesemia are all considered good candidates. That having been said, in long-term studies of patients with NAFLD (>20 years), survival was good and independent of the histologic, clinical, and biochemical characteristics at the time of biopsy; the main causes of death were cardiovascular disease and cancer (Dam-Larsen et al., 2009).

Fatty liver may also be a marker for the presence of NASH. NASH is a histologic diagnosis applied to a picture that looks very much like alcoholic liver disease, except that it occurs in the absence of alcohol abuse and generally is associated with obesity; the prevalence of NAFLD is rising as it parallels the increasing prevalence of obesity in developed countries, and it is estimated that NASH now affects up to 45% of the middle-aged population (Hjelkrem et al., 2008).

Liver enzymes, especially aminotransferases, are almost always elevated, which often helps to establish the diagnosis. Fifteen to forty percent of NASH patients will go on to develop hepatic fibrosis, with 1%–2% developing cirrhosis (Harrison and Neuschwander-Tetri, 2004). Susceptibility to NASH is greatest in patients carrying the C(-159)T polymorphism in the *CD14* gene promoter region (Brun et al., 2006). Insulin resistance may also play a role but, clinically, the only way to tell whether the disease is progressing or regressing is repeat liver biopsy. Several semiquantitative biopsy grading systems have been devised.

One scoring system, termed the NAFLD activity score, originally evaluated 14 histologic features, but the 5 features most relied upon are steatosis ($p = 0.009$), hepatocellular ballooning ($p = 0.0001$), lobular inflammation ($p = 0.0001$), fibrosis ($p = 0.0001$), and the

absence of lipogranulomas ($p = 0.001$) (Hjelkrem et al., 2008). Drugs such as amiodarone, tamoxifen, and some ARV drugs can induce both steatosis and NASH. Stavudine and zidovudine are often implicated.

Although numerous investigations suggest that reactive oxygen species (ROS) are key to the progression of fatty liver to NASH, the etiology of this disease remains uncertain; however, generation of ROS by the mitochondrial respiratory chain, particularly cytochrome P450 2E1 (CYP2E1), is clearly implicated. Increased hepatic CYP2E1 expression and activity has often been detected in the obese and NASH, but the role of this particular enzyme is not known; increased hepatic levels of fatty acids and insulin resistance may play a role. It has also been postulated that greater expression of hepatic CYP2E1 could play a significant role by inducing lipid peroxidation and causing oxidative damage to key cellular components. In addition, CYP2E1-mediated overproduction of ROS also promotes hepatic insulin resistance, which would further exacerbate steatosis. CYP2E1 is located within liver mitochondria, and its increased expression could also have detrimental effects on mitochondrial function, perhaps explaining the hepatotoxicity associated with some drugs like zidovudine. For the moment, all of these suggestions are plausible but unproven.

5.12.4.8 HIV Infection

The gastrointestinal tract is a less common target for HIV involvement than either the brain or respiratory tract (Jellinger et al., 2000). Nonetheless, liver disease is common in HIV-infected individuals, especially in IVDUs, who are likely to be already infected with HCV. The viruses themselves, as well as treatment with dideoxynucleoside analogs (didanosine and stavudine), make the occurrence of steatosis and hepatic fibrosis even more likely (Bani-Sadr et al., 2006; McGovern et al., 2006), and it has now been clearly proven that ARV therapy and high counts of CD4− T cells are associated with reduced progression of steatosis in patients co-infected with HIV and HCV. Thus every effort is now made to diagnose and prevent steatosis, particularly in those with a high body mass index and excessive alcohol intake (Woreta et al., 2011) should be made.

HAART has changed the clinical features of HIV infection, and while the number of HIV deaths is decreasing, the proportion involving liver and heart disease is increasing (Palella et al., 2006). In a review of HIV deaths occurring after HAART's introduction, the leading causes of AIDS deaths were (1) AIDS multiple causes (31%), (2) *M. avium* complex (18%), (3) *Pneumocystis* pneumonia (10%), and (4) non-Hodgkin's lymphoma (7%). Hepatic disease accounted for 19% of the deaths in this series (Krentz et al., 2005). Data from the United States are not available, but in Spain, where there are an estimated 60,000–80,000 individuals co-infected with HIV and HCV and 5000–10,000 co-infected with HIV and HBV, 10%–15% of these individuals suffer from liver cirrhosis (Miro et al., 2004).

Hepatic disease in HIV patients may be a consequence of alcoholism or previously existing viral hepatitis, a manifestation of opportunistic infections or opportunistic tumors, or a result of drug therapy or may occur due to any combination of the three (Zeremski and Talal, 2006). Some of the opportunistic liver infections include *M. avium-intracellulare*, which causes multiple granulomas obstructing the terminal branches of the biliary tree (Glasgow et al., 1988), cytomegalovirus, *Cryptococcus neoformans*, and type 2 herpes simplex virus, to name but a few (Schneiderman, 1988; Ainsworth et al., 2000). Opportunistic parasitic infections such as leishmaniasis are now common in HIV-infected patients and are usually diagnosed days after initial diagnosis of HIV, though leishmaniasis may, in fact, be the presenting symptom (Khandelwal et al., 2011).

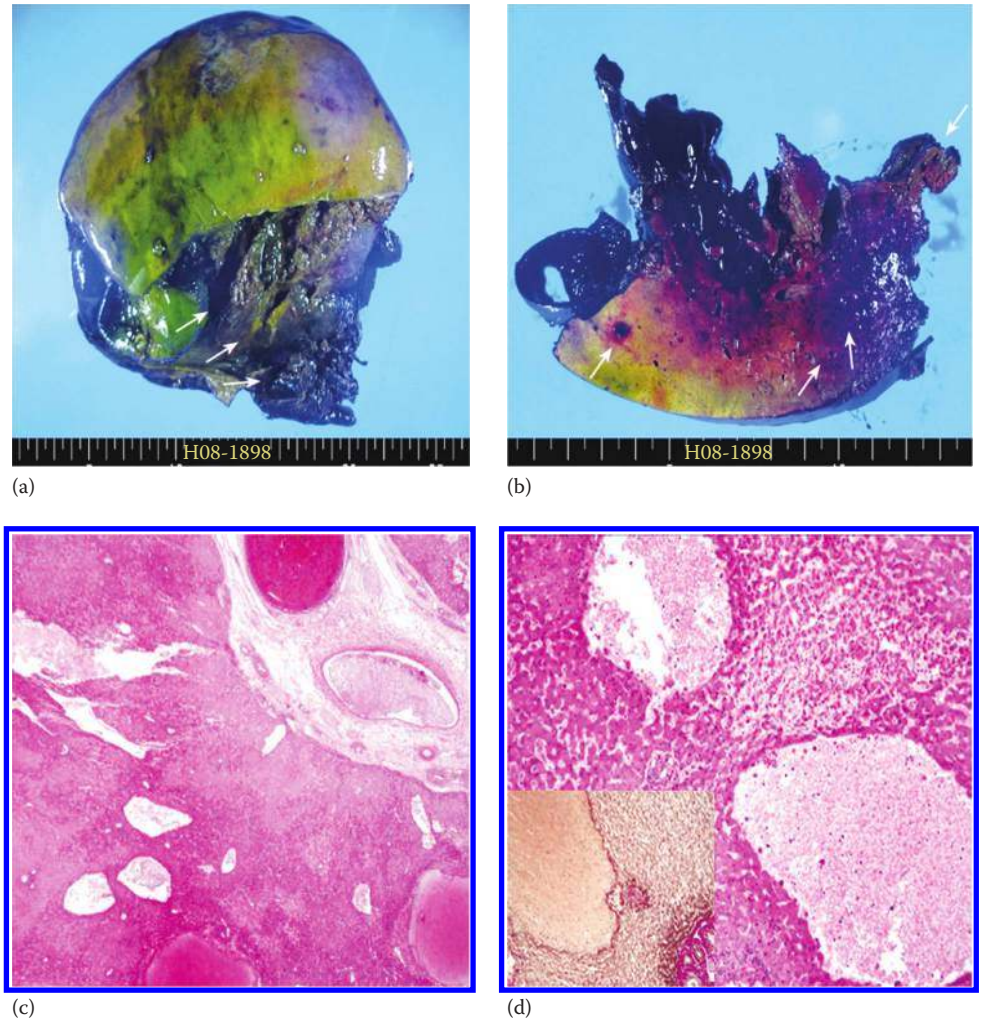


Figure 5.55 Photographs showing peliosis in a patient who had been treated with anabolic steroids for a year and died of a hepatic hemorrhage. Blood-filled lesions are randomly scattered throughout the liver, often in association with foci of hepatocellular necrosis. The condition is difficult or impossible to diagnose in life without CT or ultrasound scanning. The first sign of its presence may be massive hematoperitoneum. Gross findings: (a) resected specimen shows extensive necrosis and hemorrhage (arrows) and (b) cut surface of the liver parenchyma reveals small blood-filled cystic lesions (arrows). Microscopic findings: (c) low-magnification view shows variable-size, blood-filled cystic spaces (HE, ×10) and (d) high-magnification view shows hemorrhagic necrosis in areas adjacent to peliotic spaces without lining endothelium (HE, ×10). (From Choi, S. K. et al., *World J. Gastroenterol.*, 5, 5493, 2009. With permission.)

HIV-infected patients, like anabolic steroid abusers, may develop peliosis (Figure 5.55), a condition characterized by the presence of many small, cystic, blood-filled areas, usually in the liver but occasionally in the lungs or other organs. Blood-filled lesions are randomly scattered throughout the liver, often in association with foci of hepatocellular necrosis. The condition was first recognized in conjunction with tuberculosis, but the connection with anabolic steroid abuse has been obvious for some time (Choi et al., 2009).

5.12.4.9 Amyloidosis

Intravenous drug abusers with hepatic amyloid often are HIV infected and may well also have HCV. Nonetheless, when hepatic amyloid deposition occurs in heroin and cocaine abusers, it is almost invariably a consequence of the chronic suppurative skin lesions, a result of poor hygiene and repeated subcutaneous heroin injection. In heroin addicts, the type of amyloid deposited is unpredictable and of no diagnostic value, nor is knowledge of the pattern of deposition particularly helpful either (Osick et al., 1993). Interestingly, amyloid is not a common finding in the brains of drug abusers, but it does appear to be strongly implicated in HIV-associated dementia (Chang et al., 2011).

5.12.5 Renal Disease

5.12.5.1 Introduction

Chronic narcotics abusers develop renal disease (Table 5.34). In cocaine and heroin users, it is manifested as nephrotic syndrome, acute glomerulonephritis, glomerulosclerosis, amyloidosis, interstitial nephritis, and rhabdomyolysis. The pathophysiologic basis of cocaine-related renal injury involves renal hemodynamic changes, glomerular matrix synthesis and degradation, and oxidative stress and induction of renal atherogenesis. The predominant renal lesion in black heroin users is focal segmental glomerulosclerosis (FSGS), and in white heroin users it is membranoproliferative glomerulonephritis. Although the prevalence of heroin use in the United States has increased, the incidence of *heroin nephropathy* (Figures 5.56 and 5.57) has declined. Reports of heroin nephropathy predated surveillance of HCV and HIV, and it may well be that what had previously been thought to be a heroin-related disease is actually a virus-related disease. There is no doubt that the endothelins (ET-1) play a role, greater or lesser, in most of these disorders, especially those characterized by excessive renal vascular resistance, such as ischemic renal failure, CyA nephrotoxicity, radiocontrast nephropathy, endotoxemia, rhabdomyolysis, acute liver rejection, and others (Kon et al., 1989; Kohan, 1997).

In addition, socioeconomic conditions, cultural and behavioral practices, genetic polymorphisms may, to a large extent, determine whether or not a nephropathy develops in the first place. Administration of cocaine to animal models produces nonspecific glomerular, interstitial, and tubular cell lesions, but there is no animal model of heroin-related kidney disease. There simply are no well-designed, prospective, epidemiologic studies to assess the incidence and the prevalence of renal disease in populations of opiate users (Jaffe and Kimmel, 2006).

5.12.5.2 Heroin-Related Kidney Disease

FSGS is the term used to describe a common lesion seen in a disparate group of progressive renal diseases. Clinicians, however, use the term to describe the clinical syndrome

Table 5.34 Renal Disorders in Opiate Abusers

Focal glomerulosclerosis
Membranoproliferative glomerulonephritis
Renal amyloidosis
Necrotizing angiitis with renal involvement
Interstitial nephritis
Acute tubular necrosis due to rhabdomyolysis

Source: Compiled from world literature.

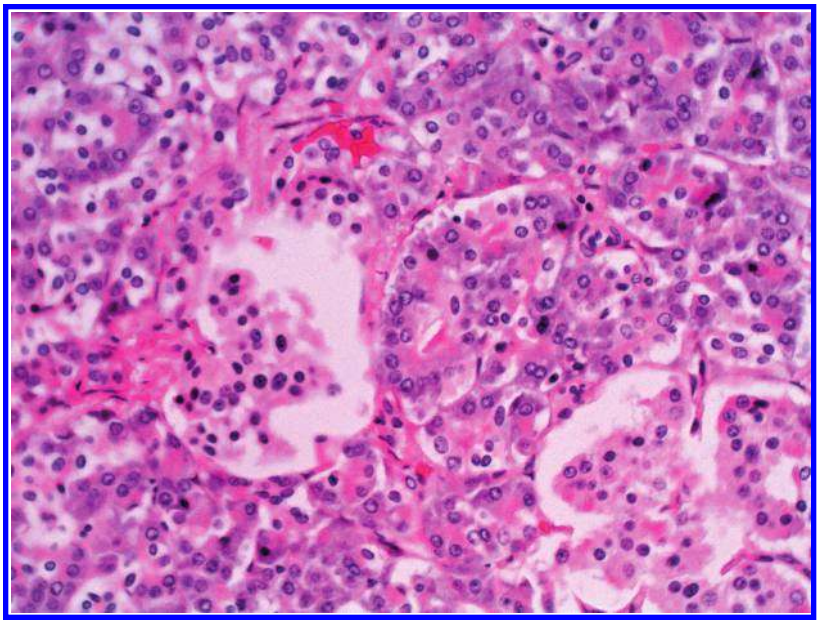


Figure 5.56 Glomerulosclerosis in a chronic cocaine abusers with no history of renal disease. Glomeruli are shrunken and the endothelial cytoplasm granular and fragmenting. The glomeruli also contain increased numbers of lymphocytes, and hyaline deposits. These morphologic changes are nonspecific. (Micrograph by author.)

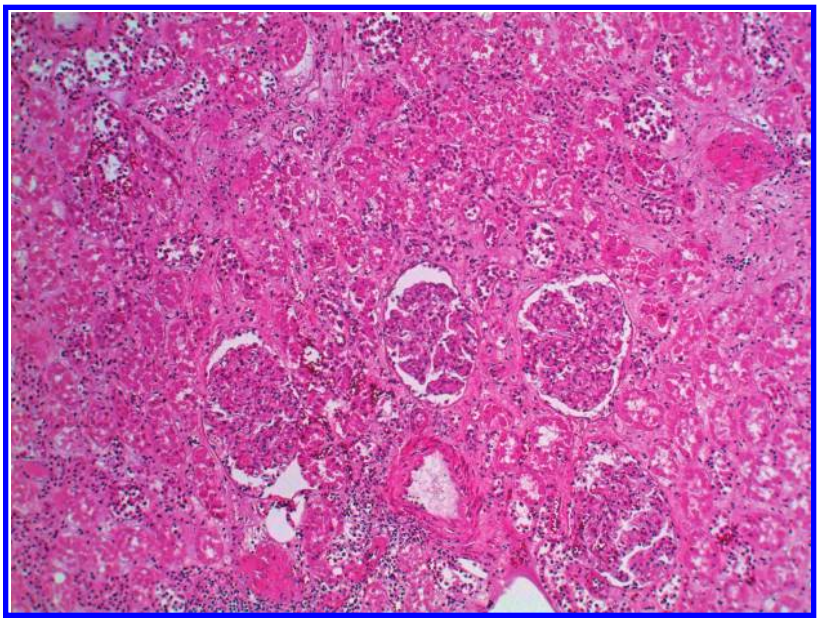


Figure 5.57 Extensive destruction of the glomeruli with scarring and shrinkage of the parenchyma. (Photograph by author.)

associated with this lesion. Different patterns of FSGS exist, and each is associated with a different disease. Once the diagnosis of FSGS has been made, the pattern present must be distinguished from other possible types of disease, and this is done by looking for the presence of five different patterns that can be seen with the light microscope.

FSGS is common in chronic drug abusers. It can also occur in conjunction with many different disorders: immune-complex mediated injury, hypertension, obesity, diabetes, reflux nephropathy, chronic interstitial nephritis (CIN), and HIV infection (Rossini and Fogo, 2004). Nonetheless, if one of the five light microscopic patterns can be identified, effective treatment may be possible.

In the early 1970s, reports began to appear describing a relentlessly progressive variety of nephrotic syndrome. It was unresponsive to therapy and terminated in renal failure within a few months to a few years (Rao et al., 1974; Cunningham et al., 1983; Dubrow et al., 1985). The syndrome occurred only in heroin abusers, primarily in blacks, and only in the U.S. The predominant histologic alteration in these individuals was FSGS (Grishman et al., 1976). By the mid-1990s, however, new cases simply stopped appearing (Friedman and Tao, 1995). Although it has never been proven, many believe that kidney failure in those individuals was an immune-mediated process. However, many different conditions can lead to a picture that is histologically indistinguishable. Infection with HIV, parvovirus B19, simian virus 40 (Moudgil et al., 1997; Li et al., 2002; D'Agati, 2003) all lead to the same morphologic picture as is seen in heroin abuse, although some distinctions may be possible with special staining and/or electron microscopy (Rossini and Fogo, 2004).

One particularly interesting fact about the timing of this outbreak in the mid-1990s was that serotyping for hepatitis C was still uncommon, and most patients who were carrying the virus had no idea that they were infected. The possibility of viral causation cannot be ruled out. In one recent biopsy study of 19 Caucasians with nephropathy secondary to heroin abuse, all 19 had serologic evidence of HCV infection, 1 had HBV surface antigen, and 3 were HIV positive. Thirteen patients (68.4%) were found to have membranoproliferative glomerulonephritis (MPGN), twelve with type I, and one with type III. Of the remaining patients, two had CIN, two had acute proliferative glomerulonephritis, one had amyloidosis, and one had granulomatous glomerulonephritis with interstitial nephritis. The researchers concluded that, in the cohort they were studying, HVC-associated membranous glomerulopathy was the most frequent pattern of nephropathy evident, indicating that the nephropathy associated with heroin abuse in Caucasians is not of the focal and segmental glomerulosclerosis type as previously reported in Africans—all of which means that a different type of therapy may well be required.

Another problem with the older reports is that in the past little effort was made to track heroin adulterants. That information is available now, and some of the chemicals that are still being added are clearly nephrotoxic (Schneider and Meys, 2011). Phenacetin is a fairly frequent heroin adulterant, and its connection with kidney disease was first reported in 1968 (Dubach et al., 1968). Similarly, acetaminophen (paracetamol) is a known cause of interstitial nephritis (Fruchter et al., 2011), which is why the U.S. FDA forced opioid makers to reduce the amount of acetaminophen they were adding to combination products.

Some other histologic features may be helpful to the nonspecialist (Table 5.35): the kidneys of heroin abusers usually show evidence of marked interstitial fibrosis with interstitial infiltrates of lymphocytes and plasma cells. In addition, Bowman's capsule may be markedly thickened. By contrast, the FSGS that occurs in HIV patients is usually devoid of cellular infiltrates, and HIV patients generally do not have interstitial fibrosis. The results of animal

Table 5.35 Differentiation of Heroin-Associated Nephropathy from HIV

Heroin-Associated Nephropathy (HAN)	Human Immunodeficiency Virus (HIV)
Mesangial hypercellularity	Mesangial hypocellularity
Interstitial infiltrates present	Interstitial infiltrates absent
Interstitial fibrosis prominent	Interstitial fibrosis absent

studies suggest that the glomerular and renal epithelial cells are the primary targets of HIV-1 pathogenesis in the kidney and that the essential pathologic process involves dysregulation of the epithelial cell cycle, with increased proliferation, apoptosis, cellular dedifferentiation, and altered cellular polarity (Genderini et al., 1990; Barisoni et al., 2000). Other studies have shown that HIV cardiomyopathy receptor-controlled apoptosis is the underlying mechanism. Similar pathways have not, as yet, been observed in humans (Twu et al., 2002).

Cases of HAN have been reported from Europe. Autopsies were performed on a group of more than 5000 drug users. Essentially all the decedents were white, and 82% were men. Almost the same number, 81.4%, were intravenous drug abusers. Median age at death was 39 years and duration of drug abuse was 17 years. Toxicology testing showed that nearly all were polydrug users and that most of them suffered from a variety of systemic illnesses. Direct renal examination disclosed a broad spectrum of pathologic alterations, mainly atherosclerotic and/or ischemic. In addition, mild interstitial inflammation, calcification of renal parenchyma, and interstitial fibrosis and tubular atrophy, with hypertensive ischemic nephropathy, were found to be the most common causes of nephropathy. Specifically, interstitial inflammation (OR, 16.59; 95% CI, 3.91–70.39) and renal calcification (OR, 2.43; 95% CI, 1.03–5.75) were seen in the severe intravenous users, whereas hypertensive and ischemic damages were associated with cocaine abuse (OR, 6.00; 95% CI, 1.27–28.44). Perhaps more important, neither specific glomerular damage indicative for heroin abuse nor changes consistent with HCV-related disease or even analgesic nephropathy could be detected (Buettner et al., 2014).

Renal disease is not rare among European heroin users. A retrospective study of 179 forensic autopsies disclosed that slightly less than two-thirds of the decedents had nonlymphocytic membranoproliferative glomerulonephritis, and half of the specimens contained deposits of IgM antibody, but none showed any evidence of focal glomerulosclerosis. In any individual case it is impossible to say whether IgM antibody deposits are a response to infection with HCV, or HBV, or to some toxic adulterant mixed with street heroin (Dettmeyer et al., 1998). Whatever the cause, progression of the lesions ultimately leads to glomerular destruction and symptomatic renal disease. Advanced lesions consist primarily of intracapillary deposits of eosinophilic, PAS-positive material involving isolated or multiple segments of the glomerulus.

Other infectious diseases can also involve the glomerulus, either directly or indirectly. Many heroin injectors with endocarditis will have focal or diffuse glomerulonephritis as a result of the deposition of circulating antigen–antibody complexes (Rao et al., 1974). The deposition of immune complexes within the glomerulus causes diffuse proliferative changes and even classic crescent formations. However, many, if not most, of the reported cases are in the older literature in individuals with staphylococcal endocarditis (Louria et al., 1967; Gutman et al., 1972).

The true incidence of glomerulonephritis in addicts has never been established, but reports are uncommon. In Sapira's (1968) autopsy study, the incidence of chronic glomerulonephritis in known addicts was 8% (Sapira, 1968). That value may no longer apply today. More recent experience suggests that the incidence of acute renal disease may be much

lower. In most cases of endocarditis, renal embolization with infarction is more likely than immune complex deposition. In either case, these lesions rarely cause significant disease. Finally, membranous nephropathy associated with chronic hepatitis B surface antigenemia is a recognized entity (Cunningham et al., 1983), and chronic HCV infection may be associated with mixed cryoglobulinemia that may in turn result in glomerulonephritis (Ramos et al., 1994; Bakir and Dunea, 2001). Like focal glomerulosclerosis in addicts, reports of cryoglobulinemia in addicts have simply disappeared, only to be replaced with HIV nephropathy, with which it shares some common traits (Genderini et al., 1990).

5.12.5.3 Necrotizing Angiitis

In 1970, Citron et al. described a polyarteritis-like syndrome occurring in intravenous drug abusers who were living in New York City; medium-sized and small arteries in most organs, as well as the arterioles in the brain, were involved. The elastic arteries, capillaries, and veins were all spared. Acute fibrinoid necrosis of the media and intima was observed, along with prominent infiltrates of eosinophils and lymphocytes. Occlusive thrombi were also present. The subacute process was marked by intimal proliferation and luminal narrowing, with saccular aneurysms, especially at vessel bifurcations. Very little evidence suggests that such a disorder ever occurs in opiate abusers or even that it occurs among today's amphetamine abusers. Most of the patients described by Citron et al. were intravenous amphetamine abusers or polydrug abusers taking combinations of amphetamine and other drugs. Of the patients Citron et al. studied, none who used only heroin developed the syndrome. Sporadic reports have been appearing ever since (Samuels et al., 1996; Niehaus and Meyer, 1998).

Although no evidence links necrotizing vasculitis with heroin abuse, there is ample evidence for a connection with methamphetamine abuse (Bingham et al., 1998) and with levamisole-adulterated cocaine (Geller et al., 2011). However, vasculitis in cocaine and methamphetamine abusers does not meet the histologic definition for polyarteritis.

5.12.5.4 Acute Renal Failure and Nontraumatic Rhabdomyolysis

Rhabdomyolysis (Figures 5.58 and 5.59) accounts for a significant proportion of morbidity and mortality among IVDUs. The connection was first noted nearly 40 years ago (Richter et al., 1971), and cases have been reported regularly ever since. There have been no reports of this disease in any of the European centers dispensing pharmaceutical-grade heroin and clean needles, suggesting very strongly that the problem is due to toxic agents mixed with the heroin or to sanitary practices in general (Gschwend et al., 2004). Rhabdomyolysis is caused by a confluence of events, including hypotension, fluid imbalance, and pressure necrosis. The result is muscle destruction and liberation of myoglobin into the bloodstream. However, in 1971 Richter et al. observed that this syndrome can occur in patients who are neither comatose nor subject to muscle compression (Richter et al., 1971), further strengthening the argument that myotoxic adulterants play a role (de Gans et al., 1985; Melandri et al., 1996).

There are, however, case reports suggesting that heroin may have direct myotoxic activity and be especially damaging to heart muscle. Some in vitro studies have shown that heroin produces dose-related increases in cardiomyocyte intracytosolic calcium which could, potentially, lead to cell destruction (Liu et al., 2007). There are also individual case reports confirming myocardial damage in heroin abusers that appears to be the result of direct myotoxicity (Melandri et al., 1991; Toth and Varga, 2009).

Whatever the etiology, the clinical course is always the same with rapid onset of oliguria followed by azotemia, acidosis, hypophosphatemia, hyperuricemia, and all of the other

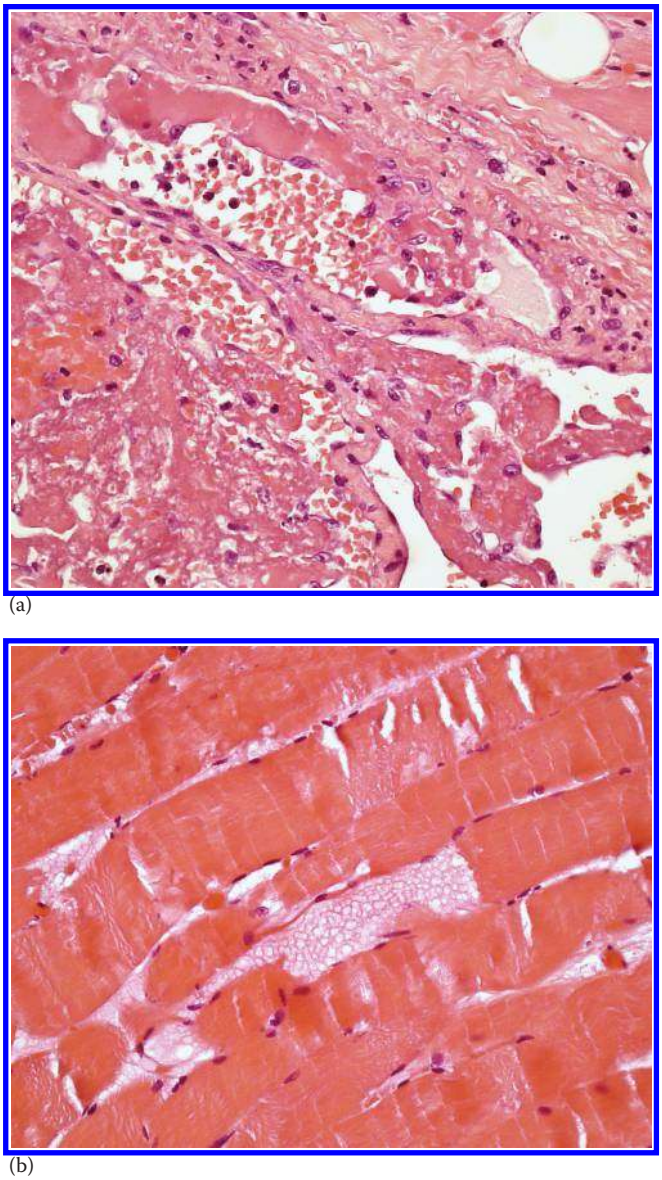


Figure 5.58 (a, b) Acute rhabdomyolysis in an intravenous heroin abuser. Both slides are from the same patient. (Courtesy of Dr. U. Oehler, Institut für Pathologie, Singen, Akademisches Lehrkrankenhaus der Universitat Freiburg, Germany.)

electrolyte and chemical disorders associated with renal failure. Once it is recognized, the condition is rarely fatal, so these patients seldom come to autopsy or even biopsy. There is no reason to suppose that the histologic changes are in any way different from those encountered in cases of traumatic rhabdomyolysis. If the patient survives for several days, autopsy will disclose yellow-orange staining of the fat, a consequence of muscle cell breakdown (see Figure 5.59).

5.12.5.5 Secondary Amyloidosis

Systemic amyloidosis is a long-term complication of multiple, different, chronic inflammatory disorders. Organ damage is a consequence of the extracellular deposition of proteolytic

Figure 5.59 Rhabdomyolysis in a heroin abuser, presumably secondary to prolonged pressure during a drug-induced near coma state. Note the characteristic yellowish color of the tissue. (Courtesy of American College of Pathologists.)

fragments of the acute-phase reactant serum amyloid A as amyloid fibrils in tissues throughout the body. Heroin users (and perhaps users of other drugs as well) who inject heroin subcutaneously (*skin popping*) have a higher chance of developing secondary amyloidosis, with the kidneys, liver, and spleen as the main targets. More than 90% of patients with renal amyloidosis will present with proteinuria, nephrotic syndrome, or impaired renal function. The underlying etiology is believed to be chronic immunologic stimulation by one or more exogenous antigens contaminating the street heroin or multiple acute inflammatory episodes occurring in response to heroin adulterants (Cooper et al., 2013).

The occurrence of renal amyloidosis in a heroin abuser was first described in a paper published in 1978 (Jacob et al., 1978). Since then it has become apparent that the incidence of renal amyloid in heroin addicts is significantly higher than the incidence of amyloid in the general population (Dubrow et al., 1985; Maury and Teppo, 1982). The incidence of this complication may be increasing, but with no adequate surveillance system, it is difficult to say. In one recent study, 70% of biopsy-proven patients with renal amyloid were heroin abusers (Manner et al., 2009).

Amyloid deposits are more commonly found in the kidneys of older, long-term abusers. Amyloid deposition results in massive proteinuria, with or without azotemia. Unlike autosomal dominant hereditary amyloidosis (Granel et al., 2006), amyloid in addicts is an acquired disease. Over 90% of addicts with renal amyloid will have clinical evidence of repeated suppurative skin lesions (Meador et al., 1979; Dubrow et al., 1985; Neugarten et al., 1986). The first reported cases of heroin-associated renal amyloid were from New York City, but there are now sporadic reports from wherever there are concentrations of heroin abusers.

Routine light microscopy of patients with renal amyloid, using either hematoxylin–eosin or PAS staining, will reveal large amounts of eosinophilic material within the glomerulus. Confirmation that the material is in fact amyloid can be obtained by Congo red staining or by using polarizing microscopy. Amyloid has a typical apple-green birefringence. Electron microscopy shows amyloid fibrils.

5.12.6 Neuropathology

5.12.6.1 Introduction

When the first descriptions of heroin toxicity were published at the turn of the late 1800s, opiates were thought to be neurotoxic (Nissil, 1897). Fatty degeneration, particularly of neurons in the deeper layers of the frontal cortex and Ammon's horn, was thought to be diagnostic for *morphinism*. Subsequent studies have shown that the changes observed were either nonspecific or artifactual, but more recent studies have soundly established that injected heroin/morphine may act synergistically with the HIV virus to cause neuronal damage (El-Hage et al., 2014).

In the 1970s it was thought that heroin abusers were uniquely prone to basal ganglion infarction (Jervis and Joyce, 1948; Strassmann et al., 1969; Hall and Karp, 1973). The nonspecific nature of that finding is also now understood. With the exception of perivascular pigment deposition within macrophages, which probably is the result of repeated intravenous injection of foreign material (Figure 5.60) (Gray et al., 1992), no one histologic lesion is diagnostic for narcotic abuse, though they may be visualized with newer imaging techniques (Wang et al., 2011b). Even when morphologic changes are evident, they are almost always a consequence of some infectious process acquired during the process of heroin injection. The better-known neuropathologic complications of narcotic abuse are listed in Table 5.36.

5.12.6.2 Hypoxic Encephalopathy

Deaths from acute opiate toxicity are usually associated with cerebral edema, meningeal congestion, and flattening of the gyri (Adelman and Aronson, 1969; Strassmann et al., 1969; Pearson et al., 1972). As a rule, these deaths occur so rapidly that morphologic evidence of cellular injury is not apparent without the use of immunohistochemical staining (Oechmichen and Meissner, 2006). With longer periods of survival, characteristic patterns of tissue necrosis emerge. The explanation is that hypoxic deaths are very quick, and there

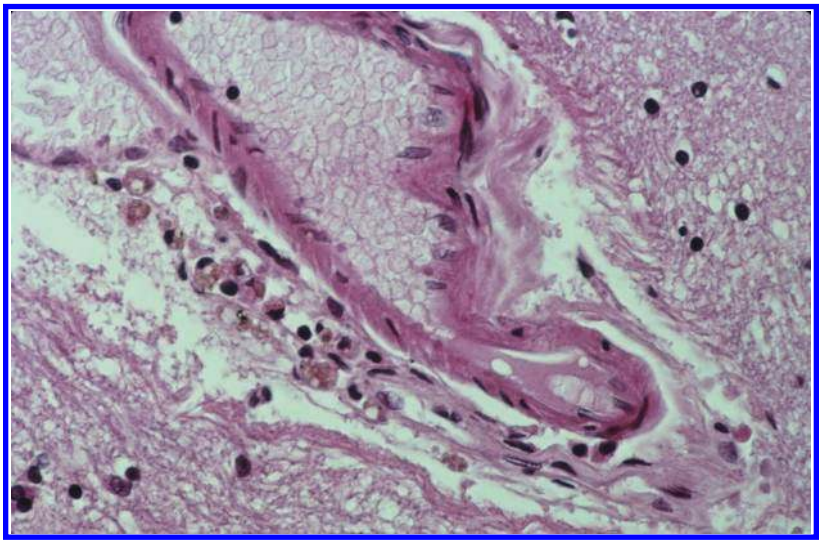


Figure 5.60 Hemosiderin-laden macrophages. Micrograph from the brain of an HIV-negative heroin addict. Similar cells are often seen in the lungs. In both locations, they appear to be the result of repeated intravenous injections of particulate matter. (Courtesy of Professor Francoise Gray, Départment de Pathologie, Hôpital Henri Mondor, Gretiel, France.)

Table 5.36 Neuropathologic Complications of Narcotic Abuse

1. Hypercapnic hypoxia
   a. Cerebral edema
   b. Venous congestion
   c. Focal hemorrhage
2. Infectious
   a. Complications of endocarditis
   b. Complications of HIV infection
      i. Encephalopathy
      ii. Opportunistic infections
      iii. Opportunistic tumors
   c. Phycomycosis
3. Spongiform encephalopathy
4. Transverse myelopathy
5. Peripheral neuropathy
6. Rhabdomyolysis
7. Stroke
8. Necrotizing angiitis
9. Parkinsonism

simply is no time for the characteristic injury patterns to become visible. The injuries seen are not so much a result of hypoxia but rather a result of the arterial hypotension that ensues because of the hypoxia (Brierley et al., 1971). In any event, the earliest visible change is neuronal microvacuolation that can be seen 2–4 h after death. Neurons maintain their shape, but vacuoles may form within the cytoplasm, then the neurons themselves become distorted, the cell body shrunken, and the cytoplasm intensely eosinophilic. This change persists for at least 6 h (Brierley et al., 1971; Graham and Adams, 1971). Should the victim survive an episode of acute hypoxia, the changes become progressively worse. Acute swelling of glial cells may occur very rapidly, but several days must elapse before there is any noticeable proliferation of astrocytes.

The three recognizable phases of neuronal death can each be related to different molecular events occurring in the dying cell. Excess activation of ionotropic glutamate receptors causes the influx and accumulation of Ca^{2+} and Na^{+} ions (Annunziato et al., 2007). That process leads to rapid swelling and subsequent neuronal death. Oxidative stress occurs due to the accumulation of reactive oxygen and nitrogen species. Finally, apoptosis, preprogrammed cell death, comes into play, though microscopically it is not immediately evident (Won et al., 2002).

Under certain circumstances, the pattern of injury may reveal a great deal about the clinical events that preceded death. A major abrupt decrease in systemic blood pressure typically produces necrosis in the arterial boundary zones between the major arteries. The area most frequently involved is the parieto-occipital region (Figure 5.61). If the drop in blood pressure is more gradual and longer in duration, then laminar necrosis of the calcarine cortex may be seen. This lesion is also prominent in the deeper layers of the cerebellum (Greenfield et al., 2002).

A pattern of continuous necrosis, often accentuated in arterial border zones, may also be encountered. The Purkinje cells of the cerebellum are particularly vulnerable to injury, as are the cells of Sommer's sector, located in the hippocampus (Adams et al., 1966). Here too, time must elapse before these patterns become apparent. If death occurs within 3–6 h,

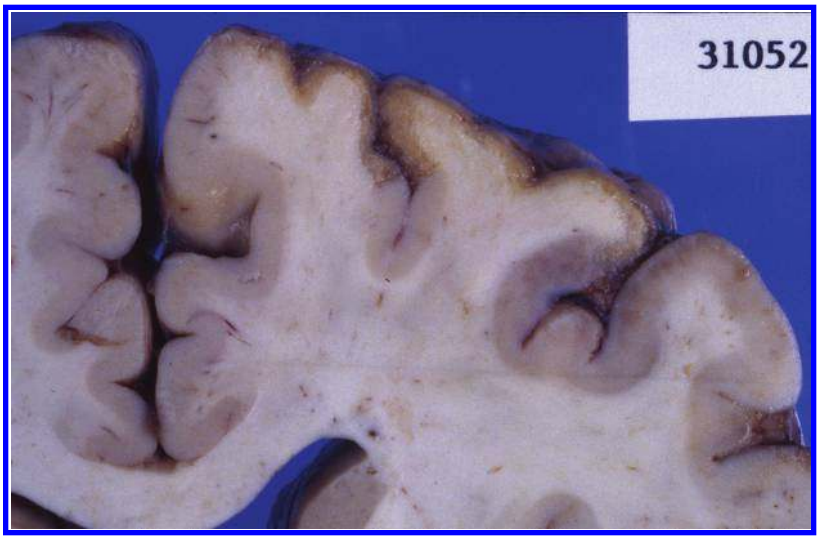


Figure 5.61 Laminar necrosis. When a drop in blood pressure is partial and sustained, laminar necrosis may be prominent.

the probability of detecting anything but chronic changes is small. With the passage of more time, typical eosinophilic degenerative changes become apparent in scattered neurons. The cells of the caudate and putamen may or may not be involved. If changes are to be detected in those nuclei, then sampling from multiple sites will be required.

These gross findings are consistent with single photon emission computed tomography (SPECT) studies of opiate addicts, where baseline perfusion reductions are apparent in the frontal and parietal cortices while, at the same time, flow to the thalamus is increased. When these same individuals are given naloxone, cerebral perfusion to these areas decreases (Krystal et al., 1995; Hermann et al., 2012; Moreno-Lopez et al., 2012).

Chronic hypoxic episodes from repeated drug overdoses predictably result in necrosis and scarring of the hippocampus. However, the diagnostic value of this observation is limited by the fact that most heroin abusers today are in fact polydrug abusers. For example, hippocampal atrophy is common in alcoholics, particularly women (Agartz et al., 1999). Amygdaloid volume seems to be a good indicator of relapse to alcoholism (Wrase et al., 2008), as is reduced cross-sectional area of the corpus callosum (Pfefferbaum et al., 1996). These acute lesions may be superimposed on preexisting chronic or subacute changes. Thus, zones of parietal–occipital necrosis may be seen along with areas of laminar necrosis, suggesting an initial acute hypotensive episode followed by prolonged hypotension and decreased cerebral flow. This type of pattern is not uncommon in heroin addicts.

5.12.6.3 Neurologic Complications of Endocarditis

Narcotics abusers develop infectious diseases because of their unhealthy lifestyles, because their injection techniques are not sterile, because chronic opiate use results in immunosuppression, and, until the introduction of levamisole as an adulterant, only very rarely because the heroin being injected is contaminated (Hagmann, 2000). Some very bizarre infections, such as aspergillosis (Morrow et al., 1983), nocardiosis (Hershewe et al., 1988), phycomycosis (Adelman and Aronson, 1969), chromoblastosis (Kasantikul et al., 1988), mucormycosis (Masucci et al., 1982), and *Burkholderia cepacia* (which is essentially confined to those who

are severely immunocompromised) (Ki et al., 2011) have all been reported. Even Subutex (sublingual buprenorphine that has been injected intravenously) has been reported as a cause (Chong et al., 2009), and some evidence suggests it may be more damaging than other opiates. Specifically, it has been reported that the injection of crushed buprenorphine tablets may result in a diffuse leukoencephalopathy (Seet et al., 2005). Generally, these exotic infections are not major causes of morbidity. There is some evidence that, as μ-opiate receptors are expressed both in the brain and immune system (in lymphocytes and phagocytes), injected buprenorphine could trigger an immunologic response to neuronal tissues within the brain, severely affecting areas that are susceptible to the effects of demyelination and ischemia. This hypothesis has not been validated in an animal model.

On the other hand, septicemia, endocarditis, and even necrotizing fasciitis are increasingly common, and all three disorders may have neurologic sequelae. In fact, the incidence of neurologic complications resulting from subacute endocarditis has changed hardly at all since the introduction of antibiotics (Ziment, 1969).

Vegetations on the aortic and tricuspid valves can shed, producing disseminated microabscesses throughout the CNS, with smaller lesions centering around septic emboli that lodge in terminal vessels, producing cerebral infarction (Louria et al., 1967; Dreyer and Fields, 1973; Grindal et al., 1978). In more severe cases, foci of metastatic suppuration may be seen throughout the leptomeninges. Intracranial hemorrhage secondary to the rupture of mycotic aneurysms can occur, but even today such events remain relatively uncommon (Jones et al., 1969; Chu et al., 2005). The main sites of bacterial infection of the brain are in the capillaries and small venules. They are usually surrounded by perivascular collections of polymorphonuclear leukocytes. As a rule, microabscesses do not produce severe or focal symptoms, and their presence may often be camouflaged by other more obvious disease processes (Biller et al., 1986).

5.12.6.4 Complications of HIV Infection

Since the first announcement of AIDS in 1981, approximately 33 million people worldwide have become infected with HIV (Schwartländer et al., 2011). In addition to the obvious detrimental systemic effects on the immune system, HIV-1 enters the brain almost as soon as the infection begins. Once established in the brain, the virus can induce a variety of severe and debilitating neurologic disorders and opportunistic infections, many of which lead ultimately to dementia. Infected peripheral macrophages (and other cell types, though macrophages predominate) infiltrate the brain and provoke a series of deleterious responses that may be very widespread. Viral and host factors, such as the viral strain and the way that the host's immune system responds, have a huge influence on how disease will progress.

Different brain imaging techniques allow the examination of the structure, biochemistry, metabolic state, and functional capacity of the brain. All of the major neurodegenerative disorders have relatively specific imaging findings that can be identified. New imaging techniques carry the hope of revolutionizing the diagnosis of neurodegenerative disease so as to obtain a complete molecular, structural, and metabolic characterization, which could be used to improve diagnosis, to stage each patient, and to follow disease progression and response to treatment. Structural and functional imaging modalities contribute to the diagnosis and understanding of different dementias. HIV-related dementia is classified as a secondary dementia, as opposed to Alzheimer's or Creutzfeldt–Jakob disease (Tartaglia et al., 2011). Whether any of these techniques will prove useful in *virtual autopsies*, even after the brain has been removed, remains to be seen.

In addition, HIV-1-dependent diseases in the periphery may have a substantial effect on CNS pathology. In the CNS, HIV-1 initiates activation of chemokine receptors, inflammatory mediators, extracellular matrix-degrading enzymes, and glutamate receptor-mediated excitotoxicity. These agents, in turn, activate numerous downstream signaling pathways that disrupt neuronal and glial function. Although treatment has improved remarkably in the last decade, thanks largely to the introduction of HAART, most of the advances have been in the treatment of peripheral disease. There still is no cure for HIV dementia (Kaul and Lipton, 2006).

HIV-1 is a neurotrophic lentivirus. When it enters the CNS of adults and children, a family of different clinical syndromes emerges. In adults the result is AIDS–dementia complex, while in children the most common effect is HIV-1-associated progressive encephalopathy. The neuropathologic findings seen in the pediatric patients include impaired brain growth, reactive gliosis, myelin pallor, calcifications of the basal ganglia, cortical and cerebral atrophy with neuronal loss, ventricular enlargement, and abnormalities of the cerebral vasculature. In adults the picture is quite different. *Penicillium marneffei* is one of the more frequent fungal opportunists. Disease is frequently more advanced and complicated by opportunistic infections (Schwartz and Major, 2006). Thus, even with HAART, HIV-infected intravenous drug abusers still can be expected to have CNS abnormalities detectable at autopsy. The lung is the organ most frequently involved by complications of HIV, though the actual incidence of brain involvement is not much lower than in the lung (Masliah et al., 2000). It is hard to say how much lower, as there has been no large autopsy series published since the introduction of HAART, but tuberculosis is now clearly established as the main HIV-associated infectious complication.

The WHO recommends cough as the trigger for tuberculosis screening in HIV-infected patients, with acid-fast bacillus smear as the initial diagnostic test, the aim being to assess the yield and cost of a more intensive tuberculosis screening in HIV-infected patients starting ARV therapy (Bassett et al., 2010). *Mycobacterium tuberculosis* is a common, devastating cause of meningitis in HIV-infected persons, and it is yet to be proved that HAART will slow its progression, but the possibility of tuberculosis meningitis must always be considered in this group of patients (Marais et al., 2011).

AIDS-associated neurologic disorders can be divided into four groups: (1) AIDS progressive multifocal encephalopathy, due to the direct effects of the virus itself; (2) opportunistic viral, fungal, parasitic, and bacterial infections; (3) opportunistic neoplastic processes, particularly primary brain lymphoma; and (4) HIV-related lymphocytic meningitis. The most frequently seen abnormality in the brains of AIDS patients is atrophy with diffuse or focal lesions in the white matter (Pantanowitz et al., 2011). Necrosis and pallor of the myelin is usually obvious and the necrosis is likely to be the most obvious within the centrum semiovale. Diffuse or focal neuronal loss in the caudate and putamen may also occur (Navia et al., 1986). In cases where diffuse white matter damage is present, multifocal microgranulomatous lesions and multinucleated giant cells can be seen.

5.12.6.5 Primary Phycomycosis, Mucormycosis, Penicillium marneffei

Fungal brain infection is usually associated with poorly controlled diabetes or the presence of some disorder, such as leukemia, that depresses immunity (Song et al., 2006). At one time all diseases caused by invasive fungi were grouped together under the name zygomycosis, a term no longer in use because the classification system has changed. Now, once the fungus responsible is identified, the disease is referred to by the name of the causative fungus: phycomycosis, mucormycosis, *P. marneffei*, etc. Common fungi found in soil and in decaying vegetation cause most fungal disease. Healthy individuals exposed to the

fungi on a regular basis have partial or complete immunity, but the immunocompromised (which would include the heroin addicted) do not and therefore are at risk.

A handful of reports have linked phycomycosis to intravenous drug abuse, usually in heroin users, though today this is not nearly so common as in HIV patients (Adelman and Aronson, 1969; Carpenter et al., 2007). The first reports of phycomycosis in the immunosuppressed and HIV-infected individuals, as opposed to those with general debilitation and immunosuppression, began to appear in 2000, only to be followed by cases of mucormycosis (Perez-Uribe et al., 2005; Agarwal et al., 2012; Bonifaz et al., 2012).

Infection begins in the nasal cavities, then invades the turbinates and the veins that drain them, extends into the paranasal sinuses, and eventually reaches the orbit. In other instances, the infection reaches the brain by a hematogenous route. It may be that the brain supplies a particularly conducive environment in which the fungus can grow. Whatever the route of infection, the result is edema, proptosis, and ultimately destruction of the trigeminal and facial nerves. At least in drug addicts, the disease follows a fulminant course. Most patients die within 2 weeks of onset. Diagnosis in life may require brain biopsy, because fungi are not detected in the CSF. Both CT and MRI scanning may be invaluable for early diagnosis (Moll et al., 1994), though the offending agents can be identified by microscopic examination of biopsy material. Lesions are usually multiple and symmetric and involve the basal ganglia. Material removed at surgery or autopsy is composed of aggregates of macrophages, lymphocytes, and multinucleated giant cells. Even routine H&E staining will show the broad, branching, nonseptate fungal mycelia (Schwartz et al., 1982).

P. marneffei (Figure 5.62) was first isolated from bamboo rats dying of disseminated mycosis in Vietnam. The new species was named *P. marneffei* in honor of Hubert Marneffe, the Director of the Pasteur Institute in Indochina (Wong and Wong, 2011). The first natural human infection was reported in 1973 from a patient with Hodgkin lymphoma who lived in Southeast

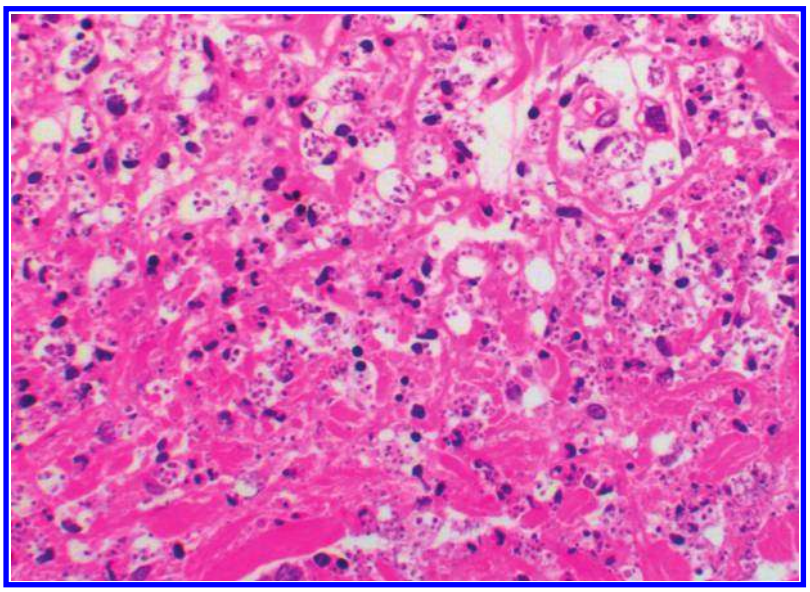


Figure 5.62 A high-power view of *P. marneffei* culture. Note the presence of 2–4 μm intracellular fission arthroconidia within the histiocytes (H&E, ×40). It was first isolated from bamboo rats dying of disseminated mycosis in Vietnam. (From *eScholarship*, the online journal of the University of California, https://escholarship.org/uc/item/6j96f72f.)

Asia (DiSalvo et al., 1973). Before the first case of HIV in Southeast Asia was reported in 1988, cases were rare. Since then, the incidence of penicilliosis has increased in parallel with the development of the HIV pandemic, and penicilliosis infection has become one of the AIDS-defining illnesses among HIV-positive patients in endemic areas (Supparatpinyo et al., 1994).

The pathology of penicilliosis varies in different organs depending on the host's immune status, but necrotizing tissue reactions are commonly observed in AIDS patients. Granuloma formation should suggest the diagnosis (Duong, 1996; Anderson and Scott, 2006). The most frequent sites of involvement are liver and lungs, but lymph nodes, bone marrow, skin, and intestines all may be involved. In the liver, histiocyte infiltration of the sinusoids and parenchyma is seen, and epithelioid granulomas may be found. Unfortunately, there seems to be no correlation between the extent of tissue involvement and any abnormalities reflected by liver function tests.

Strangely, liver function test results correlate poorly with the histologic changes often observed in the liver (Yousukh et al., 2004). Microscopic examination often reveals lymphoid depletion with histiocytic proliferation and focal necrosis (Tsui et al., 1992). A number of CNS complications have been reported in the HIV-infected individuals, including multiple brain abscesses (Noritomi et al., 2005). Multiple cystic lesions were found in the corpus callosum without mass effect (Lyratzopoulos et al., 2002). Reports of pulmonary involvement appear to be more common than those describing CNS disease.

5.12.6.6 Spongiform Leukoencephalopathy

In 1982, an epidemic outbreak of spongiform leukoencephalopathy occurred in the Netherlands (Wolters et al., 1982; Haan et al., 1983). In the initial outbreak, nearly 50 patients were involved, and the only factor common to all those affected was that they were addicts who smoked heroin. In most cases, the disorder ran a 2–3-month course. In the initial stages, motor restlessness and apathy, with obvious cerebellar signs, rapidly gave way to hypertonic hemiplegia or even quadriplegia. In some cases, patients developed myoclonic jerks or choreoathetoid movements. Onset of hemiplegia seemed to mark a turning point in the progression of the disease. Half of the patients stabilized or improved, while the other half progressed to a final, fatal stage with central pyrexia, spastic paresis, and akinetic mutism. These individuals died of respiratory failure. MRI typically disclosed symmetric lesions in the white matter of the cerebrum, cerebellum, and midbrain (Jee et al., 2009). Selective involvement of the corticospinal tract, the solitary tract, and the lemniscus medialis also has been found. In 1997, the first cases were reported in the United States, and additional cases have been reported from around the world since then (Kriegstein et al., 1999).

The cases reported from the first cluster of patients reported from the Netherlands had obvious cerebral edema with flattening of the convolutions and brain weights of 1380–2560 g. In all cases, microscopic examination showed damaged white matter filled with vacuoles (Figures 5.63a through d and 5.64a through d). In some areas, the vacuoles had coalesced to form larger cavities. Around the cavities could be seen a fine network of attenuated myelin. The number of oligodendroglia was reduced, but no myelin breakdown products were evident. Inflammatory cells were also absent. Electron microscopy done in several cases showed multivacuolar degeneration of the oligodendroglia, with swollen mitochondria and distended endoplasmic reticulum. Although not visible with light microscopy, electron micrographs showed abnormalities of the myelin lamellae and axoplasm, which also contained swollen, abnormal mitochondria. MRI studies have demonstrated

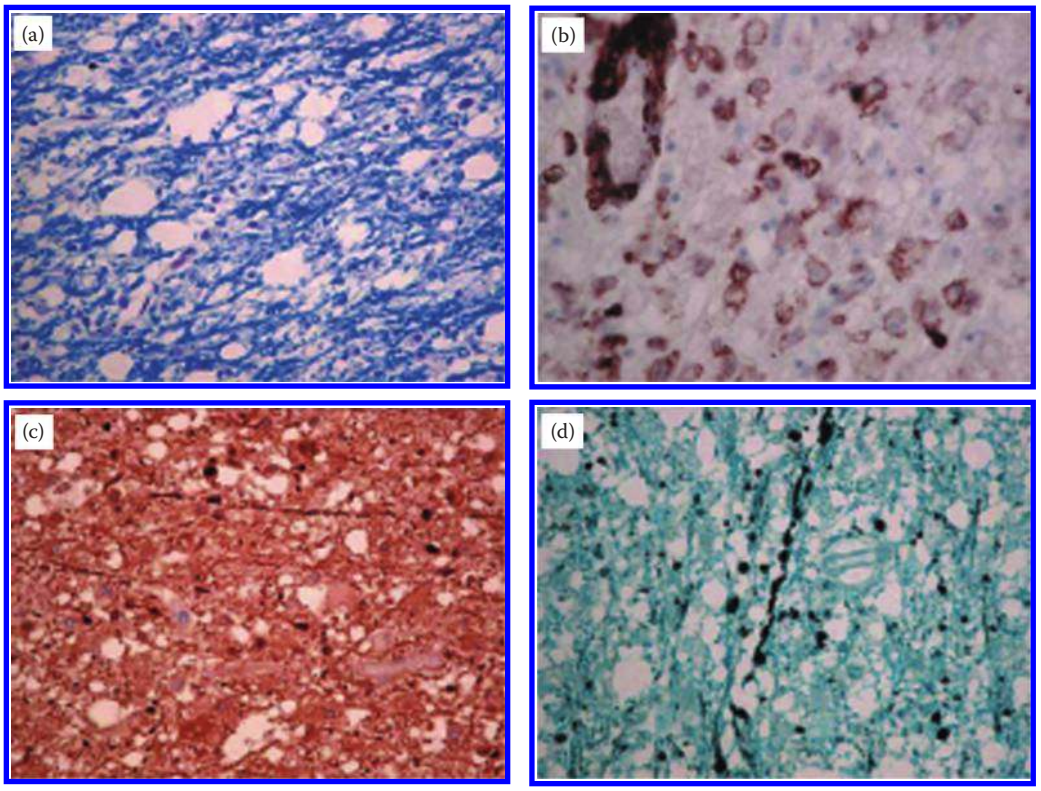


Figure 5.63 (a–d) Spongiform leukoencephalopathy. Vacuolar change in the deep frontal white matter (a) (luxol fast blue) associated with variable macrophage infiltrate (b) (common leukocyte immunostain); axonal loss (c) (neurofilament) and degenerating axons with spheroid formation (d) (amyloid precursor protein). (Reproduced from Ryan, A. et al., *J. Neurol. Neurosurg. Psychiat.*, 76(7), 1014, 2005. With permission.)

the presence of elevated lactate concentrations in white matter, raising the possibility that the underlying abnormality in these individuals may be mitochondrial dysfunction.

Very little has been discovered about this disease since it was first reported, although a succession of new case reports continues to appear (Zhou et al., 2010; Verma et al., 2011; Have et al., 2012). Unique though the appearance may be, the underlying etiology remains unknown. All the usual toxicologic possibilities have been investigated and found wanting. Chemical analyses of samples of local heroin used by the addicts affected by this disorder have shown only the usual adulterants—caffeine, lidocaine, procaine, phenobarbital, and methaqualone—and now these have been replaced largely by caffeine and acetaminophen. None of these agents has ever been shown to be neurotoxic, and no one seriously believes that a toxic adulterant is responsible for these cases. Very recent in vitro studies have demonstrated that heroin activates the extrinsic pathway for apoptosis. If true in man, this observation would explain why a plausible explanation for this disorder has remained so elusive—none of the methodologies now in use were unavailable at the time of the earlier outbreak.

The changes in these individuals are easily distinguishable from those seen in AIDS-associated progressive multifocal leukoencephalopathy (PML), a disorder that may be present in 10% or more of autopsied patients with AIDS (Aksamit et al., 1990).

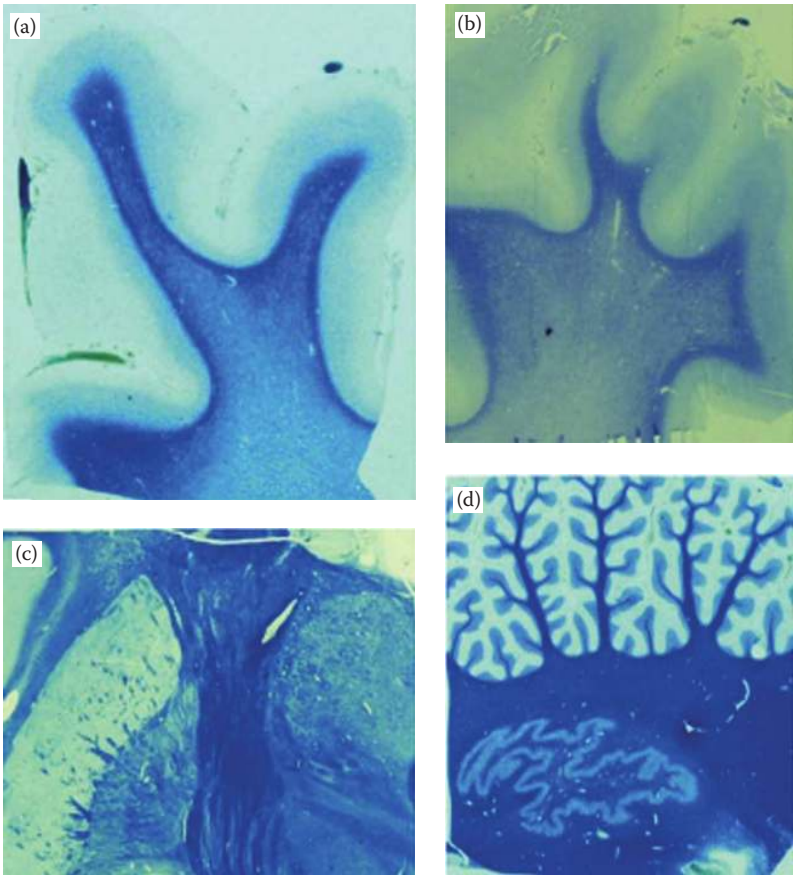


Figure 5.64 (a–d) Spongiform leukoencephalopathy. Myelin pallor in frontal (a) and parietal (b) white matter, contrasted with normal myelin staining intensity in internal capsule (c) and cerebellum (d). Note preservation of subcortical U fibers in the frontal and parietal white matter. (All stained with luxol fast blue.) (Reproduced from Ryan, A. et al., *J. Neurol. Neurosurg. Psychiat.*, 76(7), 1014, 2005. With permission.)

Before the AIDS epidemic, PML was rare, usually seen only in association with leukemia and lymphoma. PML is due to infection with JC virus, a papovavirus that most people are exposed to in childhood (Sweeney et al., 1994). The JC virus is a type of human polyomavirus (formerly known as papovavirus) and is genetically similar to simian 40 virus. It was discovered in 1971 and named after the two initials of a patient suffering from PML (Padgett et al., 1971).

In spongiform leukoencephalopathy, demyelination accompanied by oligodendroglial and astrocytic pathology is always evident. Changes tend to be widespread with predominant oligodendroglial abnormalities (Mossakowski and Zelman, 2000). Morphologic changes also occur in the microvasculature, including mural thickening, endothelial pleomorphism, and prominent perivascular collections of HIV-positive monocytes and multinucleated cells. Even with treatment, survival in patients with AIDS-associated PML is poor, with a 6-month survival rate of less than 10%. The results of some animal studies suggest that the leukoencephalopathy seen in HIV-infected humans might actually be a variant of prion disease. For the moment, however, there is no evidence to support this theory.

5.12.6.7 Transverse Myelitis

This rare entity was first described in 1926. Its etiology also remains obscure but its occurrence has been noted in conjunction with a heterogeneous group of disorders including viral infections, AIDS, systemic lupus erythematosus, smallpox vaccination, trauma, extreme physical exertion, and heroin abuse. The association with heroin abuse was first noted in 1968 (Pearson et al., 1972). When the disorder occurs in conjunction with heroin use specifically, it is sometimes called *heroin myelitis*. Since the index report, transverse myelitis in association with heroin abuse has been reported only on a handful of occasions and, in spite of the increased availability of heroin, the incidence of this disorder does not seem to be increasing; only 10 new case reports have been published since 1995 (Yang et al., 1995a,b; Bernasconi et al., 1996; Sverzut et al., 1998; Derkinderen et al., 2000; Gupta, 2011; McCreary et al., 2000; Nyffeler et al., 2003; McGuire et al., 2008; Sahni et al., 2008).

The patients first described by Richter and Rosenberg had all stopped taking heroin, many for a period of months, and they developed neurologic symptoms only after they began injecting heroin again. In all of the cases reported, onset of symptoms was quite rapid, ranging anywhere from a few hours to a few days. Victims developed flaccid paralysis and complete sensory loss ascending from the lower extremities to thoracic or even cervical levels (Richter et al., 1968). In the addict subpopulation, at least, fairly rapid improvement seems to be the norm, though residual deficits usually are seen. Myelography in acute cases is unremarkable (Arlazoroff et al., 1989). The few cases that have been studied with modern imaging techniques have all shown infection to be the cause (spinal cord abscess, spondylodiscitis, and epidural abscess). Even fewer cases have been autopsied and all they disclosed was nonspecific necrosis.

Transverse myelitis was initially thought to be the result of anterior spinal artery occlusion, but when more cases were studied, it became evident that the circulation in other territories could also be involved. Even ventral pontine disease has been observed which, when it occurs, is usually the result of an isolated vascular accident within the spinal cord (Hall and Karp, 1973). In the case of narcotic abusers, it is not clear whether that accident is the result of thromboembolic phenomena, some sort of inflammatory vascular disease, or a toxic manifestation due to some contaminant injected along with the heroin, though in today's environment the latter possibility seems very unlikely. It is clear that heroin administration decreases blood flow to specific areas of the brain (Wang et al., 1997), a condition that would favor ischemic damage as well as making the area more vulnerable to infection.

In 2009 a patient developed acute myelopathy after intranasal insufflation of amphetamines and heroin and became paraplegic. MRI imaging showed a somewhat unique pattern with selective T2 hyperintensity and intense enhancement confined to the spinal anterior horns and lumbar nerve roots and plexus, suggesting that the pathologic process began in spinal anterior horns, leading to motor neuron cell death and then death of the nerve roots and lumbar plexus as a consequence of wallerian degeneration (Riva et al., 2009). While the etiology of this disorder remains obscure, it is important to remember that transverse myelitis has been reported as a complication of many other disorders, including cytomegalovirus (Karunarathne et al., 2012), multiple sclerosis (Tono et al., 2014), and even Guillain–Barré syndrome (Topcu et al., 2013).

5.12.6.8 Peripheral Neuropathy

Unsterile injections may lead to local infection with nerve involvement, as can the injection of toxic adulterants. Neuropathy associated with rhabdomyolysis is a well-recognized entity, but nerve injury in heroin abusers is a fairly frequent occurrence anyway, though

most of the time the cause is obvious: the result of indirect trauma or a direct result of ischemia that can occur if compartment pressure rises too high (Adrish et al., 2014). Unperceived pressure or traction can also cause plexus or peripheral nerve injuries, even without muscle swelling (Kaku and So, 1990), and this is especially likely if a semicomatose heroin user lies in the same position for a number of hours.

Evidence suggests that at one time or another, all of these mechanisms come into play. Other, quite rare, forms of nontraumatic peripheral neuropathy include polyradiculopathy, plexitis, Guillain–Barré syndrome, and mononeuropathy (Hecker and Friedli, 1988; Herdmann et al., 1988; Buttner et al., 2000). A very rare syndrome of lumbosacral plexopathy accompanied by rhabdomyolysis, with onset shortly after intravenous heroin injection, may also involve the brachial plexus (Stamboulis et al., 1988; Delcker et al., 1992; Evans and Millington, 1993). In most reported cases, peripheral nerve involvement has been asymmetric.

In addition, HIV-positive patients are subject to peripheral and autonomic neuropathies, secondary to direct invasion of the nerve by the virus, though it has also been suggested that an autoimmune etiology might be possible. Distal sensory polyneuropathy (DSP) is thought to be the most common neurologic complication of HIV infection, though the risk factors for its occurrence remain poorly defined, particularly since the advent of HAART. None of the established risk factors, including CD4 cell count, plasma HIV RNA, and use of dideoxynucleoside ARVs, seem to be related to the progression of DSP, and treatment remains unsatisfactory (Simpson et al., 2006). Nerve injuries in addicts have never been studied in an autopsy series. Numerous other causes are recognized for DSP, but systematic studies are absent (Lacomis et al., 2014).

5.12.6.9 Rhabdomyolysis

Heroin had been available for nearly 70 years before it was observed that the intravenous injection of heroin occasionally gave rise to acute myoglobinuria (Richter et al., 1971). Additional cases have been reported ever since (Koffler et al., 1976; Gibb and Shaw, 1985; Yang et al., 1995a,b; Abdullah et al., 2006; O'Connor and McMahon, 2008; Kosmadakis et al., 2011; Radovanović et al., 2012; Adrish et al., 2014) but controlled studies have never been performed. The incidence of rhabdomyolysis in heroin users has never been estimated, but the number of cases does not seem to be increasing.

In some instances, the cause of muscle injury is obvious—pressure necrosis from the weight of the patient's own body while the individual is lying comatose (Schreiber et al., 1972)—but few of the reported cases can be explained in this fashion (Chan et al., 1995). Cases with unequivocal evidence of concurrent cardiac necrosis, where the etiology could hardly have been pressure necrosis, have been reported (Schwartzfarb et al., 1977; Wynne et al., 1977; Scherrer et al., 1985; Melandri et al., 1991). A direct effect of heroin or of an adulterant seems to be responsible but it has never been identified. One case report describes a young male who smoked heroin for the first time and developed transverse myelitis, rhabdomyolysis, and acute kidney injury requiring dialysis (Gupta et al., 2011).

Patients usually complain of muscle weakness, pain, and swelling that begins several hours to days after using heroin. The muscles of the lower limbs are involved more often than those of the upper limbs. Associated neurologic complaints and neuropathies occur in conjunction with heroin-induced rhabdomyolysis although it should be noted that not all neuropathies in heroin users are a consequence of rhabdomyolysis. The presence of symmetric brachial neuropathy should raise the suspicion of lead poisoning, a rare consequence of using contaminated heroin (Antonini et al., 1989).

The diagnosis of rhabdomyolysis is clearly suggested by the presence of muscle swelling and elevated creatinine phosphokinase. However, muscle swelling need not always be evident, and because myoglobin is so quickly cleared from the circulation, demonstrating the presence of myoglobin is an unreliable indicator of the severity of the problem. Early in the course of the disease, laboratory tests will disclose marked elevations of creatinine phosphokinase and aldolase. Some individuals may complain of dark urine, and about half will eventually develop full-blown renal failure, with typical laboratory findings.

Since the introduction of HAART, muscular complications of HIV infection have become more common and should not be dismissed as diagnostic possibilities (Bakir and Dunea, 2001). These complications can roughly be divided into four groups: (1) HIV-associated myopathies and related conditions including polymyositis, inclusion-body myositis, nemaline myopathy, diffuse infiltrative lymphocytosis syndrome, HIV wasting syndrome, vasculitis, myasthenic syndromes, and chronic fatigue; (2) iatrogenic conditions including mitochondrial myopathies, HIV-associated lipodystrophy syndrome, and immune restoration syndrome; (3) opportunistic infections and tumor infiltrations of skeletal muscle; and (4) rhabdomyolysis (Authier et al., 2005). (Note: for additional discussion of rhabdomyolysis, see Section 5.11.5.4.)

5.12.6.10 Stroke

Stroke occurs in heroin users, and case reports continue to be published (Somala, 2009; Esse et al., 2011; Yeung et al., 2011; Lee et al., 2012; Benoilid et al., 2013), but in spite of very substantial increases in the number of heroin abusers, the number of case reports has not increased proportionally. In most instances the etiology is obscure. It was once thought that the reexposure of addicts to heroin after a period of abstinence might lead to vascular hypersensitivity reactions (Rumbaugh et al., 1971), but the theory has never been substantiated and is no longer given credence.

Necrotizing angiitis (see Section 5.12.5.3) can certainly cause cerebral infarction (Citron et al., 1970), but there is rarely evidence for this disorder in drug abusers, either of stimulants or opiates, and the few cases that have been reported have been almost exclusively in methamphetamine abusers (Toffol et al., 1987). The decline in reported new cases of necrotizing angiitis, even among methamphetamine abusers, suggests that when cases did occur they may have been the result of some toxic contaminant mixed with the heroin (Citron et al., 1970). This is not to say that necrotizing angiitis is not a real disease, but the classification of antineutrophil cytoplasmic antibody-associated vasculitis and polyarteritis nodosa for epidemiology studies is confusing (Watts et al., 2007) and may not even apply in cases where the angiitis is drug related. As often as not, angiographic studies in heroin-abusing stroke victims will be normal (Willoughby et al., 1993). If any change is evident at all, it is likely to be spasm (Niehaus and Meyer, 1998).

Table 5.37 lists additional mechanisms that can cause stroke in opiate abusers. The same mechanisms that cause stroke in stimulant abusers could also cause stroke in opiate abusers, but barring vasoactive contaminants, vasospasm seems unlikely in opiate users because opiates share no common pharmacologic mechanisms with stimulants and do not (except for pentazocine) cause elevations in circulating catecholamines. A likely mechanism in many cases of stroke is positional vascular compression. One report describes a 35-year-old addict with dense hemiparesis. Regional flow studies demonstrated severe hyperemia of the entire carotid territory on the affected side but normal vessels on angiography. Such localized hyperemia is often seen following restoration of flow in stroke patients and after

Table 5.37 Possible Etiologies for Stroke in Opiate Abusers

Thromboembolism
Thrombocytopenia
Vasculitis
Septic emboli
Hypotension
Secondary to arrhythmia
Secondary to decreased cardiac output
Secondary to peripheral vasodilation
Positional vascular compression

cerebral spasm (Caplan et al., 1982). Generalized hyperemia is more likely to be observed after global ischemia. Stroke in these patients may be the result of an unfortunate set of circumstances. Large doses of narcotic lead to hypotension, decreased respiration, and generalized cerebral ischemia. If the carotid artery is then compressed by lying in the wrong position, perfusion may be lowered beneath some critical level, and stroke could occur in an already ischemic brain (Olson and Winther, 1990). In the absence of experimental evidence, such an explanation is speculative, but it could well account for an occasional infarct.

Hemorrhagic stroke in heroin abusers is the result of a deranged clotting mechanism, as might be encountered in cases of fulminant hepatitis or in individuals with AIDS-associated thrombocytopenia (Brust and Richter, 1976; Chevalier et al., 1995). Rupture of a mycotic aneurysm or underlying arteriovenous malformation is also possible, and the incidence of this complication seems to be increasing as the femoral route of injection has become more popular. This is in contrast to hemorrhagic stroke in cocaine users, where victims commonly bleed from a preexisting malformation or aneurysm (Manekeller et al., 2004; Chu et al., 2005).

5.12.6.11 Parkinsonism and MPPP

Although this subject continues to be the object of scientific investigation, the compound 4-methyl-α-pyrrolidinopropiophenone (MPPP) is mainly of historical interest (Archer and Fredriksson, 2012). MPPP is a potent analog of meperidine. When it is synthesized by clandestine chemists, inattention to detail occasionally results in the production of a by-product known as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Figure 5.65). MPTP is itself nontoxic, but astrocytes oxidize MPTP to a pyridinium metabolite (MPP+) that can damage neuronal cells. Dopaminergic neurons are particularly vulnerable to MPTP toxicity because they accumulate MPP+ and retain it for prolonged periods. Two pathways

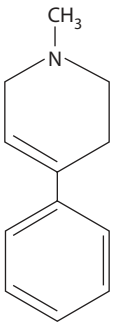


Figure 5.65 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine molecule.

of astrocytic MPP+ formation have been identified, one utilizing monoamine oxidase (MAO) and the other requiring the presence of transition metals, though experimental studies suggest MAO plays a minor role in human MPTP toxicity (DiMonte et al., 1996; Przedborski and Jackson-Lewis, 1998). Whatever the metabolic route of MPTP production, it results in selective destruction of dopaminergic neurons in the substantia nigra and the globus pallidus (Jenner et al., 1992).

There is evidence that genetic predisposition plays a role in the etiology of this disease (Miller and Federoff, 2005), but the most recent evidence indicates that MPTP induces neuronal apoptosis along the niagrostriatal tract (Liu et al., 2014). Exposure to MPTP selectively kills dopamine neurons within the substantia nigra. Genetic models incorporating mutations in the α-synuclein molecule produce symptoms that mimic those seen in patients. The presynaptic end of the neuron has been identified as a primary site of injury.

Also promising is the recent discovery that mutations in the leucine-rich repeat-2 (*LRRK2*) gene are responsible for autosomal dominant and sporadic Parkinson's disease, probably by some mechanism involving enhanced toxicity. A common *p.G2019S* mutation (rs34637584:A>G) is responsible for up to 30%–40% of Parkinson's disease in some ethnic populations. This gene interacts with human peroxiredoxin 3 (PRDX3), a mitochondrial member of the antioxidant family of thioredoxin peroxidases. Importantly, mutations in the *LRRK2* kinase domain significantly increase PRDX3 phosphorylation leading to decreased peroxidase activity and increased cell death. It appears that *LRRK2* mutants actually stimulate mitochondrial factors involved in apoptosis, causing an increase in reactive species and leading to further cell damage (Angeles et al., 2011).

If taken in sufficient quantity, MPTP can produce all the classic symptoms of parkinsonism including resting tremor, rigidity, bradykinesia, and postural instability. At least three isolated outbreaks of recognized MPTP toxicity have been reported. The first reported case occurred in 1979 (Davis et al., 1979). A graduate student who had been synthesizing and intravenously injecting MPPP for 6 months made a mistake in synthesis. Shortly after he injected the new batch of what he believed to be MPPP, he developed symptomatic Parkinson's disease. Later analysis by authorities disclosed that the student had in fact produced a mixture of MPPP and MPTP. His symptoms responded well to treatment, but he died of an unrelated drug overdose some 2 years later. Detailed neuropathologic examination of his brain disclosed degenerative changes within the substantia nigra that were confined to the zona compacta. A marked astrocytic response and focal glial scarring were present along with abundant collections of extraneuronal melanin pigment.

A second cluster of patients was reported in 1983. Four patients bought what they thought was *synthetic heroin* and within a matter of days developed striking features of parkinsonism. Analysis of material injected by these individuals showed they had been using mixtures of MPTP and MPPP (2.5%–3.2% MPTP, 0.3%–27% MPPP) (Tetrud et al., 1989). Since the original report, 22 additional cases with less florid symptoms have been identified, all stemming from exposure to product from the same clandestine lab that had been operating in Northern California (Tetrud et al., 1989). The results of follow-up epidemiologic studies indicate that during the 3-year period from 1982 to 1985, over 500 individuals were exposed to MPTP, probably all from the same clandestine lab (Ruttenber, 1991).

Additional cases stemming from exposure to products from other sources were reported in 1983 and 1984 and continue to be reported episodically (Kramer et al., 1998). The first of these was in a non-drug-abusing chemist exposed to MPTP at work. He developed classic symptoms of parkinsonism that responded to treatment. The most recent

reported case was in a polydrug-abusing chemist who responded to initial treatment but died of unrelated causes 2 years later. This individual preferred to snort his drug, but his parkinsonian symptoms were no less severe than those of the intravenous users. When he accidentally drowned, examination of his brain was perfunctory, and the substantia nigra was never even examined (Wright et al., 1984).

Except for the fact that different age groups are involved (average age in the 30s vs. average age in the 60s), there is little to distinguish parkinsonism occurring after MPTP exposure from parkinsonism in the general population. Initial symptoms may be mild or quite severe, though some evidence suggests that tremor is somewhat less common in the drug abusers. It is an open question whether additional new cases are likely to be encountered. Only sporadic seizures of samples containing MPTP have been reported. The most recent was in 1985, the same year that production of MPTP was made illegal. A closely related analog of MPTP called 1,2-phenylethyl-1,2,5,6-tetrahydropyridine can be generated as a by-product of PCP production and may well possess the same neurotoxicity as MPTP, but no case of parkinsonism attributable to PCP contamination has been reported.

A distressing new report describes evidence that some underground chemists are inadvertently producing MPPP when they synthesize the newly popular cathinone derivatives such as mephedrone and methylone (Brandt et al., 2011).

5.12.6.12 Seizures

Seizures have been attributed to a long list of different opioids: meperidine (Szeto et al., 1977; Goetting and Thirman, 1985), fentanyl (Katz et al., 1988; Fujimoto et al., 2003), sufentanil (Brian and Seifen, 1987), tramadol (Koussa et al., 2003), pentazocine (Jackson et al., 1971), normeperidine in those without renal failure (Gordon et al., 2000), and even high-dose morphine (Hagen and Swanson, 1997), as well as extradural morphine (Borgeat et al., 1988), the latter occurring only in known epileptics.

A single case report described a seizure in a 7-year-old with sickle cell disease after the administration of codeine (Borgeat et al., 1988). Given what is known today about codeine metabolism, one suspects that the child was a UM, polymorphic for CYP2D6. Another case report describes seizures after oxycodone administration in individuals known to be seizure prone (Klein et al., 2005). The mechanism by which opiates can produce seizures is not known. What is known, however, is that opiate-induced seizures usually occur in individuals with preexisting seizure disorders (Tempelhoff et al., 1990).

Of interest is the recent emergence of reports of tramadol-related seizures from the Middle East, where tramadol has become an extremely popular illicit drug of abuse (Yarkan Uysal et al., 2011; Farajidana et al., 2012; Hassanian-Moghaddam et al., 2013). Tramadol-induced seizures are generally considered benign.

5.12.7 Hormonal and Immune Alterations

The idea that opioids modulate the immune system is not new. In the late nineteenth century, Joan Cantacuzene used morphine to suppress cellular immunity and lower the resistance of guinea pigs to bacterial infection. While exogenous opioids mediate immunosuppression, endogenous opiates exert opposite actions. Acute and chronic opioid administration is known to have inhibitory effects on humoral and cellular immune responses including antibody production, natural killer (NK) cell activity, cytokine expression, and phagocytic activity (Table 5.38).

Table 5.38 Immune Abnormalities in Opiate Abusers

Depressed E-rosette formation (in vitro)
Depressed cutaneous sensitivity
Depressed mitogenic response
Elevated CD4 cells
Elevated CD4/CD8 ratio
Elevated levels of CD4 receptor
Elevated neopterin levels
Elevated soluble interleukin-2 receptors
Elevated γ-interferon levels

Source: Adapted from Pillai, R. et al., *Arch. Toxicol.*, 65(8), 609, 1991.

Opiates behave like cytokines, modulating the immune response by interaction with their receptors in the CNS and in the periphery. Potential mechanisms by which central opiates modulate peripheral immune functions may involve both the hypothalamic–pituitary–adrenal axis and the autonomic nervous system. The presence of opioid receptors outside the CNS is increasingly recognized. These receptors have been identified not only in peripheral nerves but also in immune inflammatory cells.

The immunosuppression mediated by opiates may explain the increased incidence of infection in heroin addicts, and there is ample experimental evidence suggesting that it does. Unfortunately, the epidemiologic data are, at best, equivocal, and further studies will be required before firm conclusions are possible, particularly when discussing any possible connection with HIV and AIDS (Wang et al., 2011a). The fact that peripheral immunosuppression is mediated at least in part by opioid receptors located in the CNS and that intrathecally administered opioids do not exert the same immunosuppressive effects may have important clinical implications for those patients receiving long-term opioid therapy for malignant and nonmalignant pain.

Heroin abusers are subject to a number of hormonal alterations, mostly involving sexual and reproductive functions. Studies have demonstrated decreased levels of both testosterone and luteinizing hormone, with testicular atrophy and impotence (Mendelson and Mello, 1982). Opiates induce hyperprolactinemia in both experimental animals and chronic opiate abusers (Tolis et al., 1978). Compared to non-drug-using controls, long-term heroin users have decreased levels of parathyroid hormone and decreased levels of testosterone. As a consequence, they have abnormal bone and mineral metabolism, with decreased vertebral bone density (Pedrazzoni et al., 1993). The etiology of these changes is unknown, but there is some evidence that opiates may act directly on the pituitary. When compared to controls, the response of β-endorphin and ACTH to metyrapone administration in addicts is significantly blunted, suggesting that the chronic stimulation of opiate receptors in some way impairs the function of the anterior pituitary gland (Vescovi et al., 1990). This notion is also supported by the observation that pituitary volume in healthy men addicted to heroin and cocaine, when assessed by MRI, is nearly twice as great as the volume observed in healthy controls (Teoh et al., 1993).

Long before the advent of HIV, it was generally agreed that heroin addicts had higher rates of both opportunistic infection and cancer than the population at large (Sapira, 1968). Studies performed in the early 1900s proved that morphine acts directly on lymphocytes (Atchard et al., 1909) but, of course, no one had any idea why, let alone how endogenous opioids act to decrease immunity. During the early years of the twentieth century, following

the advent of intravenous narcotic abuse, other abnormalities of the immune system were also recognized. These included generalized lymphadenopathy (Halpern and Rho, 1966), elevated serum immunoglobulins (Kreek et al., 1972), lymphocytosis (Sapira, 1968), and abnormal T-cell rosette formation (McDonough et al., 1980).

It has become increasingly evident that morphine alters the immune response of most of the major cell types involved. Its actions include depressing the activity of NK cells, depressing T-cell function (manifested by either inhibition or induction of delayed-type hypersensitivity reactions), altering cytotoxic T-cell activity, and causing abnormal expression of T-cell antigen (Eisenstein and Hilburger, 1998). Both acute administration and chronic administration of opioids inhibit humoral and cellular immune responses, including antibody production, NK cell activity, cytokine expression, and phagocytic activity. Opiates behave like cytokines, modulating the immune response by interaction with their receptors in the CNS and in the periphery.

Morphine downregulates phagocytic cell function both in human PBMCs and human polymorphonucleocytes. Not only does exposure to morphine inhibit phagocytosis, but it also disrupts chemotactic responses and interleukin production, and at the same time it inhibits the generation of activated oxygen intermediates and activation of the arachidonic acid cascade. How all these actions are accomplished is not known, but the existence of an in vivo neural–immune control mechanism seems to be increasingly likely (Eisenstein and Hilburger, 1998).

Opioids suppress immune function by acting within the CNS to increase the activity of the hypothalamic–pituitary–adrenal axis and activate the sympathetic nervous system. Catecholamine and adrenocorticoid production are responsible for many of the observed immunomodulatory effects that occur following opioid administration. In general, the sympathetic nervous system has been shown to play a role in regulating lymphocyte proliferation and NK cell activity as well as several other parameters of immune function (Hall et al., 1998).

The lifestyles of addicts are partially responsible for some of their immune abnormalities. Chronic infection with HIV and other viruses contributes. Hepatitis C is now the most common chronic blood-borne infection (from 35% to 95% of all injecting drug users in any given area), and infection with this virus is associated with many nonspecific immune changes (Dalekos et al., 1993; Garfein et al., 1998). In addition to indirectly controlling mast cell function via cytokine release, opiates can also bind directly to specific receptor sites on the mast cell membranes (Fjellner and Hagermark, 1984). One result is that histamine release can lead to bronchospasm, hives, and flushing.

Opiate-induced histamine release is IgG antibody related, and it has occasionally been referred to as *pseudoallergy* (Biagini et al., 1992). However, it is now clear that opioids bind to G-coupled proteins on mast cell walls and that their stimulation leads to histamine release (Ramkissoon et al., 2006). Urticaria seen in heroin users is simply histamine-induced dermal edema occurring secondary to vasodilatation. Histamine produces a prototypic, short-lived urticaria, but other molecules, including prostaglandins, leukotrienes, cytokines, and chemokines, are also produced by activated mast cells and also play a role. Urticaria in addicts occurs after binding of IgG autoantibodies to IgE and/or to the receptor for IgE molecules on mast cells. Mast cell activation can also result from type III hypersensitivity reactions via binding of circulating immune complexes to mast cells expressing Fc receptors for IgG and IgM.

Under some circumstances, even T cells cause histamine release. When that occurs, the process is referred to as a type IV hypersensitivity reaction. Nonimmunologic urticarias due

to mast cell activation are also possible; these mechanisms, however, play no role in the narcotic user (Hennino et al., 2006). IgM antibodies specific for morphine and codeine have also been demonstrated. In two studies, IgM antibodies were detected in 50%–60% of addicts tested (Gamaleya et al., 1993). Most addicts have elevated serum immunoglobulins, especially IgM. The immunoglobulin elevations are thought to be a consequence of repeated injections of antigenic material (Gamaleya et al., 1993). This abnormal state reverts when heroin use is discontinued, unless, of course, chronic HCV infection has supervened. The immunosuppression mediated by opiates may explain the increased incidence of infection in heroin addicts. Opiates may also promote HIV infection by decreasing the secretion of α- and β-chemokines (important inhibitory cytokines for the expression of HIV) and at the same time increasing the expression of chemoreceptors CCR5 and CCR3, co-receptors for the virus (Vallejo et al., 2004), but this proposition is far from proven, at least in clinical medicine.

Finally, what may be the most important alteration produced by long-term opiate abuse is testosterone suppression. Epidemiologic data in recent years suggest that up to five million men with chronic nonmalignant pain suffer from opioid-induced androgen deficiency in the United States alone (Elliott et al., 2011).

5.12.8 Bone and Soft Tissue Disorders

5.12.8.1 Introduction

Fibrous myopathy was a recognized complication of both chronic pentazocine abuse and chronic meperidine abuse, and even heroin abuse (Levin and Engel, 1975; Rousseau et al., 1979; Mastaglia, 1982), but there have been no new reports of pentazocine or meperidine myopathy in more than 30 years. Controlled studies of heroin users in methadone replacement programs have also shown osteopenia, which does not improve within the first year of abstinence. This change is, no doubt, due to the demonstrated decrease in testosterone concentrations among those chronically treated with opioids.

The chronic use of sustained-action opioids produces similar hypogonadotrophic hypogonadism in both men and women. Dehydroepiandrosterone sulfate deficiency, indicating adrenal inhibition, is present in most men and women chronically consuming sustained-action oral or transdermal opioids. Concentrations of gonadotrophin, androgen, and estradiol in women who chronically consume opiates for nonmalignant pain are 40%–60% lower than those in normal women. Additional lowering of free testosterone levels is associated independently with oral estrogen replacement and low body mass index. Thus chronic opiate abuse poses a danger of osteopenia and pathologic fracture in both women and men (Daniell, 2002).

At the same time, concentrations of osteoresorption marker (type I collagen cross-linked telopeptide) and osteoformation markers (osteocalcin and propeptide of type I collagen) are increased when opiate users are compared both to methadone users and drug-free controls. The serum testosterone levels in heroin-addicted MMT men are significantly decreased (3.3 nmol/L), but after 1 year of treatment osteoresorption measures return to normal, though testosterone levels do not (Wilczek and Stepan, 2003).

These changes occur because the μ-receptors are also located on human osteoblasts. In tissue culture, human osteoblast-like cells express the three main types of opioid receptors. Osteocalcin synthesis is significantly inhibited by high concentrations of the μ-agonists such as morphine, but it is restored to normal when osteoblastic cells are incubated simultaneously with naloxone, the narcotics antagonist. So far as is known, no opioid agonist has any effect on alkaline phosphatase secretion (Perez-Castrillon et al., 2000).

Female heroin users infected with HIV develop significantly worse osteoporosis than HIV-infected women who are not heroin users (Teichmann et al., 2003). Over the last decade, it has become clear that skeletal integrity is strongly influenced by the immune system. Faulty regulation of skeletal renewal and bone loss is a common feature of inflammatory conditions associated with immune activation. Bone loss is also associated with conditions of immunodeficiency, including HIV infection, which is associated with a high rate of bone fracture (Ofotokun and Weitzmann, 2011).

However, the fact remains that most bone and soft tissue disorders seen in opiate abusers are infectious in origin and the main reason that drug abusers are hospitalized (Cherubin et al., 1972). This situation has changed very little in the last half century (Harris and Young, 2002; Lucas, 2005; Kaushik et al., 2011). It is mundane conditions such as cellulitis, soft tissue abscess, and septic thrombophlebitis that most often bring the abusers to medical attention.

5.12.8.2 Bone and Joint Infections

In most instances, the source of bone and soft tissue infections is either the solution used to dissolve the drug or the abuser's own skin flora (Tuazon et al., 1974). Once a contaminated needle is introduced into the body, infection may spread locally or hematogenously. With repeated injection, insoluble substances may accumulate or disseminate. Typically, this leads to abscess formation at the injection site, cellulitis, necrotizing fasciitis, and chronic ulceration (Harris and Young, 2002; Warner and Srinivasan, 2004). The pattern of sites most frequently infected and the organism responsible for the infection vary according to local custom and necessity.

In the past, the skeletal sites most frequently infected were the vertebral column and sternoarticular joints (Goldin et al., 1973; Gifford et al., 1975; Bayer et al., 1977) but reports of these complications seem to be diminishing. This may be a direct consequence of needle exchange programs. In more recent studies the extremities, especially the left knee (Chandrasekar and Molinari, 1987), were found to be involved much more often than the sternoarticular joint. The shift seems to be due to the facts that more addicts are injecting themselves in the groin and that infection is most likely to occur in the structures closest to the injection site. Because most individuals are right handed, the left side is most frequently injected.

In early studies, *Pseudomonas aeruginosa* was responsible for most (more than 80%) of the joint and bone infections in intravenous drug abusers (Waldvogel and Papageorgiou, 1980), but any number of organisms may be responsible. More recently, *Clostridium* species have been responsible for widespread illness, particularly in subcutaneous and intramuscular injectors. Infectious discitis caused by *Enterobacter cloacae* has been described in both HIV-positive and HIV-negative IVDUs (Marce et al., 1993), as have infections with *Candida*; however, the number of reported cases seems to have declined markedly, possibly because of public health interventions (Derkinderen et al., 2000).

Heroin users occasionally develop osteomyelitis of the cervical spine (Silvani et al., 1987), an infection that almost never occurs in non–drug abusers. More often than not, the infectious agent is *Staphylococcus*, introduced when the addict injects into the great veins of the neck (Endress et al., 1990). In life, CT scanning will show an inflammatory reaction about the carotid sheath, with prevertebral soft tissue masses adjacent to the areas of bone destruction. *Candida* bone infections, on the other hand, almost never involve the cervical spine. While such infections may occur in intravenous abusers, they are more commonly seen in immunosuppressed patients in general and those with indwelling catheters

in particular (Eisen et al., 2000). The only real difference between IVDUs and immunosuppressed patients with pyogenic spine infections is that the former are more likely to present with paraplegia and infection is almost always cervical. In non–drug users infection of the thoracolumbar spine is far more common (Wang et al., 2012).

Almost all cases of *Candida* osteomyelitis have involved the lower lumbar area. The most reasonable explanation seems to be that infection spreads into the endplate of the vertebral body, which is supplied by ventral branches of the spinal arteries. *C. albicans* is usually the responsible agent (Almekinders and Greene, 1991) but the more exotic infections also must be considered (Owen et al., 1992).

The prevalence of tuberculosis is increased in heroin users, especially those who are HIV seropositive. Extrapulmonary involvement, with or without obvious lung lesions, is seen in 15%–20% of cases (Alvarez and McCabe, 1984; Abdelwahab et al., 2006a,b), and in many of those the extrapulmonary site involved is osteoarticular, usually the vertebral bodies and their intervertebral discs. The involvement of the bony arch usually produces a compression syndrome. Fortunately, the involvement of the vertebral arch is rare, but it has been reported in intravenous heroin users (Martos et al., 1989). Pott's disease is also a rarity, but it does occur. Clinically, tuberculous osteomyelitis of the spine can be distinguished from pyogenic or fungal infection by its less indolent onset. Patients with Pott's disease can be expected to present with fever, back pain, weight loss, and night sweats.

5.12.8.3 Soft Tissue Infections

Skin and soft tissue infections are common among intravenous abusers, but there is nothing to distinguish their appearance from similar lesions in non-drug users. The bacteriology of these infections is controversial, with conflicting results being reported from different centers. In one series, most infections were polymicrobial, and only 19% had isolates of *S. aureus*, the remainder being anaerobes, including *Clostridium* and *Bacteroides* spp. (Webb and Thadepalli, 1979). Other series have also described polymicrobial infections, with *S. aureus* present in almost every case, along with enteric Gram-negative aerobes and oropharyngeal organisms (Orangio et al., 1984). In more recently published series, *Streptococcus* seems to be as common as any of the more unusual agents (Mackenzie et al., 2000). *Eikenella corrodens*, a Gram-negative anaerobe, part of the normal flora in the mouth, is occasionally seen when addicts use their saliva to dilute or dissolve their drug for injection (Zumwalt and Franz, 1983). Femoral drug injection often leads to infection and bacteremia, sometimes resulting in iliofemoral thrombosis (Mackenzie et al., 2000).

Of 42 surgically treated addicts with soft tissue lesions from "skin popping lesions," 30 (71.43%) grew *Staphylococcus* species. Of these 30, 11 (36.67%) culture specimens were identified as methicillin-sensitive *S. aureus* and 19 (63.33%) as methicillin-resistant *S. aureus*. Of the remaining 12 culture specimens, 8 (19.05%) grew *Streptococcus* species, 2 (4.76%) displayed growth of multiple organisms (including *Haemophilus parainfluenzae*, *Clostridium baratii*, *E. cloacae*, diphtheroid bacilli, and *E. corrodens*), and 2 (4.76%) showed no bacterial growth (Pirozzi et al., 2014).

5.12.8.4 Fibrous Myopathy

This condition occurs primarily with pentazocine abuse and is the result of a foreign body reaction with crystallization of the drug within the muscle (Levin and Engel, 1975), but since that drug has decreased in popularity, so has the incidence of fibrosis. There have been no new reports of this disorder since 2008. When it occurs, woody infiltration,

cutaneous ulcers, and abnormal pigmentation can be seen surrounding areas of repeated pentazocine injection. Clinically, the syndrome is marked by limitation of motion, neuropathic symptoms, and even muscle and joint contractures (Kim and Song, 1996). The contractures and neuropathic symptoms are secondary to nerve damage and reflex sympathetic dystrophy. Microscopically, birefringent crystals may be found in the areas of most intense induration (Adams et al., 1983), so blocks of these tissues should always be obtained at autopsy. Even on gross examination, it is apparent that destroyed myocytes have been replaced with dense, fibrotic tissue. Inflammatory infiltrates may or may not be present. Dystrophic calcification may be sufficiently pronounced to detect even with postmortem x-ray.

5.13 Bioanalytic Considerations

5.13.1 Overview

The overall state of the body and the part of the body being tested impact substantially on any interpretation. In addition, the cessation of blood flow and associated changes in blood hematocrit, water content, postmortem hemolysis, and pH in blood may affect concentration although opioids tend not to have significant differences between concentrations seen in blood and serum, which is often the case with other drugs.

Most of the various opioids are reasonably stable in solution and in biological specimens, and no special precautions need to be taken. Just as with other drugs, it is wise to refrigerate specimens as soon as possible after they have been collected, and where days may elapse before analysis, freezing them to at least −20°C is recommended.

There are some data suggesting that the prolonged storage (several months or more) of some opioids (e.g., buprenorphine and the methadone metabolite EDDP or 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine in particular) can result in lower concentrations in preserved blood and urine (Dugan et al., 1994; Moody et al., 1999). Opiates appear to be stable in urine frozen for 1 year at −20°C. Sufentanil concentrations decline by 10% when plasma is left at 4°C for 1 h (Dufresne et al., 2001). Instability has also been observed in plasma samples kept at −25°C in less than a day.

Glucuronides of opioids are the least stable of all common metabolites and can be hydrolyzed back to the precursor molecule. In the case of morphine glucuronides, morphine itself can be reformed. In Sections 5.8 and 5.9 it was emphasized that putrefaction or contamination of the blood from liver can cause hydrolysis. This issue takes on significance in decomposed bodies, where several days to weeks elapse until discovery of the body (Skopp et al., 2001). Similar considerations also apply to other glucuronides. Of course toxicology data derived from decomposed bodies are unreliable, making interpretation of concentrations even more difficult.

When death investigations are focused on specific opioids, blood concentrations cannot be interpreted in isolation (even if collected from a peripheral site). In almost all cases the determination of a cause of death is based on the circumstances of the death, the available medical history, and postmortem findings, not the drug concentration measured in any specific part of the body; specific concentrations are rarely required and even less often diagnostic.

Occasionally, morphine concentration may be more accurately assessed if morphine glucuronate or other metabolites in other tissues, that is, liver or brain, are also measured.

Death from intravenous heroin injection can sometimes occur within minutes of injection. In these cases morphine will be detectable in blood but may be not detectable in urine, or only at very low concentrations, but often 6-acetylmorphine will be seen since it is excreted first by the kidneys.

5.13.2 Preferred Analytic Methods

5.13.2.1 Initial Testing

Opiates and some of the opioids can be measured in biological specimens using immunoassay methods. The *kits* that exist for *opiates* are usually designed for morphine with significant cross-reactivity for codeine and 6-AM. These are kits designed for urine (EMIT, CEDIA, etc.) or ELISA kits designed for blood screening. They are most often used in workplace drug-screening programs designed to detect illicit use of heroin. In that situation cutoffs such as 2000 ng/mL are used for morphine in the United States, but lower cutoffs are used in most other countries (typically 300 ng/mL). A kit also exists for the urinary detection of 6-AM itself. Depending on the maker of the kit and the antibody used in production, there may also be cross-reactivities with other morphine-like drugs such as oxycodone, dextromethorphan, and pholcodine. Immunoassay kits exist for a number of other opioids including methadone, fentanyl, oxycodone, and buprenorphine. These tend to be more specific than other *opiate* kits but, as with all immunoassays, other substances will cross-react, which means that confirmation testing will be required (Holler et al., 2004; Miller et al., 2006; Leino and Loo, 2007).

Immunoassay-based screening can also be used for oral fluid testing (Niedbala et al., 2001) in the same way as for blood since concentrations are similar or higher than the corresponding concentration in blood; however, special kits are required for the hair due to the lower concentrations of opioids in the hair (Musshoff et al., 2012). In a forensic setting it is advised not to use cutoffs designed for workplace use, rather the detection limit of the test calibrator.

Several different immunoassays would be required to screen for the presence of most relevant opioids (i.e., those listed in this chapter). For that reason laboratories now are often using chromatographic-based screening for this class of drugs. The advantage is that only one test is required and it can also detect other relevant metabolites or drugs that may be present in the specimen (even other, unsuspected drugs).

LC-MS/MS for screening has become the most popular approach since sample preparation is minimal and sensitivity down to ng/mL levels is achieved with minimal sample volumes (Gergov et al., 2009; Dowling et al., 2010). The additional advantage of LC-MS is that the actual opioid is identified, provided at least two key transitions (multiple reaction monitoring [MRM]) are used in the monitoring and if calibrations are performed simultaneously with detection of the opioid.

More recently, the availability of affordable high-resolution MS has made it possible to screen for drugs where identification is based on accurate molecular mass in conjunction with retention time. This is known as time of flight-MS (TOF-MS) and it can avoid the need for a standard (in this case no retention time matching can occur). The advantage of this approach is that no preconceptions of possible drug presence need to be made. With LC-MS/MS using MRM, only a targeted range of drugs is included for the analysis. If this technique is used, a confirmation step will still be needed to prove the drug assignment.

5.13.2.2 Confirmation Testing

The forensic standard is the use of MS. This can either be GC-MS or LC-MS/MS (Maurer, 1992; Musshoff et al., 2006; Gustavsson et al., 2007). When GC-MS is used, morphine and other dihydroxy opioids require derivatization to improve sensitivity and prevent loss at the injection port. Typical derivatives are perfluoroacyl esters.

When results are reported it is vital that relevant metabolites are also detected and quantified. Examples of important opioid metabolites are *O*-desmethyltramadol, morphine (from codeine), 6-AM, normeperidine, norpropoxyphene, and, for kratom exposure, various products associated with exposure to this plant.

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Dissociative Anesthetics 6

6.1 Introduction to Dissociative Anesthetics

The drugs described in this chapter—phencyclidine (PCP), ketamine, γ-hydroxybutyrate (GHB), dextromethorphan (DXM), *Salvia divinorum*, and propofol—are hallucinogens. All save *Salvia* are *N*-methyl-D-aspartate (NMDA) channel blockers (Jordan et al., 2006). In vitro evidence suggests that none of them binds to the D_2 dopamine receptor, as do stimulant drugs such as cocaine and methamphetamine. While the psychological profiles produced by their use appear to be grossly similar, *Salvia* is a pure κ blocker that operates via a completely different mechanism than other hallucinogens. Propofol is an agonist at γ-aminobutyric acid (GABA)-A receptors, occasionally exerting hallucinogenic effects, depending on dosage. DXM is a cough remedy that shares many of the hallucinogenic properties of other NMDA blockers, but only when used to excess.

Salvia is a pure hallucinogen. It has no known anesthetic effects. Patients who are given PCP or ketamine do experience anesthetic effects; they remain conscious but exhibit no apparent response to surgical pain. The same is also true of GHB. None of these anesthetic agents produces muscle relaxation and neither does propofol. As a consequence, when any of these drugs is used at surgery (either human or animal), other agents must be administered to produce muscle relaxation. *Salvia* induces neither anesthesia nor muscle relaxation. *Salvia* abuse has become more widespread since the last edition of this book, possibly because it remains a legal substance in most jurisdictions. It can easily be purchased over the Internet (or even grown in the backyard). The first *Salvia*-related fatalities have yet to be reported, but occasional episodes of death and morbidity have been attributed to the other dissociative drugs, most notably that of the famous pop star, Michael Jackson.

In 1999, only 93 PCP-related deaths were reported in the Drug Abuse Warning Network (DAWN) survey (SAMHSA, 2000), and even fewer were attributed to ketamine. The Substance Abuse and Mental Health Services Administration (SAMHSA) report from December 2013 cites information from the Drug Enforcement Administration (DEA) indicating that, save for one or two "hot spots" (New York and Chicago), use has declined to negligible levels (SAMHSA, 2013). Neither *Salvia* nor GHB has ever been mentioned in any federal report dealing with death or morbidity. Clearly, deaths from GHB overdose do occur, but they must be relatively uncommon to rate so little mention. Deaths from PCP derivatives may be more common than deaths from PCP itself. Beginning in the late 1990s, other new PCP-derived drugs appeared on European illicit

markets; PCMPA, PCMEA, and PCEEA. These new derivatives were sold under their own name or mixed in with other existing designer drugs (Rosner et al., 2005). Propofol was never a controlled substance, but is likely to be rescheduled by the time this edition is published. Nonetheless, it remains a pillar of trauma surgery and is often safely used for outpatient surgery.

Only one PCP analog is known to be available on the black market, *N*-(1-phenylcyclohexyl)-3-ethoxypropanamine. It was first synthesized as a reference compound for scientific use. Very little is known about the pharmacological properties of this compound (Maddox et al., 1965). However, given its molecular structure, its actions are likely to be similar to those of PCP or ketamine, acting as an antagonist at NMDA receptors and exerting psychotomimetic as well as anesthetic effects. Another PCP metabolite, known as *N*-(1-phenylcyclohexyl)-amine, a known metabolite of PCP and other PCP-derived compounds, has been shown to produce a long-lasting, dose-dependent effect on the efflux of dopamine in the rat (Takeda et al., 1986). The meaning of this observation, if any, is unclear, but it might well explain the mixed symptoms displayed by many of the users.

The emergency room (ER) component of the 2009 DAWN report places the number of PCP-related ER visits at 12/100,000 persons, compared to 0.6/100,000 for GHB and 0.2/100,000 for ketamine (SAMHSA, 2010).

No deaths from *Salvia* or propofol were listed in any of the official drug surveys, although, as mentioned, cases of propofol-related deaths have been reported (Kirby et al., 2009; Han et al., 2013).

PCP is the most commonly abused member of the group, and probably the most dangerous as well, but its use is mainly localized to North America. Massive doses of PCP can cause rhabdomyolysis and death, while large doses of GHB and propofol can induce transient (but potentially fatal) respiratory paralysis. However, taken as a group, the principal toxic effects exerted by these drugs are psychiatric. Considerable attention has been devoted to the use of GHB in drug-facilitated sexual assault, but the magnitude of the problem is difficult to assess and probably overblown by the media. The few studies that have been performed suggest that the use of these drugs to facilitate sexual assault is very low (ElSohly and Salamone, 1999; Du Mont et al., 2010).

Methamphetamine and PCP both induce psychotic states nearly indistinguishable from schizophrenia, and ketamine does the same (Krystal et al., 1994). NMDA glutamate receptor antagonists, including PCP, ketamine, GHB, and DXM, all produce a state of dose-dependent disinhibition where NMDA antagonists abolish GABAergic inhibition. GABAergic inhibition probably explains the addictive nature of propofol and the evidence of sexual excitation sometimes seen on emergence from propofol sedation; where there is simultaneous and excessive release of acetylcholine and glutamate, the NMDA receptor itself becomes less functional. Progressive loss of the NMDA receptor function, even when induced by relatively low doses of NMDA antagonist drugs, can lead to specific forms of memory dysfunction, with or without clinical psychosis. If NMDA function is severely reduced, the result can be a clinical syndrome very similar to a psychotic exacerbation of schizophrenia. Large doses of NMDA inhibitors, administered over a long time, can lead to histopathologic changes (Farber, 2003).

6.2 Phencyclidine (Table 6.1)

6.2.1 General

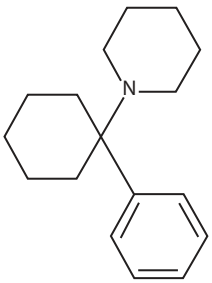
Synthetic PCP alters the state of consciousness and causes hallucinations and also symptoms of dissociation. It has been an abused drug for many years, particularly in localized regions within the United States. It is available in liquid or powder form. Pharmacologically, it is related to ketamine, and for a few years, it was actually used as anesthetic in humans. It is still used as an anesthetic for animals. PCP-like analogs include thienylcyclohexylpiperidine (TCP), phenylcyclohexylpyrrolidine (PHP), phenylcyclopentylpiperidine (PCPP), cyclohexamine (PCE), and dizocilpine (MK-801) (Mozayani et al., 2003).

6.2.2 Availability and Epidemiology

PCP, either alone or in combination with another drug, was listed as the 34th most frequent cause of drug-related deaths reported in the DAWN surveys for 1998 and 1999. In 1999,

Table 6.1 Physiochemical Properties and Pharmacokinetics of Phencyclidine

Chemical Name	1-(1-Phenylcyclohexyl) piperidine
Physiochemical properties, structure, and form	CAS 77-10-1 (base). CAS 956-90-1 (HCl). MW, 243.4. pK_a, 8.5. Protein binding 60%–80%.
Synonyms	Angel dust, busy bee, cadillac, CJ, crystal, elephant tranquilizer, embalming fluid, hog, killer weed, ozone, PCP, peace, rocket fuel, supergrass, snorts, T, wack.
Pharmacokinetic parameters	Bioavailability (oral), 72%. C_{max}, 1 mg: 3 ng/mL at 2.5 h. $T_{½}$, ~24 h. V_d, 6–8 L/kg.
Common blood concentrations	Range approximately 2 mg in active users.
Metabolism and metabolites	Hepatic inactivation by hydroxylation and glucuronidation producing a 4-hydroxypiperidine (PCHP) and a 4-cyclohexanol (PPC).
Excretion	Approximately 75% is excreted in the urine within 10 days, the remainder in the feces, 4%–19% as unchanged PCP.
Postmortem artifacts	Very likely to elevate postmortem.
Interactions	Phencyclidine is metabolized in part by CYP3A4 and inactivates CYP2B6 and may affect clearance of other drugs, such as cyclophosphamide.
Key papers	Cook et al. (1982a,b); Tran et al. (2008); Shebley et al. (2009).



98 deaths were reported, accounting for 0.84% of all the drug-related deaths reported to the government that year. More recent data suggest that the number is substantially higher. The 2006 National Survey on Drug Use and Health, which only polls adults older than 12 years of age, found a lifetime use prevalence of 2.7%. In 2005, 0.1% (an estimated 187,000 people) had tried PCP at least once, which amounts to at least 30,000 people having used PCP (SAMHSA, 2008).

Another source of information is the National Aviation Safety Board. Their findings showed the prevalence of drug violations in the most recent testing was 0.64% (95% confidence interval [CI]: 0.62%–0.65%) in random drug tests and 1.82% (95% CI: 1.47%–2.24%) in postaccident tests. The odds of positive-testing employees being involved was almost three times the odds for those who tested negative (odds ratio 2.90, 95% CI: 2.35%–3.57%), with an estimated attributable risk of 1.2%. In the same survey, marijuana accounted for 67.3% of the illicit drugs detected. The proportion of illicit drugs represented by amphetamines increased progressively during the study period, from 3.4% in 1995 to 10.3% in 2005 ($p < 0.0001$), but only 0.64% of these individuals were confirmed positive for PCP (Li et al., 2011) according to National Institute on Drug Abuse (NIDA) rules.

6.2.3 History

PCP (1-(1)-phenylcyclohexyl piperidine) was discovered by Parke Davis pharmacologists in 1956 (Greifenstein et al., 1958). It was first sold as an intravenous anesthetic called Sernyl (Collins et al., 1960). In recommended doses, PCP produces neither respiratory nor cardiovascular depression and, at least in animals, appears to be devoid of cellular toxicity (Chen and Weston, 1960). Human use of PCP was discontinued after it was discovered that 10%–20% of patients given PCP became delirious and/or unmanageable for many hours after surgery (Greifenstein et al., 1958). In 1978, PCP was transferred to schedule III under the Controlled Substance Act. In the United Kingdom, legal production was discontinued entirely, and PCP was labeled as a class C drug in January 2006. In Canada and Hong Kong, it is classified as a schedule I drug.

The first reports of recreational abuse came from California during the late 1960s; however, the drug rapidly developed a reputation for causing antisocial, violent behavior and use seemed to quickly tail off (Fauman et al., 1976). Abuse remained prevalent during the 1970s and early 1980s. Interest in this drug is no longer very great, although availability is increasing. Criminal groups produce most of the PCP available throughout the United States, especially in California. Still, the number of laboratories seized amounts to less than a dozen each year. Whether the PCP derivatives will become popular outside of Europe remains to be seen.

6.2.4 Clandestine Laboratories

There are several ways to make PCP (Allen et al., 1993). The synthetic route preferred by clandestine chemists begins with the condensation of 1-phenylcyclopentylamine with pentamethylene (Kalir et al., 1969). It is also possible to make PCP from piperidine, although acquiring this compound is becoming increasingly difficult as its purchase now falls under international controls. The direct conversion of piperidine to PCP is also possible (the piperidine ring is the central core of the PCP molecule). The synthesis is simple and the economics attractive. Given an average street dose of 1–10 mg, 1 kg of piperidine can be converted to anywhere from 100,000 to 1,000,000 doses (INCB, 1999).

Many different PCP derivatives have been produced, some psychoactive and some not. The resultant psychological effects are at least partly related to how well the synthetic derivative binds the σ receptor (Loustau-Then et al., 1997). Some of these derivatives are used in positron emission scanning in order to help localize σ receptors and to study the effects of prolonged drug use.

Street drug may be anywhere from 50% to 100% pure. The ethyl ether and other volatile solvents used in the production process give off a distinctive odor that often gives away the location of the laboratory. The fumes are also quite explosive, which makes producing illicit PCP a risky affair. 1-Piperidinocyclohexanecarbonitrile (PCC) appears in poorly synthesized batches as a by-product of the manufacturing process. When present in significant concentrations (10%–25%), it may induce abdominal cramps, bloody emesis, diarrhea, or even coma. PCC is an unstable compound and is degraded fairly quickly to piperidine. As a result, contaminated batches of PCP can sometimes be recognized by the strong fishy odor of piperidine. On heating (smoking), PCC liberates hydrogen cyanide, so the possibility of cyanide poisoning in PCP smokers must also be considered.

PCP analogs are prepared in the same way as PCP. They include TCP, PHP, PCPP, and 1-(1-phenylcyclohexyl)pyrrolidine, phenylcyclohexyldiethylamine, PCE, and phencyclohexylamine (Giannini et al., 1985; Cho et al., 1991).

6.2.5 Routes of Administration

PCP can be smoked, snorted, injected, or swallowed. Results of animal studies indicate that the effects produced are essentially the same regardless of whether the drug is smoked or taken intravenously (Meng et al., 1996). That observation probably explains why smoking PCP has become the preferred route of ingestion. In some parts of the United States, cigarettes soaked in PCP were very popular during the 1980s. PCP-laced cigarettes were called *Sherms*, after the Nat Sherman Tobacco Company, which manufactured the brand of cigarette used to soak up the solution. The term may still be in use but there are many other synonyms, and the drug is referred to by different names in different areas. Parsley leaves or marijuana leaves are sometimes soaked in PCP, with little apparent difference in result. Studies on human volunteers who smoked 100 mg of (^{3}H)-phencyclidine indicate that most of the PCP that is smoked is absorbed.

6.2.6 Pharmacology

PCP is a noncompetitive antagonist of glutamate-type NMDA receptors (Su, 1991). In low doses, PCP binds to a specific receptor in the NMDA channel. This may explain why low doses produce only mild inebriation. At higher doses, PCP acts as an indirect agonist at σ sites and can produce long-lasting psychotic episodes. It has an as yet uncharacterized effect on the dopamine system. This effect probably explains PCP's behavioral effects; interaction with the σ opioid system produces the dysphoria and other unpleasant side effects.

In many ways, the effects of PCP resemble those of methamphetamine. The resemblance is explained by the ability of both drugs to block dopamine reuptake. On a weight-per-weight basis, PCP is nearly as potent a reuptake inhibitor as methamphetamine. And, like methamphetamine, PCP also causes the release of stored catecholamines, though in this respect, at least, it is much less potent than methamphetamine (McMahon and Cunningham, 2003; Takamatsu et al., 2006). In the rat model, PCP has a biphasic course

of action. High doses lead to an initial increase in brain glucose metabolism at 3 h, followed by decreased glucose utilization at 24 h, and a return to normal at 48 h.

Low doses of PCP causes no initial changes in glucose metabolism, but at 24 h glucose uptake is depressed and remains so for some time (Gao et al., 1993). PCP also inhibits ATP-sensitive K^+ channels in both heart and brain, increasing the inward Ca^{2+} current and blocking the outward K^+ current (Kokoz et al., 1994). Evidence also exists that σ_1 receptors in the heart are directly coupled to K^+ channels within intracardiac neurons and that activation of σ_1 receptors depresses the excitability of intracardiac neurons, the net result being that parasympathetic input to the heart is decreased (Zhang and Cuevas, 2005).

PCP is implicated in the pathophysiology of multiple neurologic and neuropsychiatric disorders including Parkinson's disease, schizophrenia, alcoholism, and stroke (Waterhouse, 2003). In animals, treatment with PCP induces apoptosis of striatal neurons, particularly neurons that project to the globus pallidus. The mechanism that causes cell death in these neurons is not clear, but animals that are subject to this type of neural damage also show evidence of early gene activation (*c-fos*), a process that has come to be associated with apoptosis (Griffiths et al., 1999). Paradoxically, PCP also is neuroprotective in some settings. The mechanism by which PCP protects neurons against ischemia has never been established, although NMDA receptors appear to be involved.

Evidence indicates that glutamate-induced excitation is responsible for the mechanisms of neural cell damage and that inhibition of NMDA receptors by MK-801 (a substance closely related to PCP) might play an important role in neuroprotection. Several studies have shown that administration of MK-801 significantly reduces the volume of ischemic damage of the brain after middle cerebral artery occlusion (Bomont and MacKenzie, 1995). In spite of the promising data, clinical trials in humans with PCP-like drugs have been universally disappointing.

PCP attaches to σ receptors throughout the body, not just those found in the central nervous system (CNS) but also those found on membranes from endocrine, immune, and peripheral tissues. Sigma stimulation is thought to be responsible for many of the unpleasant side effects associated with opiate use and could possibly explain why in vitro studies have shown that lymphocyte function is depressed after exposure to relatively low doses of PCP (Thomas et al., 1993). In addition to PCP, cocaine, pentazocine, DXM, and even anabolic steroids all bind to σ receptors, which may explain certain similarities in the behavioral effects of these drugs.

Abusers seeking medical treatment after PCP use present with a variety of symptoms. These include violence (35%), agitation (34%), and bizarre behavior (29%). About half the patients are alert, while the other half present with lethargy, stupor, or even coma. Seizures, generalized rigidity, hypoglycemia, localized dystonias, catalepsy, athetosis, nystagmus, and hypertension have all been described. Cardiac arrest is rare (McCarron et al., 1981b). In one collection of case reports, the most frequent symptoms reported tended to be mania, depression, or schizophrenia. Each subject had toxic psychosis and/or acute delirium (Yago et al., 1981).

In cases of PCP overdose, death appears to be a consequence of respiratory and cardiac depression. In the dog model of extreme PCP intoxication, respiratory failure is followed by a combination of hypoxia, hyperpyrexia, and acidosis. If the animals are paralyzed, convulsions and hyperthermia are prevented, but respiratory and cardiac depression still occurs. At the highest doses, death seems to be entirely due to myocardial depression

(Davis et al., 1991). These results should be extrapolated to humans only with great caution, because reports of massive overdose (blood concentration > 1800 ng/mL) in humans do not mention myocardial compromise (Jackson, 1989).

6.2.7 Disposition

6.2.7.1 Blood Concentrations and Elimination

Peak blood concentrations occur 15–20 min after smoking a dose of 69 μg, but researchers have observed that a second peak occurs slightly later, suggesting delayed release of PCP from the lungs. The maximum concentration achieved in the aforementioned quoted smoking study was 1.5 ng/mL. The mean half-life of the smoked PCP is approximately 24 h (Cook et al., 1982b), although a slightly lower half-life has been reported in other studies (Cook et al., 1983). Oral absorption leads to a bioavailability of 72%. Volunteers given 1 mg of PCP orally had average PCP concentrations of 2.7 ng/mL. Plasma concentrations after 1 mg given intravenously were 2.9 ng/mL (Cook et al., 1982a). See Table 6.1 for a summary of PCP's main physiochemical and pharmacokinetic properties.

Peak plasma levels occur at 2.5 h after PCP is orally administered, although levels are near maximal at 1.5 h. After both oral and intravenous administration, a 1–2 h plateau period follows, during which plasma levels remain relatively stable (Cook et al., 1983). Skin absorption does occur and can result in positive urine test results, possibly at levels exceeding NIDA cutoffs. In one study, a crime lab chemist was found to have a PCP level of 28 ng/mL (Pitts et al., 1981). Just how relevant all of these measurements are to the problems of clinical intoxication is not obvious. The amount of PCP used for the volunteer studies was probably very small when compared to the amounts taken by abusers. When PCP was first introduced as a legal anesthetic, sophisticated techniques for measuring blood levels were not available. Now that such techniques exist, ethical considerations prevent the administration of PCP in quantities that accurately reflect street practices.

Blood concentrations of PCP in patients presenting at ER have ranged from 0.3 to 143 ng/mL (mean 15 ng/mL) (Yago et al., 1981). Nonfatal intoxications in 20 cases had PCP blood concentrations ranging from 6 to 240 ng/mL (median 60 ng/mL) (Pearce, 1976). Intoxication in humans is not apparent when blood levels are less than 3 ng/mL, and clinical correlations between blood levels and physical findings, except for systolic blood pressure, are generally poor. In 70 cases where PCP was the cause, or a partial cause, of death, 90% of decedents had blood concentrations that ranged from 10 to 300 ng/mL (Budd and Liu, 1982).

In a smaller series of five PCP-related deaths, concentrations at autopsy ranged from 8 to 2100 ng/mL. Plasma concentrations in 10 individuals with clinical evidence of intoxication were lower, ranging from less than 10 ng/mL up to 812 ng/mL. A case report from 1989 described a man who swallowed two balloons full of PCP and promptly lapsed into a coma. The man's previous medical history remained unknown until day 11 when the man passed the two PCP-filled balloons. One balloon ruptured while the patient was still comatose. The maximum blood level on the third hospital day was 1879 ng/mL. The blood level at the time he passed the two balloons was not recorded, but the level in his cerebrospinal fluid at the time was 245 ng/mL, and the plasma level the day before was nearly 1000 ng/mL (Jackson, 1989).

Episodes of fatal PCP intoxication from direct toxicity (as opposed to homicides and trauma deaths where PCP is an incidental finding) are uncommon (Noguchi and

Nakamura, 1978; Budd and Liu, 1982; Poklis et al., 1990; Li and Smialek, 1996) and, in any event, blood levels in patients dying directly from the effects of PCP overlap with the blood levels seen in victims of accidental deaths (Poklis et al., 1990). Maternal–fetal relationships have not been studied in depth, but the few papers that have been published have shown that PCP crosses the placenta easily and concentrates in the fetus (Aniline and Pitts, 1982).

6.2.7.2 Metabolism

PCP is metabolized by hydroxylation at position 4 on the cyclohexane ring and/or on the piperidine moiety, and the resulting metabolites are pharmacologically inactive. The metabolites undergo glucuronidation and are excreted in the urine (Cook et al., 1983).

Several cytochrome P450 (CYP) isozymes contribute to PCP metabolism—mainly CYP3A4 although other isozymes are also involved (Laurenzana and Owens, 1997). For example, it has been shown that CYP2B enzymes may hydroxylate the phenyl ring, after which there is spontaneous decomposition to piperidine and an electrophilic quinone methide intermediate, which can react with glutathione (Driscoll et al., 2007; Shebley et al., 2009). These compounds may attach to other macromolecules and cause toxicity.

PCP may also suppress CYP isozymes, including CYP2C11 and members of the CYP2D subfamily (Hiratsuka et al., 1995; Shelnutt et al., 1997). PCP plasma concentrations may be altered when other drugs are taken at the same time. In a dog model of PCP intoxication, concurrent administration of PCP with marijuana resulted in higher blood and brain PCP concentrations than when PCP was given alone. Alcohol, on the other hand, does not exert this effect (Godley et al., 1991). This synergy may explain why PCP and marijuana are frequently detected in the same urine specimens. PCP appears in saliva, and saliva concentrations appear to correlate well with blood levels (McCarron et al., 1981b).

6.2.7.3 Excretion

PCP is lipid soluble with a relatively high volume of distribution. It is 60%–70% protein bound, although just which proteins are involved is not known; less than one quarter of PCP is bound to albumin (Busto et al., 1989). PCP is subject to enterohepatic recirculation. Measurable levels may persist in bile for months (Aniline and Pitts, 1982).

Recovery of PCP and its metabolites in urine and feces is incomplete. Hydroxylated derivatives, accounting for less than 50% of a total dose, can be recovered from the urine; however, there is little fecal excretion (Cook et al., 1983). The window for detection of PCP in the urine is variable. In experimental animals, the half-life for PCP is only 3–5 h (Woodworth et al., 1985), but in humans, it is much longer. After oral administration, PCP's terminal half-life may approach 24 h, which means that PCP should still be detectable in the blood for 5 days, and for at least as long in the urine (Cook et al., 1982a). Usually, less than 10% is excreted unchanged in urine although, like the amphetamines, excretion is pH dependent (Domino and Wilson, 1977; Perez-Reyes et al., 1982). PCP is also excreted into the stomach, and continuous gastric suction helps speed its removal from the body (Aniline and Pitts, 1982).

6.2.7.4 Other Tissues

PCP has been detected in vitreous humor at about half the concentration of that found in blood (Cox et al., 2007; Jenkins and Oblock, 2008). It is also present in hair where it is relatively easy to detect. Measured concentrations depend on the amount of melanin in the hair (Nakahara et al., 1995; Sakamoto et al., 1998; Slawson et al., 1998). PCP can also be found in saliva and sweat (Cook et al., 1982b; Cook et al., 1983; McCarron et al., 1984).

6.2.8 Toxicity by Organ System

6.2.8.1 Neurologic Disorders

Many papers have described the psychological changes produced by PCP exposure, and even more has been written on the neuropathologic changes produced in experimental animals (Shin et al., 2008). Human autopsy studies make no mention of any consistent or unique neuropathologic changes. Most of the human autopsy studies on PCP deaths were reported before it was known that PCP induced cell apoptosis. Indeed, most of the reports were published before the process of cell apoptosis was even recognized. Apoptosis occurs because damaged neurons are programmed to self-destruct. This process can be recognized by the observation of condensed and fragmented cell chromatin contained within an intact cellular membrane. It can also be identified by immunofluorescent *TUNEL* staining. This immunostain is commonly used to detect DNA fragmentation produced by apoptotic signaling cascades (Gavrieli et al., 1992).

In spite of gaping deficits in our knowledge, there is fairly broad agreement on how PCP, and other NMDA antagonists, is able to alter human behavior. NMDA antagonists abolish GABAergic inhibition, leading to the simultaneous and excessive release of acetylcholine and glutamate, causing a progressive decrease in NMDA receptor function. Underexcitation of NMDA receptors, induced by even relatively low doses of NMDA antagonist drugs, can produce specific forms of memory dysfunction without clinically evident psychosis. More severe loss of NMDA function can result in a clinical syndrome very similar to a psychotic schizophrenic exacerbation.

Sustained and severe NMDA hypofunction is associated with well-characterized neuropathologic features (Farber, 2003). These include vacuolization of neurons in the posterior cingulate and retrosplenial cortices. Changes can be seen within 4 h of 1 mg/kg subcutaneous injection. Some evidence suggests that the changes resolve, and tolerance to the effects develops with repeated usage (Olney et al., 1989; Gao et al., 1993). Evidence also indicates that PCP can cause damage to Purkinje cells of the cerebellar vermis (Nakki et al., 1995). It is conceivable that these transient changes could account for cases of fatal status epilepticus that have been reported in human abusers (Kessler et al., 1974; McCarron et al., 1981a).

6.2.8.2 Cardiovascular Disorders

In experimental models, at least in the frog model of PCP intoxication, the action potential of ventricular muscle is altered (D'Amico et al., 1983). The action potential of atrial muscle is prolonged in the guinea pig (Temma et al., 1985). There are no published reports suggesting that PCP can block hERG currents, and there are no reports of QT prolongation or torsades de pointes associated with PCP, either in animal or human studies. It may be that the in vitro data are not representative of effects observed in vivo. Alternatively, it may be that some other effects produced by PCP mask any action it exerts on the QT interval. For example, PCP causes tachycardia in humans (Barton et al., 1981). Increased heart rate may counteract QT prolongation by reversing the normally observed rate dependence of hERG/IKr blockade.

Most cases of sudden death in police custody involve abusers with many years' history of polydrug abuse who have already undergone left ventricular remodeling. At least two reported deaths thought to involve neck hold restraint actually involved intoxicated PCP abusers (Barton et al., 1981; Mercy et al., 1990). Neck hold restraint produces carotid

sinus stimulation and reflex bradycardia. It has been suggested that if hERG/IKr is blocked by PCP, the reverse rate dependence of the blockade could mean that QT prolongation is exaggerated at slower heart rates. The same might apply to cocaine and possibly even methamphetamine (Sheridan et al., 2005).

Most of the studies on the cardiovascular effects of PCP were done more than 30 years ago, using experimental animals given very large doses by continuous infusions, and their relevance is questionable. In any event, these studies showed that PCP increases heart rate, cardiac output, blood pressure, and temperature (Davis et al., 1991). Other in vitro and animal studies have shown that, even in low doses, PCP inhibits the calcium-dependent ATPase located in cardiac sarcoplasmic reticulum. This action would effectively disrupt intracytosolic calcium homeostasis, thereby decreasing cardiac output (Pande et al., 1998), but whether this sequence actually occurs in humans is not known. What is known now, but was not known then, is that ~35% of sudden unexplained death cases and ~20% of sudden infant death syndrome cases may be explained by mutations in cardiac ion channels (*cardiac channelopathies*) (Mizzanti et al., 2014; Wang et al., 2014). Without DNA resequencing, it would be very difficult to say, absent any obvious signs of toxicity (such as rhabdomyolysis), that PCP and not some genetic aberration is the case of death.

6.2.8.3 Renal Disorders

In a published case series of 1000 PCP-intoxicated patients, 2.2% were found to have rhabdomyolysis, but only three of these patients had renal failure requiring dialysis (McCarron et al., 1981a,b). Renal failure usually occurs only in deeply comatose patients who are experiencing convulsions or rhabdomyolysis (Cogen et al., 1978; Hoogwerf et al., 1979; Fallis et al., 1982; Zhu et al., 2004).

6.2.9 Postmortem Toxicology

6.2.9.1 Preanalytic Considerations

PCP is a stable molecule (Giorgi and Meeker, 1995). However, PCP concentrations have a tendency to decrease gradually when stored; an 18% loss was observed after 3 years when stored at −20°C (Grieshaber et al., 1998).

6.2.9.2 Preferred Analytic Methods

PCP is a relatively easy drug to detect in biological fluids. Commercial immunoassays are available for both blood and urine analyses (Moore et al., 1999; Kerrigan and Phillips, 2001). In workplace settings, the urine cutoff is 25 ng/mL; however, in the forensic setting, much lower reporting limits are recommended. Some interference with PCP immunoassays has been noted with venlafaxine and tramadol (Bond et al., 2003; Hull et al., 2006).

Confirmatory analysis needs to be conducted using some form of mass spectrometry. Gas chromatography–mass spectrometry (GC–MS) methods are plentiful and require no prior derivatization (Vorce et al., 2000; Ferguson and Garg, 2010). Alternatively, liquid chromatography–mass spectrometry (LC–MS) can be used (Chimalakonda et al., 2010). In both GC–MS and LC–MS, it is common to target other drugs of abuse in addition to PCP. Similar methods have also been described for the detection of PCP in oral fluid (Sergi et al., 2009).

Table 6.2 Blood and Tissue Levels in Seventy Fatal Cases of PCP Intoxication

Blood (ng/mL)	Urine (ng/mL)	Liver (ng/mL)	Bile (ng/mL)	Brain (ng/mL)	Kidney (ng/mL)
100–2400	100–7600	100–7820	100–1690	30–710	400–900

Source: Adapted from Budd, R.D. and Liu, Y., *J. Toxicol. Clin. Toxicol.,* 19(8), 843, 1982.

6.2.9.3 *Interpreting Blood and Tissue Concentrations*

As with many substances, the detection of PCP in blood and urine indicates exposure but does not provide proof of an effect (impairment, toxicity, fatality, etc). To reach such a conclusion, the analytic result must be interpreted in the context of the case and clinical or pathologic information (Table 6.2).

Given PCP's high lipid solubility and relatively long half-life, it will persist in the body for long periods of time, particularly in fat tissues (James and Schnoll, 1976). Detection in blood and urine could occur for at least 1 week following last use. In one case report, a police chemist who reportedly had daily contact with PCP still had a plasma level of 70 ng/mL 6 months after leaving the laboratory (Aniline and Pitts, 1982). Therefore, PCP will be subject to postmortem redistribution, which strongly implies that any postmortem blood specimen will have a higher concentration than would be obtained in perimortem blood. In any case, blood (and other tissue) concentrations of PCP capable of causing behavioral alteration (including impaired driving) overlap with concentrations that might be life threatening. The interpretation is very much driven by the circumstances and supporting information.

6.3 Methoxetamine (Figure 6.1)

MXE (mket, or 2-(3-methoxyphenyl)-2-(ethylamino)cyclohexanone) is a PCP analog that has been detected in a number of places around the world including the United Kingdom, EU, and the United States (Morris and Wallach, 2014). The drug has been known as Haze.

It is a noncompetitive NMDA receptor antagonist as well as a dopamine reuptake inhibitor, and an agonist at dopamine D_2, serotonin 5-HT_2, muscarinic cholinergic, σ_1, and opioid μ and κ receptors (Coppola and Mondola, 2012). MXE, like its analogs, can reverse depression in patients.

The drug was first reported to the EU through its monitoring center from a UK report in late 2010, and first reports of intoxication with this drug appeared in 2011. Clinical features of acute toxicity are suggestive of a *dissociative/catatonic* state similar to that seen with ketamine, with tachycardia and hypertension (Wood et al., 2012). GC–MS was used to detect this compound. Serum concentrations ranged from 0.09 to 0.2 mg/L. In another

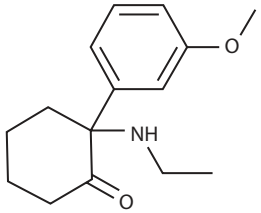


Figure 6.1 Molecular structure of methoxetamine.

series of reported hospitalizations following use of this drug, serum concentrations of MXE were 0.1–0.5 μg/g (Wikstrom et al., 2013). In some of these cases, other drugs were also detected.

Acute neurologic toxicity has also been associated with this drug (Shields et al., 2012). A 19-year-old male presented with severe truncal ataxia, nystagmus, incoordination, and reduced consciousness several hours after nasal insufflation. Features of cerebellar toxicity persisted for 3–4 days before gradual recovery. Two more patients aged 17 and 18 years presented with severe cerebellar ataxia, imbalance, and reduced conscious level 40 min after nasal insufflation of MXE. Both had slurred speech, incoordination, and cerebellar ataxia that resolved within 24 h. In these cases, serum MXE concentrations ranged from 0.16 to 0.45 mg/L. No other drugs were detected.

MXE was believed to be more bladder friendly than ketamine, which is associated with bladder and renal toxicity with regular use; however, 3-month chronic studies in mice were associated with inflammatory cell infiltration, tubular cell necrosis and glomerular damage, and other adverse changes suggesting that MXE is no safer (Dargan et al., 2014).

The drug can be measured by conventional GC–MS or liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Imbert et al., 2014).

6.4 Ketamine (Table 6.3)

6.4.1 General

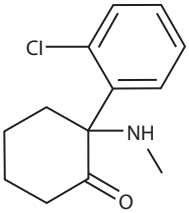
Ketamine is a chiral molecule, and the racemic mixture is widely used as an anesthetic. (*S*)-ketamine is the more active enantiomer and has slightly different actions than either (*R*)-ketamine or (*R, S*)-ketamine. It tends to be used in specialized surgery, especially as an adjunct to other anesthetics in pediatric surgery. It is a noncompetitive antagonist of the NMDA receptor and enhances the antinociceptive effects of conventional opioid analgesia (Baker et al., 2002). There is growing evidence that ketamine also exerts potent anti-inflammatory effects (Mei et al., 2011; Welters et al., 2011), a property making it especially desirable in vascular surgery, though concerns have been raised that it might be responsible for neuroapoptosis (Persson, 2010). Like the other drugs discussed in this chapter (save propofol), it produces a dissociative state characterized by profound analgesia in which patients are in a hypnotic amnesic state with eyes open but unresponsive to commands. Perhaps because of this action, it also has been subject to significant abuse.

6.4.2 Availability

Statistics relating to abuse of ketamine within the United States are hopelessly outdated. The most current data available from the U.S. government are contained within the DAWN reports; however, these compilations are published many years after the data reported were first collected. The 1999 report contains 21 ketamine mentions, with an insignificant increase in the number of deaths from 3 to 16 deaths reported a year earlier, ranking ketamine in the 71st position on the DAWN drug-related deaths lists in 2000 and accounting for 0.18% of all drug-related deaths reported to the federal government that year (Kissin et al., 2000). According to the DAWN's 2005 report, there were only 275 ketamine-related ER visits in the preceding year, and none was fatal (SAMHSA, 2006).

Table 6.3 Physiochemical Properties and Pharmacokinetics of Ketamine

Chemical Name	α(±)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone
Physiochemical properties, structure, and form	Free base or HCl salt. CAS 6740-88-1 (base). CAS 1867-66-9 (HCl). MW, 237.7. pK_a, 7.5. Protein binding, 20%–50%.
Synonyms	Jet, super acid, special K, green, K, cat valium, KitKat, vitamin K, ket.
Brand names	(−)-Ketamine, (S)-(−)-Ketamine, (S)-Ketamine, CI 581 base, CLSTA 20, Esketamine, Ketaject, Ketalar, Ketalar base, Ketanest, Ketolar.
Pharmacokinetic parameters (oral)	Bioavailability: nasal 50%, rectal 50%, oral 17%. $T_{½}$, 3–4 h.
Common blood concentrations	>400 ng/mL produces analgesia; concentrations >1000 ng/mL produce anesthesia.
Metabolism and metabolites	Dealkylation, hydroxylation, conjugation to norketamine (active), hydroxyl metabolites, and glucuronides.
Urinary excretion	Two percent excreted unchanged, 2% norketamine, 16% dehydronorketamine (DHNK), 80% conjugates.
Postmortem artifacts	Likely to increase blood concentration significantly after death.
Interactions	Potentially dangerous interactions with numerous drugs exist, particularly propoxyphene, dextromethorphan, memantine, and other NMDA antagonists (propoxyphene was withdrawn from the U.S. market in 2010). Clarithromycin increases plasma concentrations over threefold when coadministered with ketamine.
Key papers	Wieber et al. (1975); Malinovsky et al. (1996); Hallak et al. (2011).



Ketamine is often used as a methamphetamine adulterant, and sometimes, it is sold as tablets misrepresented as ecstasy. Occasionally, it is even used to adulterate real ecstasy. There is concern about increased rates of ketamine abuse in East and Southeast Asia. These concerns are reflected by the proportion of ketamine seizures within Southeast Asia, especially since the area still accounts for to 86% of global production (8.2 metric tons, or more than double global ecstasy seizures in Southeast Asia alone) in 2008. According to the United Nations (UN), Asia alone accounted for 99% of global ketamine seizures in 2009. Most of the ketamine sold in Southeast Asia is produced in the region.

The growing use of ketamine is of particular concern in Hong Kong and China, just as the demand for high-quality MDMA (3,4-methylenedioxymethamphetamine; ecstasy) appears to be decreasing. The number of registered drug users who prefer ecstasy-group substances within China has declined by 40% since 2004. At the same time, the number of ketamine users has doubled. According to reports from the UN, this change in preferences is probably price driven. Between 2007 and 2009, the average price per pure gram of ketamine in Hong Kong was just HK$144, making it a cheap substitute for the increasingly expensive ecstasy or methamphetamine. Illicit diversion was the primary source of ketamine. However, in 2009, China reported seizing two illicit laboratories, each processing hydroxylamine hydrochloride, the immediate chemical precursor to ketamine, together

with 8.5 metric tons of finished ketamine (UNODC, 2010). China is now a net exporter of illicit ketamine to the United States and Canada. In January 2011, police reported having confiscated more than a ton of ketamine being carried on a ship in Vancouver harbor (Anon, 2011). As ketamine is not under international control, however, the extent of manufacture and consumption is probably underreported.

6.4.3 Epidemiology

There are data regarding use of ketamine in the gay community but no reliable epidemiologic data relating to use among the heterosexual population. In New York City, ongoing intercept surveys conducted annually at lesbian, gay, and bisexual community events are used to monitor trends in club drug use. The latest report covers the years from 2002 to 2007 and studied nearly 7000 men. It was found that recent use of ecstasy, ketamine, and GHB had all decreased significantly over the period. Crystal methamphetamine use initially increased in 2002 but then decreased for the remainder of the period. Use of cocaine and amyl nitrates remained consistent. A greater number of HIV-positive (vs. HIV-negative) men reported recent drug use across the study years. Downward trends in drug use in this population mirror trends that have been observed in other groups (Pantalone et al., 2010).

6.4.4 History

Ketamine was first introduced as an anesthetic agent in 1965 (Domino et al., 1982) (Figure 6.2). It is classified as a dissociative anesthetic with a structure and actions closely related to those of PCP. Even though it was used for many years as an anesthesia adjunct

Figure 6.2 Ketamine is still available as a veterinary anesthetic, and some is diverted to the illegal market. This drawing shows a woman holding a bottle of Ketaset, presumably with the intent of swallowing some.

(Ketalar, Parke-Davis), in 1999, the DEA placed ketamine, including all of its salts, isomers, and salts of isomers, into schedule III of the Controlled Substances Act (CSA) (21 U.S.C. 801 et seq.).

It continues to be sold in the United States as a veterinary anesthetic under the names Ketajet, Ketaset, and Vetala. Although Ketalar is no longer sold in the United States, it is widely sold in Europe and even offered for sale in the United States by Internet pharmacies. Ketamine has a chiral center at the C_2 carbon of the cyclohexanone ring, allowing the existence of both (+) and (–) isomers. Veterinary ketamine preparations are supplied as mixtures containing equal amounts of both isomers.

6.4.5 Clandestine Laboratories

In the past, illicitly used ketamine was illegally diverted from legitimate suppliers. To produce street drug, commercial anesthetic ketamine was allowed to evaporate, and the resultant crystals were scraped into a fine powder and then packaged. No clandestine ketamine laboratory has ever been discovered in the United States. Virtually all the ketamine illicitly consumed in the United States is made in Southeast Asian and Chinese laboratories.

6.4.6 Routes of Administration

Ketamine can be administered via almost any route. The route chosen depends on the intent. In addition to intramuscular and intravenous routes, epidural and intrathecal administration has been employed, but with variable results. In one study, 50 mg of intrathecally administered ketamine provided adequate anesthesia, but only of short duration (Bion, 1984). Other studies have shown that epidural morphine was more effective and longer lasting than ketamine (Kawana et al., 1987). Oral ketamine administration is also possible and, because of first-pass effects and the formation of norketamine (the metabolite has some psychoactive properties in its own right), anesthetic effects are observable at lower blood concentrations. Rectal administration has an even more rapid onset and ketamine has been used as an induction agent in children (Idvall et al., 1983). When used as a recreational drug, ketamine is usually inhaled through the nose (insufflated).

6.4.7 Pharmacology

The analgesic effects of ketamine can be explained by its ability to bind to μ opioid and σ receptors. But, like PCP, ketamine also blocks the NMDA receptor, a receptor classified by some as a subtype of the σ opiate receptor. The exact mode of ketamine's action is still not known, but new and surprising actions, such as an anticytokine effect, continue to be discovered (Welters et al., 2011). Even at a subanesthetic dose, ketamine is still a potent analgesic. Some research points to possible effects on muscarinic receptors, not to mention voltage-dependent ion channels, particularly L-type calcium channels (Wong and Martin, 1993).

Until recently, ketamine was produced only as a racemic mixture. The (*S*) form has a four times greater affinity for the NDMA receptor than the (*R*) form, but the opposite applies with the σ receptor. The pharmacokinetic differences between the two have never been studied. The pure (*R*) form is now sold under the name of esketamine and is increasingly being used in Europe.

6.4.8 Disposition

6.4.8.1 Metabolism

Ketamine is metabolized in the liver by the P450 system. In humans, CYP3A4 is the principal enzyme responsible for ketamine *N*-demethylation to form norketamine. At therapeutic concentrations, CYP2B6 and CYP2C9 make only a minor contribution to ketamine *N*-demethylation (Hijazi and Boulieu, 2002). Norketamine is then hydroxylated, conjugated, and excreted in the urine (Adams et al., 1981; Reich and Silvay, 1989).

6.4.8.2 Blood Concentrations and Elimination

Ketamine is very lipid soluble and, therefore, has a very large volume of distribution (between 3 and 5 L/kg) (Moffat et al., 2004). The elimination half-life is a little over 2 h (Domino et al., 1982). The pharmacokinetics in children is not very different from adults, although children do form more norketamine than adults (Grant et al., 1983).

The pharmacokinetic behavior of ketamine after intravenous injection may be described in terms of an open two-compartment model. The α phase of ketamine's serum half-life is about 11 min and the β phase 2.5 h. The half-life of ketamine and its metabolites in urine is comparable to that in serum. The duration of anesthesia correlates with its rapid metabolic breakdown and elimination, but is also, to a degree, a reflection of its high volume of distribution (Wieber et al., 1975).

Norketamine concentrations after intranasal and rectal administration were measured after a 3 mg/kg dose. In the intranasal group, mean plasma concentrations of ketamine peaked at 20 min at a concentration of 0.5 mg/L. After rectal administration, concentrations peaked within 21 min at a mean concentration of 2.1 mg/L. Plasma concentrations of norketamine peaked at approximately 2 h after nasal ketamine, more rapidly than after rectal administration of ketamine, and were always higher than ketamine concentrations (Malinovsky et al., 1996).

Bioavailability is high after intramuscular injections, which is one of the reasons this agent is so attractive for use in the battlefield setting. Taken orally, first-pass effects result in lower blood concentrations but, somewhat surprisingly, a more rapid onset. The explanation has to do with hepatic formation of norketamine, the active ketamine metabolite that has approximately one-third the activity of the parent compound. Given orally, a 5 mg/kg dose of ketamine produces analgesia with a plasma concentration of 0.4 mg/L, 30 min after administration (Grant et al., 1983).

When ketamine is given intravenously, doses only half as great as those given orally result in plasma concentrations more than twice as high as oral administration (Wieber et al., 1975). Even in modest doses, ketamine in recovering alcoholics induces symptoms very similar to those seen after giving ethanol. Chronic alcohol consumption is thought to increase NMDA receptor function and to account partially for the seizures and other evidence of neurotoxicity seen in chronic alcoholics (Fidecka and Langwinski, 1989).

In 20 detoxified alcoholics, an intravenous dose of 0.1 mg/kg produced peak ketamine concentrations of approximately 75 ng/mL after 80 min. When the dose was increased to 0.5 mg/kg, peak concentrations occurred at the same time but were much higher (400 ng/mL) (Krystal et al., 1998). Clarithromycin increased the concentration of ketamine more than threefold (and decreased concentration of norketamine) since it inhibits CYP3A4 (Hagelberg et al., 2010). Caution is needed when other drugs that affect CYP3A4 are coadministered.

6.4.8.3 Excretion and Urine Concentrations

Studies of ketamine urinary excretion indicate that, over a 72 h period, little unchanged drug and norketamine are present (2.3% and 1.6%, respectively). The predominant metabolite found in the urine is dehydronorketamine (DHNK) (16%), along with conjugates of hydroxylated ketamine metabolites (80%) (Adamowicz and Kala, 2005).

In urine collected from hospitalized children who had received ketamine as an anesthetic, ketamine was detectable with GC–MS for up to 2 days after drug administration (29–1410 ng/mL); norketamine was detected for up to 14 days. Using a LC–MS method, ketamine could be detected for up to 11 days (Adamowicz and Kala, 2005). In a group of presumed recreational ketamine users, urine concentrations of ketamine, norketamine, and DHNK were 6–7,744 ng/mL, 7–7,986 ng/mL, and 37–23,239 ng/mL (unconfirmed), respectively (Moore et al., 2001).

6.4.8.4 Distribution

What little is known about tissue distribution comes from postmortem case reports. In a homicide attributed (in part) to ketamine, concentrations of ketamine were as follows: blood 1.8 mg/L, urine 2.0 mg/L, brain 4.3 mg/kg, spleen 6.1 mg/kg, liver 4.9 mg/kg, and kidney 3.6 mg/kg (Moore et al., 1997). In another report of two deaths associated with ketamine, there were marked ketamine concentration differences in heart and femoral blood, confirming that redistribution had occurred (Lalonde and Wallage, 2004). There exists a small body of data derived from animal experiments, but its relevance to the human condition is not known (Watterson et al., 2012).

Ketamine has been shown to be present in the milk of cows given ketamine at approximately twice the concentration observed in plasma (Sellers et al., 2010). Oral fluid has also been used to assess exposure to ketamine because concentrations of ketamine are readily measurable in that medium (Cheng et al., 2007). Hair can also be used to detect past exposure to the drug. In one study, ketamine and norketamine concentrations in forensic hair samples were 0.2–5.7 ng/mg and 0.1–1.2 ng/mg, respectively (Harun et al., 2010). Some studies have attempted to use bone deposition as a way to estimate time of death, but the values recorded are too variable to be relied upon (Watterson and Donohue, 2011).

6.4.9 Toxicity by Organ System

Ketamine is classified as a "dangerous drug," but it has quite an extraordinary safety profile. A 1996 study on the use of ketamine as an anesthetic in the developing world surveyed 122 physicians about their experiences in operating on 12,000 patients. Pulse oximetry was used in fewer than 10% of the cases, and intermittent vital signs were taken in less than half. One unexplained pediatric death occurred during unmonitored recovery, and one adult suffered cardiac arrest after a failed intubation attempt. Apnea, possibly related to ketamine, was reported in 10 patients and laryngospasm in six. The Red Cross in its field hospitals (Lenz and Stehle, 1984) reported similar experiences. Even in the case of a substantial overdose, the main effect seems to be prolonged sedation. Green et al. described nine cases of inadvertent ketamine overdose in children. Three of the children received five times the recommended dose, five received 10 times the ordered dose, and one child was given a dose 100 times greater than ordered (all by intravenous or intramuscular route). All nine experienced prolonged sedation (3–24 h). Except for prolonged sedation, no adverse outcomes were noted (Green et al., 1999). More recently, cases of ketamine-related

laryngospasm have been reported, especially in children and often in the ER setting (Dolansky et al., 2008; Green et al., 2010; Burnett et al., 2012).

6.4.9.1 Neurologic Disorders

Ketamine dependence has been reported, particularly among hospital workers with ready access to the drug (Ahmed and Petchkovsky, 1980; Jansen and Darracot-Cankovic, 2001). Ketamine has the advantage, at least so far as hospital workers are concerned, of having a short half-life and relatively rapid renal clearance, making detection less likely. Intrathecal and epidural ketamine is sometimes used in the management of chronic pain, generally without significant side effects (e.g., no respiratory depression or urinary retention) (Yaksh, 1996). Small doses, on the order of 50 mg, produce complete pain relief for at least 1 h. Large doses result in longer pain-free periods. However, there is at least one report of isolated lymphocytic vasculitis of the spinal cord presumably related to ketamine administration (Stotz et al., 1999). Intrathecal ketamine therapy is usually reserved for patients with terminal illness, so the true incidence of this complication remains unknown.

Mounting evidence suggests that ketamine is an effective antidepressant. In a recent blinded, controlled trial, 17 individuals with depression were studied. All suffered from moderate to severe depression, and all had failed to respond to at least two types of conventional drug treatments. Depression improved within 1 day for 12 of the 17 who received ketamine. These patients showed a 50% reduction in their symptoms, according to the Hamilton Depression Rating Scale. Overall, while 9 of the 17 patients had a 50% reduction in their depression within the first 2 h of ketamine treatment, only one person receiving the placebo experienced the same effect in this period of time. The antidepressant effects of ketamine lasted for 1 week in four people and at least 2 weeks in another two subjects. The striking results are thought to be a consequence of NMDA antagonism (Zarate et al., 2006; Maeng et al., 2008; Li et al., 2011; Kupfer et al., 2012). A randomized controlled trial of intranasal ketamine in 20 patients with major depressive disorder showed unquestionable rapid antidepressant effect with minimal side effects (Lapidus et al., 2014).

6.4.9.2 Cardiovascular Disorders

Ketamine is used for anesthesia induction in trauma victims because, unlike other anesthetic agents, ketamine causes catecholamine release and an increase in blood pressure, and the increase may be of considerable magnitude (Tanaka and Nishikawa, 1994). This property makes ketamine a frequently chosen anesthetic for inducing trauma patients. In one of the homicides described in Section 6.4.10.3, where death was due to chronic ketamine poisoning, there was striking fibrosis of the cardiac myofibers and hyaline degeneration of small arteries in the heart (Tao et al., 2005). No similar case has ever been reported.

S-(+)-ketamine possesses anti-inflammatory potential, and cardiac anesthesia with *S*-(+)-ketamine may have beneficial effects in attenuating the systemic inflammatory response that occurs subsequent to surgery. The combination of sufentanil (also known to be cardioprotective) and ketamine is becoming more popular, and detection of the two in a postsurgical death may be merely incidental (Welters et al., 2011). As blood circulates through the bypass pump, there is activation of the inflammatory pathways. One recent study measured blood concentrations of interleukin-6 (IL-6) after giving 0.25 mg/kg of ketamine along with a general anesthetic. Normally, IL-6 concentrations begin to rise immediately after surgery, returning to baseline after 8 days, but no increase ever occurred in the ketamine-treated patients (Roytblat et al., 1998).

6.4.9.3 Renal Disorders

Ketamine is reported to cause lower urinary tract symptoms (LUTS), and published accounts of its deleterious effects are increasing. Several hundred reports and letters describing the syndrome are in the literature. The increased popularity of this compound seems to have gone "under the radar," but the results of a recent British survey found that the lifetime use of ketamine was 1% in those aged 14 years and above, with many reporting increasingly frequent usage in recent years (Degenhardt and Dunn, 2008). In general, affected patients present with LUTS, and the only clue to the diagnosis will be the patient's admission of ketamine abuse (Misra et al., 2014).

Little is known about the pathophysiology of this disease other than the fact that the bladder wall is infiltrated with mast cells and eosinophils. Jhang et al. reported that the severity of the histologic changes and the intensity of the discomfort experienced may be a consequence of elevated serum immunoglobulin in E concentrations (Jhang et al., 2014).

6.4.10 Postmortem Toxicology

6.4.10.1 Preanalytic Considerations

Ketamine and its metabolites are known to be stable in plasma samples stored at −20°C for up to 2 months (Idvall et al., 1979). It was even more stable in plasma samples stored at −20°C for 3 months (Gross et al., 1999).

Ketamine, norketamine, and DHNK were stable in serum when left at 4°C for 2 days and when frozen at −20°C for 10 weeks, but DHNK serum concentration decreased significantly when blood was not centrifuged almost immediately (Gross et al., 1999).

6.4.10.2 Preferred Analytic Methods

Commercial immunoassays exist for ketamine and are generally quite reliable. These include enzyme-linked immunosorbent assay methods for blood (Huang et al., 2007). Ketamine is relatively easy to detect since it is readily extracted from biological fluids and does not require derivatization for GC–MS. Norketamine should also be measured (Kim et al., 2008; Lin et al., 2010; Rohrig et al., 2010). Modern LC–MS/MS methods are capable of detecting ketamine and many other drugs of abuse in one chromatographic system (Sergi et al., 2009; Lin et al., 2010; Wohlfarth et al., 2010). A chiral method is also available (Moaddel et al., 2010). Ketamine can also be detected in hair by GC–MS (Kim et al., 2010).

6.4.10.3 Interpretation of Blood Concentrations

In the living, plasma concentrations of approximately 0.4 mg/L or more are associated with analgesia and concentrations greater than 1 mg/L with anesthesia. Compared to other anesthetic agents, ketamine appears to possess little intrinsic toxicity. In a published review of 87 ketamine-positive deaths that occurred over a 2-year period, almost all of the positive test results were found in hospitalized patients following surgical procedures or burn treatment patients, and not a single case of death could be attributed to intoxication with ketamine (Gill and Stajic, 2000).

A 1994 report is of interest. It describes a homicide committed by injecting a man with a massive amount of ketamine (Licata et al., 1994). The resultant blood concentration was 27 mg/mL, urine 8.5 mg/mL, bile 15 mg/mL, brain 3.2 mg/kg, liver 6.6 mg/kg, and kidney 3.4 mg/kg. Given the extreme lipophilicity of this drug and the relatively low concentration

observed in the brain (at least when compared to the other tissues), death must have been very rapid, but not so rapid that there was no time for conversion to norketamine, which was detected in all samples, proving that the decedent had lived long enough to metabolize at least some of the drug.

Another bizarre case was reported in 1995. It involved a homicide caused by chronic ketamine poisoning. The victim was a 34-year-old married woman with no previous medical history who died in her own home. Investigation revealed that her husband had chronically poisoned her with ketamine over a period of about 1 year. The ketamine concentration was 3.8 mg/L in blood and 1.2 mg/L in urine, and drug was detected in the gastric contents (Tao et al., 2005).

Ketamine rarely causes death by itself, and if it does, peripheral blood concentrations are likely to be well over 5 mg/L in the case of acute intoxication. Drug interactions and presence of significant natural disease are more likely to be present in a forensic case. As always, the interpretation of the significance of the results cannot be made on tissue concentrations alone.

6.5 Gamma-Hydroxybutyrate and Related Substances

6.5.1 Introduction

GHB is a simple hydroxy acid and a significant drug of abuse. It also exists as the lactone, called γ-butyrolactone or GBL (where the hydroxyl group and carboxyl form an internal cyclic ester), which the body readily converts to GHB. In solution, GHB and GBL are in equilibrium and rapidly interconvert with each other (Ciolino et al., 2001).

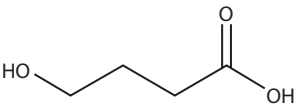
1,4-Butanediol is a GHB precursor and can be rapidly metabolized to GHB (one of the hydroxyl groups is oxidized to a carboxylic acid). It is sometimes known as fantasy (Palmer, 2004). In the United States, GHB is approved for the treatment of narcolepsy and sold under the brand name Xyrem (Tunnicliff and Raess, 2002). A summary of the main physiochemical and pharmacokinetic properties of GHB is shown in Table 6.4.

6.5.2 Incidence

GHB-related ER visits increased significantly during the 1990s, but the number of deaths attributable to GHB today has declined, at least within the United States, to a level that is difficult to estimate. The White House Office on Drug Control Policy lists GHB with other "club drugs" but has nothing specific to say about it. A study published in 2007/2008, reviewing data collected through 2003, found that both the California Poison Control System (CPCS) and DAWN were seeing ever-diminishing numbers of GHB cases, consistent with the notion that "a true decrease in case incidence is likely" (Anderson et al., 2006). Whether that is also the case in other parts of the world is not known. The lack of information is partly explained by the fact that GHB is not detected by any of the standard urine screening tests performed at hospital ER, is almost never part of standard postmortem toxicology screens, and is difficult to detect due to its very short half-life. Nonetheless, use in Europe and Asia appears to be increasing. According to the UN 2011 World Drug Report, seizures of GHB increased fourfold in Europe over the period 2005–2009. European seizures accounted for almost 80% of the world total.

Table 6.4 Physiochemical Properties and Pharmacokinetics of GHB

Chemical Name	4-Hydroxy-butyric acid or γ-hydroxy-butyric acid or sodium salt
Physiochemical properties, structure, and form	May be sold as liquid, powder, or capsules. It is freely soluble in water. CAS 591-81-1 (acid). 502-85-2 (Na salt). MW, 104.1.
Synonyms	Fantasy, Georgia home boy, G, grievous bodily harm or GBH, G-Riffick, lay, liquid ecstasy, liquid G, liquid X, mils, nature's Quaalude, scoop, thunder nectar; also available as legal drug in the United States as sodium oxybate (Xyrem as a 500 mg/mL solution).
Pharmacokinetic parameters	Elimination $T_{½}$, 0.5–1 h. Oral bioavailability, ~25%. Protein binding: negligible.
Common blood concentrations in drug users	Usually maximum at <200 mg/L rapidly declining to under 10 mg/L.
Metabolism and metabolites	Oxidation and metabolism through Krebs cycle producing succinic semialdehyde and succinic acid.
Urinary excretion	Rapidly excreted as unchanged drug, <5% unchanged.
Endogenous production	Blood concentrations up to 5 mg/L; urine up to 10 mg/L.
Postmortem artifacts	GHB is produced postmortem in peripheral blood up to ~100 mg/L, but not in urine.
Interactions	Reduces clearance of ethanol due to competing metabolism, hence increases effects of both drugs; inhibitors of P450 enzymes, such as the HIV-1 protease inhibitors ritonavir and saquinavir, can reduce clearance of GHB.
Key papers	Harrington et al. (1999); Couper et al. (2004).



6.5.3 Epidemiology

In the past, nearly two-thirds of GHB-related ER visits were due to *overdose* and one-third to *unexpected* reactions. Sixty percent of ER visits were said to have involved the use of multiple other drugs, usually GHB in combination with ethanol (76%), cocaine (6%), marijuana (5%), and MDMA (4%) (Woodworth and DEA, 1999).

A recent study in Australia found that GHB was the third most common drug (28%) found in patients presenting for emergency treatment (following amphetamines and alcohol) (West et al., 2008). In this group of patients, GHB use was most commonly associated with alcohol and amphetamines, and only 17% of the visits were occasioned by the ingestion of GHB. The blood concentrations of GHB in these presentations ranged from 18 to 265 mg/L (mean 130 mg/L). This observation makes sense because studies have since shown that ethanol potentiates the sedative and respiratory depressant effects of GHB (Morse and Morris, 2013).

6.5.4 History

GHB was first synthesized late in the nineteenth century, but it was not until the 1960s that French researchers, in the process of attempting to create a GABA analog that

could freely cross the blood–brain barrier, discovered the abuse potential of this drug (Tunnicliff and Raess, 2002). GHB is not a true GABA agonist, rather it is a naturally occurring inhibitory neurotransmitter (Bessman and Fishbein, 1963). GHB rapidly crosses the blood–brain barrier and produces almost immediate sedation. European surgeons began using GHB as an anesthetic adjunct in the early 1960s, but GHB does not produce any analgesia, so opiate administration or general anesthesia and paralytic agents (if the chest or abdomen are to be entered) are also required (Kleinschmidt et al., 1997). Because the use of additional analgesic drugs was required and because large doses of GHB cause seizure-like activity (Dyer, 1991), GHB anesthesia never really became popular in the medical community.

Abuse of GHB first began in 1977 when Japanese researchers observed that GHB could stimulate the release of human growth hormone (Takahara et al., 1977). The observation was of mild interest to the medical community but of very great importance to weightlifters and body builders who were convinced, quite correctly, that treatment with growth hormone could increase strength and endurance (Neely and Rosenfeld, 1994). During most of the 1990s, GHB and GBL were easily obtained at health food stores and gyms, and they continue to remain readily available over the Internet. GHB is a schedule III drug, meaning that suppliers can be prosecuted under federal law, but the lactone is a widely used solvent, found in such diverse products as engine degreasers and nail polish, making attempts at regulation nearly futile.

A popular children's toy, Bindeez or Aqua Dots, was recalled when it was found that 1,4-butanediol (1,4-B), which is metabolized into GHB, had been substituted for the nontoxic plasticizer 1,5-pentanediol during the bead manufacturing process. The toy was recalled after children were admitted to hospital with decreased levels of consciousness associated with vomiting and seizures (Gunja et al., 2008; Ortmann et al., 2009; Suchard et al., 2009).

During the early 1990s, GHB made the transition from gyms and health clubs to bars and dance clubs, where it had become popular as a mild intoxicant (Williams et al., 1998). Many investigators have studied the role of GHB in drug-facilitated sexual assault. The most recent meta-analysis shows that GHB is detectable in 0.2%–4.4% of reported sexual assaults. This same analysis also showed that a wide range of other drugs were likely to be present in cases of drug-facilitated sexual assault, many of them much more frequently than GHB. It appears that not only is use of this drug declining but that its popularity as a "date rape" drug is declining as well (Nemeth et al., 2010). Small amounts of GHB/GBL are present in alcoholic beverages derived from grapes: red wine vermouth (8.2 mg/L), sherry (9.7 mg/L), red wine (4.1–21.4 mg/L), and white wine (<3–9.6 mg/L) (Elliott and Burgess, 2005).

6.5.5 Clandestine Synthesis

GHB can be synthesized as sodium or potassium salt. It has a salty or soapy taste and is thermally unstable, reverting to the lactone when heated. Analysis and identification of GHB are complicated because it has no distinctive chromatographic properties, no distinguishable ultraviolet characteristics, and is easily produced by the hydrolysis of GBL, an extremely popular industrial solvent.

In theory, GHB can be produced either by acid or base cleavage (Figure 6.3), but most of the formulas circulating on the Internet, and most of the recipes disseminated in "underground" magazines, use base cleavage for the primary reaction (Suner et al., 1997). The difficulty with this approach is that too much sodium hydroxide may be used, leading

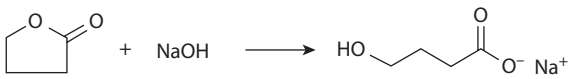


Figure 6.3 GHB can be produced by either acid or base cleavage, but most of the recipes disseminated in "underground" magazines use base cleavage for the primary reaction.

to the formation of a corrosive mixture. Burns and erosion of the lips, mouth, and esophagus may result. One of the most widely used formulas instructs would-be producers to heat one quart of GBL to its boiling point and then add one pound of sodium hydroxide crystals. The instructions say that the resultant solution should then be neutralized to a pH of between 6 and 7. If the instructions are actually followed, a 50% GHB solution is produced, which, in turn, is diluted to give a 20% solution. One teaspoon of this 20% solution will contain about 1 g of GHB (Sanguineti et al., 1997).

6.5.6 Routes of Administration

GHB is rapidly and completely absorbed when taken orally. Intravenous abuse is unheard of.

6.5.7 Disposition

6.5.7.1 Metabolism

Endogenous GHB is produced from GABA, which is first converted by GABA aminotransferase to succinic semialdehyde (Figure 6.4) (Roth and Giarman, 1969). The semialdehyde is then converted to GHB by $NADP^+$-dependent reductase (Anderson et al., 1977). Metabolic breakdown is accomplished by oxidation back to succinic semialdehyde, which is then shunted into the Krebs cycle (Doherty and Roth, 1978).

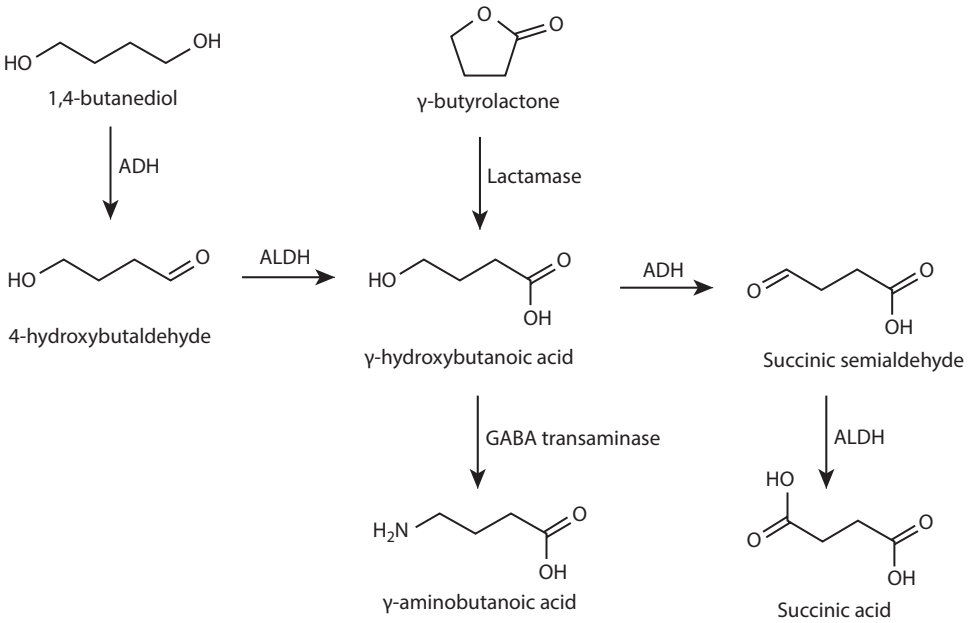


Figure 6.4 Metabolic pathways of GHB. *Note:* ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

In the fetus, GHB dehydrogenase converts succinic semialdehyde to succinic acid (Kaufman et al., 1979). In adults, it appears that GHB-ketotranshydrogenase is responsible for the conversion (Nelson and Kaufman, 1994). There is no evidence for in vivo conversion of GHB to GBL (Ferrara et al., 1993). A rare genetic disease is recognized in which GHB accumulates because of a deficiency of brain succinic semialdehyde dehydrogenase (Jakobs et al., 1984; Rating et al., 1984).

GHB and GBL are also metabolites of tegafur, a 5-fluorouracil prodrug used for treatment of some cancers. The two compounds are generated by metabolism of the furan ring on tegafur (Yamamiya et al., 2010). The maximum GBL concentrations in plasma of patients given a tegafur–uracil combination are quite low with C_{max} about 0.15 mg/L at 1 h (Emi et al., 2007). The HIV-1 protease inhibitors, because of their ability to inhibit the P450 system, particularly ritonavir and saquinavir, can reduce clearance of GHB leading to possible toxicity. Simultaneous use of GHB and MDMA may lead to toxicity via the same mechanisms (Harrington et al., 1999).

6.5.7.2 Absorption and Elimination in Blood

The blood/plasma ratio is about 1:2. Absorption and elimination have been studied following oral administration of single doses of GHB at 125, 250, and 500 mg/kg and at lower doses of 20 and 40 mg to healthy volunteers (Palatini et al., 1993; Brenneisen et al., 2004). Both the oral absorption and elimination of GHB are capacity-limited processes: the greater the dose, the slower the absorption and the longer the elimination period. The elimination half-life of GHB is of the order of 20–60 min. Liver cirrhosis causes significant reduction in clearance, thereby lengthening the half-life, but not enough to cause accumulation on repetitive dosing (Ferrara et al., 1996).

Peak plasma GHB concentrations occur 20–40 min after oral administration. Plasma samples collected from volunteers after being given either 250 or 500 mg/day of GHB had mean peak concentrations of 550 mg/mL (range, 240–880 mg/L) and 900 mg/L (range 510–1580 mg/L). Multiple dose regimens do not lead to accumulation of GHB. When GHB was given twice in one night to six narcoleptic patients who had been chronically taking GHB nightly, maximum plasma concentrations were reached in about 40 min, and the mean GHB half-life was 53 (SD 19) min. In some patients, GHB elimination was capacity limited when administered at a fixed dose of 3 g twice nightly at 4 h intervals (Scharf et al., 1998). Narcoleptic patients given a bedtime dose of 4.5 g of sodium oxybate for 8 weeks showed a small increase in C_{max} and area under the concentration curve (AUC) (13% and 16%, respectively) but reported no serious adverse events (Borgen et al., 2004). GHB is essentially eliminated from plasma within 6 h.

Single oral doses of sodium oxybate (Xyrem®) (40, 50, 60, and 72 mg/kg) given to eight volunteers (3.2, 4.0, 4.8, and 5.8 g for 80 kg persons) produced mean peak GHB plasma concentrations of 79, 83, 114, and 130 mg/L, respectively. These concentrations were associated with dose-related increases of euphoria and liking, followed by mild–moderate symptoms of sedation with impairment of performance and balance (Abanades et al., 2006).

Pharmacokinetic parameters in healthy volunteers are essentially the same as those in alcohol-dependent patients with compensated alcoholic liver disease (Palatini et al., 1993). The half-life of GHB in humans is known to be approximately 20 min, with a clearance rate of 14.0 mL/min/kg (Palatini et al., 1993). No exogenous GHB is detectable in the blood of the living after 8 h, and none in the urine after 12 h (Hoes et al., 1981). GHB users often

coingest ethanol. That practice may be dangerous because, in animal studies, high concentrations of either drug affect the metabolism of the other. When large doses of GHB are given, ethanol elimination is reduced (Hoes et al., 1981), an effect that would almost certainly lead to higher ethanol concentrations and probably to increased GHB toxicity. The picture becomes even more complicated when other drugs are used in addition to alcohol, as is often the case (Liechti et al., 2006).

6.5.7.3 Urinary Excretion

Peak urine concentrations occur within 3–4 h of ingestion (Ferrara et al., 1993). The administration of 50 mg/kg GHB to 16 adult volunteers alone, and combined with 0.6 g/kg ethanol, resulted in significant variability between individuals and GHB concentration peaked at about 150–200 mg/L at the 0–3 h urine collection. Coingestion of ethanol did not significantly affect renal clearance of GHB, but urine GHB concentrations were lower in the first 3 h when ethanol and GHB were coingested. Twelve percent of the samples collected from 3 to 6 h, 81% of samples collected from 6 to 12 h, and all of the urine specimens collected from 12 to 24 h had GHB concentrations below a 10 mg/L cutoff (Haller et al., 2006).

Endogenous GHB production has also been measured in 207 urine samples from individuals who reported they did not use GHB. Urine concentrations ranged from none detected to 2.7 mg/L. Males (n = 130) and females (n = 77) had the same average endogenous GHB concentration of 0.3 mg/L (LeBeau et al., 2006). In a different study, urine samples collected from 1126 women were analyzed and found to have GHB concentrations ranging up to 5.5 mg/L (mean 0.84 mg/L). GHB concentrations are independent of urinary pH (within the range 4.6–9.3), age (within the range 18–35 years), body mass index (within the range 13.8–36.3), and race. Adjusting GHB concentrations with respect to urinary specific gravity had little effect on the mean value (0.91 mg/L) and range (up to 8 mg/L) (Brailsford et al., 2010). Currently, a urine cutoff of 10 mg/L is widely applied to distinguish endogenous production from exogenous exposure (Brailsford et al., 2010). Pregnant women have slightly higher urinary GHB concentrations, but these should still fall below 10 mg/L (Raknes et al., 2010).

6.5.7.4 Hair Measurements

Low concentrations of GHB can be detected in the hair. The color of the hair appears to have little effect on the amount of GHB detected (Goulle et al., 2003; Kintz et al., 2003). Humans produce small amounts of GHB endogenously, explaining why, in one study, background concentrations of GHB in whole hair ranged from 1.9 ng/mg (with a mean value just under 1 ng/mg) up to 8 ng/mg (mean 1.2) (Goulle et al., 2003). Background concentrations of up to 12.0 ng/mg have been reported by others (Kintz et al., 2003).

GHB administered to a healthy 53-year-old white male at oral doses of 30, 45, and 60 mg/kg caused a rise in GHB concentration in beard hair at 24 h, but only at the two higher doses tested. The three 3 cm proximal segments showed elevated concentrations of GHB (1.22, 1.27, and 1.66 ng/mg, respectively) when compared with the basal physiologic level of GHB in this same person (mean = 0.62 ng/mg, SD = 0.15) (Goulle et al., 2003).

In still another report, elevated GHB concentrations were detected 7 days after a sexual assault at 3.1, 5.3, and 4.3 ng/mg within the last 3 mm proximal segments, whereas the mean physiologic level determined in the same woman was 0.71 ng/mg

(Goulle et al., 2003). In another case, concentrations peaked at 5 ng/mg when exposure was likely whereas distal hair had a concentration of 1 ng/mg (Rossi et al., 2009). A bodybuilder using GHB daily had a concentration of 14 ng/mg in a 2 cm long hair segment (Goulle et al., 2003).

GHB was not detected in the hair shaft following an acute overdose, but it was detected in plucked root bulbs that still had the outer root sheath attached (2221 ng/mg) and also in the root bulbs themselves after washing and removal of the outer root sheath (47 ng/mg) (Kalasinsky et al., 2001). Similar high concentrations of GHB were seen in another case where GHB was ruled the cause of death (Kintz et al., 2005). Collectively, the data show that GHB concentrations can be diagnostic of exogenous exposure, but care must be exercised to take into account background concentrations.

6.5.7.5 Oral Fluid Measurement

In one study, concentrations of GHB found in oral fluid were only one quarter to one-third as high as those found in plasma, although the ratio varied twofold over a 6 h time period (Abanades et al., 2007). Peak concentration of GHB in oral fluid (203 mg/L: SD 92) was detected at 10 min following a 25 mg/kg dose (Brenneisen et al., 2004), while the peak of 93 mg/L occurred at 30 min after a 50 mg/kg dose (Abanades et al., 2007).

6.5.7.6 Vitreous Humor Detection

Endogenous postmortem vitreous humor GHB concentrations appear to range up to 7 mg/L (Marinetti et al., 2005), though higher concentrations have been reported. Kintz described a man who died of intentional overdose. GHB concentrations were 2937, 33,727, 1800, and 2856 mg/L in femoral blood, urine, bile, and vitreous humor, respectively (Kintz et al., 2005). Back extrapolation to plasma concentration is not possible.

6.5.8 Preanalytic Considerations

Long-term freezer storage appears to prevent formation of GHB in postmortem blood, vitreous humor, urine, or bile, even when no preservative is present (Marinetti et al., 2005a). Urine samples collected from subjects who had never used GHB ($n = 31$) and stored under refrigeration (5°C) without any added preservatives showed significant elevations in GHB concentration as storage time increased (LeBeau et al., 2007). However, none exceeded the 10 mg/L threshold at 6 months of storage. Similarly, urine preserved with sodium fluoride (1%) and stored for up to 1 year at room temperature developed increased levels of GHB but no greater than 10 mg/L (Kerrigan, 2002). Sodium azide had no effect on arresting formation of GHB.

When sodium fluoride was not added to postmortem blood samples, substantial rises in GHB concentration, up to 100 mg/L, occurred within 4 months even though the sample had been stored at 4°C. This increase was followed by a gradual decrease in the following months (Berankova et al., 2006). The addition of sodium fluoride appeared to reduce rate of increase. GHB in plasma was found to be stable when stored frozen at −20°C for up to 9 months, and when stored at room temperature for 48 h, and after three freeze/thaw cycles. It was also found to be stable in processed samples stored at room temperature for 5 days and for 15 days at −20°C (Chen et al., 2003). Little change in GHB concentration occurred post collection when stored at −20°C (Moriya and Hashimoto, 2004).

6.5.9 Postmortem Formation of GHB In Situ

It is clear from the preceding that some GHB can be formed within the collection tube. Whether or not this occurs, and to what degree it occurs, depends on the source of specimen, type of preservative used, storage time, and temperature. Significant amounts of GHB are formed in the body after death, before any collection takes place. It is possible that formation of GHB is facilitated by bacteria (Elliott et al., 2004; Moriya and Hashimoto, 2004), but it would be difficult or impossible to determine the actual mechanism in any given case. Fieler et al. (1998) found no GHB in blood taken from 20 living non-GHB users (Fieler et al., 1998). However, blood from 25 postmortem cases had GHB concentrations ranging up to 168 mg/L, though the value of this observation is somewhat limited in that the site of blood collection was not specified and was likely to be from the heart.

Endogenous GHB concentrations in autopsied nonusers are significantly higher in femoral venous blood (4.6 mg/L) than in vitreous humor (0.9 mg/L), bile (1.0 mg/L), and urine (0.6 mg/L). GHB concentrations are similar in blood samples taken from different sites, suggesting little redistribution from neighboring tissues occurs (Moriya and Hashimoto, 2005). This original observation was confirmed in at least one other report where the cardiac blood/femoral blood ratio was found to be 1:2 (Kintz et al., 2005).

A study to assess postmortem blood GHB concentrations in nonusers found values that ranged from 0 to 43 mg/L (mean 9.8), and a positive correlation was observed between concentration and postmortem interval ($r = 0.571$). No correlation was found between concentration and storage interval at 4°C (no preservative was used) (Moriya and Hashimoto, 2004). The highest GHB concentration observed was 43 mg/L (postmortem interval of approximately 36 h). In another postmortem study of nonusers, the mean concentration of GHB was 12 mg/L (range 2–29 mg/L) and 13 mg/L (range 4–25 mg/L) in unpreserved and sodium fluoride–preserved samples, respectively (Elliott, 2004).

In still another postmortem study, blood taken from the heart in nonusers was found to have GHB concentrations of up to 409 mg/L, although in most cases, concentrations were less than 50 mg/L. In this particular study, the concentration in every sample taken from the femoral region was less than 50 mg/L. On the basis of this observation, it has been suggested that it is possible to distinguish exogenous exposure from postmortem production and endogenous amounts (Kintz et al., 2004). A threshold of 100 mg/L might be a safer option; however, caution still needs to be taken when there is no evidence of GHB use.

6.5.10 Forensic Case Data

GHB is commonly abused in combination with other drugs acting on the CNS (Caldicott et al., 2004). For example, in Sweden, 21 of 23 deaths attributed to GHB were found to involve other substances as well, particularly alcohol and opioids (Knudsen et al., 2010).

Concentrations of GHB that suggest exogenous exposure in blood and urine are about 5 and 10 mg/L in living persons. Couper and Logan (2000) found concentrations of 3.2 mg/L in the blood of a sexual assault victim, 33 and 34 mg/L in two driving under the influence cases, and 130 and 221 mg/L in two overdose victims who were successfully resuscitated (Couper and Logan, 2000). In a second study from the same group, GHB was identified in the blood of 13 subjects arrested for impaired driving, and the blood concentrations ranged from 26 to 155 mg/L (mean 87 mg/L, median 95 mg/L) (Couper and Logan, 2001).

In a study of 226 GHB-related deaths, 213 had suffered cardiorespiratory arrest, and 13 had fatal accidents. Seventy-eight deaths (35%) had no cointoxicants. Sixteen deaths

Table 6.5 Postmortem GHB Tissue Concentrations in a 35-Year-Old Male

Tissue	Concentration
Urine	1665 mg/L
Brain	102 mg/kg
Vitreous fluid	48 mg/L
Femoral blood	461 mg/L
Heart blood	276 mg/L
Liver	52 mg/kg

Source: Data derived from Mazarr-Proo, S. and Kerrigan, S., *J. Anal. Toxicol.,* 29(5), 398, 400, 2005.

involved *supplements*, and one involved pharmaceutical GHB. The striking finding of this study was the extreme variation in postmortem blood GHB formation, which ranged from 18 to 4400 mg/L (median, 347 mg/L), in deaths negative for other cointoxicants (Zvosec et al., 2010). These values are comparable to those seen when GHB is used as an adjunct to anesthesia; plasma concentrations of 260 mg/L are associated with deep but reversible coma (Helrich et al., 1964).

There is no simple guide to fatal concentrations because GHB may be formed postmortem (or lost if death has been delayed, i.e., the person was in a prolonged coma prior to death) (Caldicott et al., 2004; Mazarr-Proo and Kerrigan, 2005). One case report described the fatality of a 35-year-old male who was witnessed ingesting unknown amounts of GHB (Mazarr-Proo and Kerrigan, 2005). Postmortem concentrations are listed in Table 6.5.

Overdoses and deaths have been recorded after the use of 1,4-butanediol, the precursor to GHB/GBL (Lora-Tamayo et al., 2003), but such deaths would be the result of the GHB formed from 1,4-butanediol, not the butanediol. This interpretation is supported by the findings in a case report that described a young male found unresponsive, convulsing, and with constricted pupils after consuming a bottle labeled "Hurricane." The product was sold as an *organic* sleep aid that could also be used to treat panic attacks. The active ingredients were similar to ink jet cleaner, containing 1,4-butanediol. He woke 5 h later and was discharged (Yambo et al., 2004).

6.5.11 Toxicology

6.5.11.1 Preferred Methods of Measurement

Numerous methods for the detection of GHB in plasma and other specimens have been published. They are predominantly GC-based methods. Silyl derivatives using GC–MS are most common (Couper and Logan, 2000; Kankaanpaa et al., 2007; Bodson et al., 2008; De Paoli and Bell, 2008). More recently, LC–MS/MS has been used and it allows simultaneous identification and quantification of GHB, GBL, and other drugs of abuse more readily than GC–MS (Wood et al., 2004; Johansen and Windberg, 2011).

6.5.11.2 Interpreting Data

Even in the living, GHB blood concentrations depend on a large number of difficult to quantitate variables. Death investigations pose additional complications that must always be considered.

Clearly, as with any forensic matter, the context of the case is paramount. Witnesses to ingestion of GHB and other substances will be most helpful. High postmortem blood concentrations alone do not necessarily prove that the drug was taken or that it caused the death. Indeed, death from GHB is relatively uncommon, and when it does occur, it is usually in conjunction with the use of ethanol and/or other CNS depressants.

Similarly, the absence of GHB in the urine of a sexual assault victim 12 h after the event does not mean that GHB was not administered; the clearance of GHB is so rapid that by 12 h, even if concentrations had previously been very high, the actual measured concentration will have fallen below what might be considered endogenous. Conversely, if seizures and respiratory arrest occur after the witnessed ingestion of GHB and no other anatomic cause is apparent, then it is likely that GHB exposure has produced these symptoms. In short, the cause of death, or impairment, cannot be determined solely by reference to isolated tissue measurements. Knowledge of the history and autopsy findings is vital.

6.6 Pregabalin (Figure 6.5)

Pregabalin (available as Lyrica and chemically (*S*)-3-(aminomethyl)-5-methylhexanoic acid) is an anticonvulsant drug for use as an adjunct treatment of seizures as well as for treatment of neuropathic pain such as from diabetic neuropathy, postherpetic neuralgia, and central neuropathic pain. It has structural similarity to GHB and can be detected in biological specimens with similar methods, such as by LC–MS/MS (Dahl et al., 2012).

Pregabalin binds to the alpha-2-delta subunit of the voltage-dependent calcium channel in the CNS, decreasing the release of glutamate, noradrenaline, substance P, and calcitonin gene–related peptide.

A single dose of 100 mg capsule (or solution) produces a mean maximum plasma concentration of about 4 mg/L within 1 h (Bockbrader et al., 2013). At clinical doses of 150–600 mg/day, the average steady-state plasma pregabalin concentrations range from 1.5 to 6.0 mg/L. The terminal elimination half-life of pregabalin is about 6 h (Bockbrader et al., 2010). Pregabalin undergoes almost no metabolism in humans and is largely excreted unchanged in urine.

The drug has been associated with abuse, particularly in persons dependent on or withdrawing from opioid abuse (Gahr et al., 2013; Grosshans et al., 2013; Papazisis and Tzachanis, 2014). In Finland, in a review of autopsy cases, pregabalin was found in 316 cases, and drug abuse was associated with 48% of these cases including toxicity. The median blood concentration of pregabalin in those cases thought to be abusing was 15 mg/L; it was 5.8 mg/L in the other cases. Pregabalin was often associated with opioid use (Hakkinen et al., 2014).

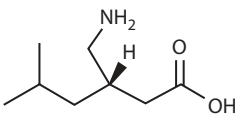


Figure 6.5 Molecular structure of pregabalin.

6.7 *Salvia divinorum*

6.7.1 Introduction

S. divinorum is an herbaceous, mint-like plant native to Mexico (Figure 6.6). It has been used in traditional medical and spiritual practices by the Shaman Aztecs in the same way as "magic" mushrooms (*Psilocybe* spp.). It is not to be confused with common *Salvia* (*officinalis* spp.).

Natives call the plant *ska pastora* or *ska Maria pastora*, meaning "leaves of the shepherdess." It is traditionally ingested as a water infusion or by eating the fresh leaves (González et al., 2006). Ethnologists report that among native peoples, it is a second-class drug, used only when psilocybin is in short supply. Interest in *Salvia* has greatly increased in recent years among recreational users. *Salvia* use has spread to Europe and North America and occupies a similar niche to other natural hallucinogenic drugs, just as ayahuasca (DMT) did a decade ago, though the actual extent of use in the United States and Europe remains completely unknown (Halpern, 2003, 2004). One recent study suggested that abuse of this drug may be much more common than had previously been supposed (Casselman et al., 2014).

S. divinorum is grown domestically and also imported from Mexico and Central and South America. The Internet is used to promote and distribute it around the world. It is readily available in *head shops*, where it is sold as seeds, plant cuttings, whole plants, fresh and dried leaves, extract-enhanced leaves of various strengths (e.g., 5×, 10×, 20×, 30×), and liquid extracts purported to contain salvinorin A.

Figure 6.6 *Salvia divinorum*—a psychotropic mint whose leaves were originally used for medicinal and religious purposes by Mazatec shamans in the Mexican State of Oaxaca. It is now used as a hallucinogen. (Retrieved from Wikipedia Commons November 18, 2014. Photo made available and published under the Creative Commons Attribution-ShareAlike 3.0 Unported license by user and author Phyzome, original upload date May 7, 2005. License can be found here and no changes were made to the image: http://creativecommons.org/licenses/by-sa/3.0/legalcode.)

The diterpenoids salvinorin A and salvinorin B are two of many different diterpenes contained in the plant, but salvinorin A appears to be active only when taken orally; it needs to be absorbed across the oral mucosa in order to avoid inactivation in the gut (Siebert, 1994; Mendelson et al., 2010).

6.7.2 Epidemiology

According to the "National Survey on Drug Use and Health Report," published by SAMHSA in February 2008, an estimated 1.8 million U.S. citizens aged 12 or older reported having used *S. divinorum* in their lifetime. Nearly one-third of those had done so in the past year. Use was more common among young adults (18–25 years old) as opposed to older adults (>26 years of age). Young adults were three times more likely than youths aged 12–17 to have used *S. divinorum* in the past year. Use is more common in males than females.

An Internet survey of 500 users found that they were predominantly male (93%) with a mean age of 23 ± 9, range 13–68 years. They reported that, on average, they had used *Salvia* 13 ± 23 times (range 1–250). Ninety-two percent of users reported having smoked the plant, 61% had used a concentrated extract, and 37% reported using dried leaf; effects were estimated to last 14 ± 13 min (Baggott et al., 2004).

The 2009 National Survey on Drug Use and Health included data from nearly 70,000 respondents aged 12 and older living in all 50 U.S. states and the District of Columbia. Due to survey design, separate analyses were conducted among adolescents and adults. Findings indicated that 1.66% of adolescents (respondents aged 12–17) and 5.08% of adults (respondents aged 18–34) reported the use of *Salvia* at some point in their lifetime. Correlates of use among adolescents included age, gender, income, peer and parent attitudes toward substance use, and other forms of drug use. Correlates of use among adults included age, gender, race, religiosity, marital status, criminal involvement, and other forms of substance use (Ford et al., 2011).

An epidemiologic study of 42,179 Canadian adolescents, aged 12–17 years, during the years from 2008 to 2009, found that overall 3.8% of adolescents reported using *Salvia* in the past year and 6.2% had used the substance in their lifetime. Surprisingly, in this study, the prevalence of 12-month *Salvia* use was higher than use of cocaine and amphetamine, but lower than use of ecstasy, cannabis, and other hallucinogens. In addition, findings suggested that low self-esteem seemed to be an important predictor of *Salvia* use (Currie, 2013).

6.7.3 Pharmacology

Salvinorin A is a highly selective full agonist at the κ-opioid receptor (KOR) (Roth et al., 2002; Butelman et al., 2004; Chavkin et al., 2004; Teksin et al., 2009) (Figure 6.7). It is, in fact, the only known nonnitrogenous κ-opioid selective agonist. It rivals synthetic lysergic acid diethylamide in potency (Teksin et al., 2009), though unlike all other hallucinogens, salvinorin A does not interact with the 5-HT_{2A} receptor, the molecular target thought to be responsible for the actions of classic hallucinogens (Sheffler and Roth, 2003).

After smoking or chewing the leaves, the onset of action is relatively rapid, on the order of 30 s for smoking and 5–10 min for buccal absorption after ingestion. When smoked, the effective dose is 200–500 μg (Siebert, 1994; Valdes, 1994).

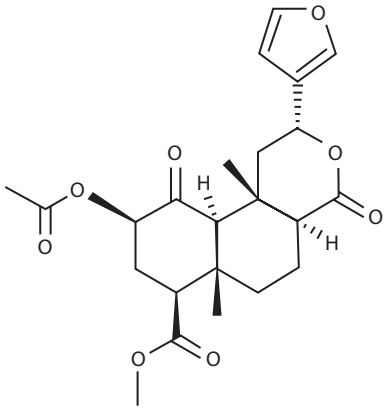


Figure 6.7 Salvinorin A molecular structure.

Salvinorin A is rapidly converted to salvinorin B, an inactive metabolite of salvinorin A (Valdes et al., 2001) that possibly shares metabolic pathways with cocaine, heroin, tetrahydrocannabinol, and MDMA. It is finally metabolized by esterases in the blood (Schmidt et al., 2005b). Pharmacokinetic studies in rhesus monkeys show that the elimination half-life of salvinorin A is rapid (57 ± 25 min) (Schmidt et al., 2005b), explaining its short duration of action. Positron emission tomography (PET) studies performed in baboons indicated extremely rapid uptake and distribution of [^{11}C] salvinorin A throughout the brain. The highest concentrations were observed in the cerebellum and visual cortex, which may explain the observed hallucinogenic and physiologic effects when *Salvia* is smoked (Hooker et al., 2008).

PET studies have shown that, compared to controls, rats given intraperitoneal-labeled salvinorin A showed the expected increase of the drug in areas rich in κ receptors (periaqueductal gray matter, stria terminalis, and hypothalamus). But activation was also seen in areas not normally thought to be rich in κ receptors. It appears that neural circuits affected by salvinorin A may not be limited to κ receptor cells, but that the drug also triggers brain circuits that mediate the effects of salvinorin on cognition, mood, and fear (Hooker et al., 2009).

6.7.4 Pharmacokinetics

Salvinorin pharmacokinetics has not been studied in humans, only primates. When salvinorin A (0.032 mg/kg) was injected as an intravenous bolus in rhesus monkeys (n = 4, 2 male, 2 female), the elimination $T_{½}$ was rapid (57 ± 25 min) for all animals. Pharmacokinetic differences (distribution $T_{½}$, elimination $T_{½}$, and AUC) were observed between males and females, suggesting potential sex differences in its pharmacologic effects. Salvinorin B, the presumed major metabolite, is observed to accumulate in vivo; however, in the studies that have been performed, it never reached the limit of detection (Prisinzano, 2005; Schmidt et al., 2005a).

In rats, the plasma profile and brain uptake of salvinorin A is also rapid with an apparent T_{max} occurring at 10–15 min after intraperitoneal administration. The elimination of salvinorin A was relatively fast with a $T_{½}$ of 75 min and clearance (Cl/F) of 26 L/h/kg. The distribution was higher than that of any known abused drug (V_d of 47 L/kg), but the brain to plasma ratio was very low (Teksin et al., 2009). Two users who smoked 75 mg of leaves of *S. divinorum* were found to have more salvinorin A in oral fluid than in urine (2.4 and 11 ng/mL) and saliva (11 and 25 ng/mL) (Pichini et al., 2005).

6.7.5 Preanalytic Considerations

Salvinorin A rapidly degrades in plasma to the deacetylated form (salvinorin B) and the lactone-ring-open salvinorin A and salvinorin B. This degradation is inhibited by sodium fluoride, an esterase inhibitor (Tsujikawa et al., 2009).

6.7.6 Analysis

Salvinorins can be measured in blood (plasma) and urine by GC–MS (Pichini et al., 2005) and by LC–MS/MS (Schmidt et al., 2005b; McDonough et al., 2008). Both liquid–liquid and solid phase extraction techniques have been employed.

6.7.7 Clinical Considerations

Persistent psychosis has been reported in association with *Salvia* use (Przekop and Lee, 2009), but this appears to be a rare complication and 8-week controlled human studies have not confirmed the original case report (Addy, 2012). In animal experiments, salvinorin A has the ability to impair learning and memory function (Braida et al., 2011).

Recently, attention has been focused on possible medical applications for salvinorin A. Besides being a potent KOR agonist, there is some evidence that it may interact with cannabinoid CB1 receptor in the brain. In animal models of inflammation, salvinorin A (0.1–10 pM) reduced lipopolysaccharide-stimulated nitrite, TNF-α, and IL-10 (but not IL-1β) production, as well as the production of iNOS (but not COX-2). Salvinorin A's protective effect on nitrite levels was reversed by the administration of naloxone. It appears that salvinorin A, acting via KOR and CB1 receptors, exerts ultrapotent actions on macrophages and also shows moderate anti-inflammatory effects in vivo (Aviello et al., 2011), suggesting that salvinorin A might be of therapeutic value. The forensic significance of this observation, if any, is unclear. Other recent studies have shown that, in animal models, salvinorin A acts as an anti-inflammatory in models of colitis (Fichna et al., 2011). As in the studies on inflammatory reaction discussed earlier, the effect is at least partially mediated by interaction with the CB1 cannabinoid receptor. No deaths from *Salvia* abuse have ever been reported.

6.8 Dextromethorphan (Table 6.6)

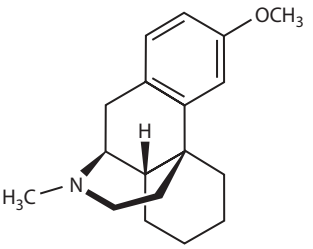
6.8.1 General Considerations

DXM is the *d* isomer of the opiate agonist levorphanol, a codeine derivative. It is an effective cough suppressant, which, along with guaifenesin, is the principal ingredient found in many over-the-counter (OTC) cough and cold remedies. It is not a controlled drug. The FDA approved DXM for OTC sale in 1958, as it was thought to have less abuse potential than codeine, even though it still possessed a potent cough suppressant effect.

The CSA specifically excluded DXM from any of the schedules in 1970 because there was no evidence of opiate-like abuse potential. However, the DEA did maintain the option to add DXM to the CSA. In fact, attempts were made to schedule it in 2010, but they were rebuffed by irate clinicians (Traynor, 2010). During the 1960s and 1970s, DXM was sold under the brand name of Romilar. In 1973, Romilar was taken off the shelves; young people had discovered it could be used as a recreational drug.

Table 6.6 Physiochemical Properties and Pharmacokinetics of Dextromethorphan

Chemical Name	(+)-3-Methoxy-17-methyl-(9α,13α,14α)-morphinan
Physiochemical properties, structure, and form	Free base. CAS 125-71-3. MW, 271.4. pK_a, 8.3.
Synonyms	Robo, skittles, triple C's, CCC rojo, Dex, DXM, poor man's PCP, tussin, vitamin D. DXM abuse is called "robotripping," Tripple Velvet, or "tussing." Users might be called "syrup heads" or "robotards." In the United States alone, the compound is contained in at least 140 different OTC products, and many prescription products in syrup, tablet, spray, and lozenge forms.
Brand names	Coricidin, Robitussin DM, Tylenol, NyQuil, and Vicks® brands are the best known. There are many others.
Pharmacokinetic parameters (oral)	T_{max}, 2 h. C_{max}, 3 ng/L DXM (30 mg) dextrorphan 11 ng/mL (60 mg). $T_{½}$, 2–7 h. Bioavailability, ~11%.
Common blood concentrations in drug users	Few data are available, but likely to exceed 0.1 mg/L in blood for DXM.
Metabolism and metabolites	Hepatic CYP26 (*O*-demethylation), conjugation, renal excretion to active dextrorphan and 3-methoxymorphinan and 3-hydroxymorphinan and conjugates.
Urinary excretion	Less than 2.5% as parent drug, mainly as glucuronides of dextrorphan and 3-hydroxymorphinan.
Postmortem artifacts	DXM is likely to elevate postmortem; at least, twofold would be expected.
Interactions	Any drug that inhibits activity of CYP3A4 will affect clearance, that is, some antiretrovirals, some selective serotonin reuptake inhibitors, and any drug with significant serotonin activity.
Key papers	Silvasti et al. (1987); Logan (2009); Monte et al. (2010); Burns and Boyer (2013); Butwicka et al. (2013).



Even though it is primarily abused in the form of an OTC cough remedy, it is with increasing frequency mixed with faux and real MDMA tablets. DXM has gradually replaced codeine as the most widely used cough suppressant in the United States. DXM production is tracked by some federal agencies, but it is still not a controlled drug. Few relevant data are gathered about use of the drug, either on the number of deaths due to DXM ingestion, or on the number of people seeking treatment for DXM abuse. Analysis of recently seized drugs suggests that dealers sometimes sell pure DXM in a powdered form (usually contained in capsules) to be abused by inhalation. The *l* form of DXM (levorphanol

or levomethorphan) also has narcotic properties (it is a potent μ opioid agonist) but is not commercially available in the United States. Differentiation between the *d* and *l* forms is a forensic issue because, even though DXM is not a controlled substance, its isomer, levomethorphan, is listed as a schedule II controlled substance.

6.8.2 Patterns of Abuse

Abuse of DXM occurs in all age groups but is most prevalent among adolescents. A 6-year retrospective study, from 1999 to 2004, of the CPCS showed a 10-fold increase in the rate of DXM abuse cases in all age groups and a 15-fold increase in the rate of abuse cases in adolescents. In 2004, CPCS reported 1382 DXM abuse cases. Extrapolating these figures to the national level suggests there may be millions of users, but without additional information, the conclusion must remain speculative.

The DAWN report for 2004 estimated there had been 12,584 emergency department (ED) visits involving pharmaceuticals containing DXM, amounting to 0.7% of all drug-related ED visits in the preceding year. The rate of ED visits resulting from nonmedical use of DXM for individuals aged 12–20 years was 7.1/100,000 population versus only 2.6 or fewer per 100,000 for other age groups. The rate of ED visits resulting from any type of use of DXM among those aged 12–20 was 10.3/100,000 population compared with 4.3 visits per 100,000 for the population overall.

Perhaps not surprisingly given the age group (18–20 years), alcohol was implicated in about a third (36%) of ED visits involving nonmedical use of DXM and in 13% of visits for those aged over 12 (Ball and Albright, 2006). The 2009 *Monitoring the Future* report indicated that the annual prevalence of nonmedical use of cough and cold remedies among students in 8th, 10th, and 12th grades was 2.6%, 5.0%, and 6.3%, respectively (National Institute on Drug Abuse, 2009).

6.8.3 Synthesis

Clandestine laboratories do not produce DXM. Except when DXM is added to MDMA or sold as faux MDMA (usually in conjunction with a piperazine—see Section 3.7.4), abusers consume various OTC products containing DXM. This may occasionally lead to the ingestion of multiple other agents such as diphenhydramine and chlorpheniramine. However, methods for extracting DXM from cold medications are readily available. One process is referred to as the *Agent Lemon* technique. A cold medication is mixed with equal amounts of ammonia and naphthalene (cigarette lighter fluid). The aqueous layer is discarded and the process repeated. Lemon juice is used to acidify the mixture, the oily layer discarded, the aqueous layer allowed to crystallize, and then the crystals are taken orally (Hendrickson and Cloutier, 2007). Abusers of DXM have also developed a simple acid–base extraction technique to *free base*, or extract, the DXM from the unwanted guaifenesin, coloring agents, sweeteners, and alcohol that are typically included in combination cold preparations.

6.8.4 Routes of Administration

There is no evidence that this drug is ever taken parenterally. Insufflation (snorting) is apparently an unpleasant experience; hence, use and abuse are almost entirely oral. There have been experiments where DXM was given intravenously to human volunteers, both

to determine pharmacokinetic behavior and to evaluate its antihyperalgesic effect. A dose of 0.5 mg/kg (40 mg to an 80 kg person) caused no ill effects and only minor side effects (Duedahl et al., 2005).

6.8.5 Pharmacology

DXM is a noncompetitive NMDA receptor antagonist with weak activity at the μ opioid receptor. The active metabolite, dextrorphan, has similar properties to DXM, but it is a weaker σ opioid receptor agonist and a stronger NMDA receptor antagonist (Miller, 2005) than the parent compound. Intoxication with dextrorphan produces symptoms that are hard to distinguish from those produced by any other NMDA blocker or dissociative anesthetic. A normal dose of DXM is 15–30 mg. It is claimed that hallucinatory effects may be experienced with doses as low as 100 mg and frank psychosis at higher levels (Roberge et al., 1999; Miller 2005). Ingestion of hundreds of milligrams has been reported, with few or no significant side effects.

6.8.6 Disposition

6.8.6.1 Metabolism

CYP2D6 enzyme catalyzes *O*-demethylation of DXM to form dextrorphan, which is then conjugated and excreted in the urine (Lutz et al., 2004). DXM is also metabolized by CYP3A4/5 to form inactive 3-methoxymorphinan or 3-hydroxymorphinan, which then undergoes glucuronidation and excretion (Takashima et al., 2005). Approximately 5%–10% of the U.S. population is deficient in the needed enzyme, and therefore these individuals metabolize DXM much more slowly than others. *Slow metabolizers* may require 17–22 h to metabolize the same amount of DXM that can be metabolized by *extensive metabolizers* in 1–4 h (Eichhold et al., 2007).

6.8.6.2 Blood Concentrations and Elimination

There is extensive first-pass metabolism of DXM (oral bioavailability about 11%) leading to low plasma concentrations on the order of 1–5 ng/mL (Barnhart and Massad, 1979; Silvasti et al., 1987; Logan, 2009; Logan et al., 2012). The concentration of dextrorphan is similar to that of the parent DXM; however, conjugated dextrorphan is over 100 times higher than the concentration of DXM (Silvasti et al., 1987).

The intravenous administration of 0.5 mg/kg DXM to volunteers (approximately 40 mg over 30 min infusion) produced a C_{max} of 80 ng/mL with an elimination half-life of 3.1 h (Duedahl et al., 2005). When oral doses of 60 mg were given to Chinese male volunteers, the plasma dextrorphan C_{max} was only 14 ng/mL (SD 8) at 2.1 h. The half-life was 3.8 h (SD 1.8) (Liu et al., 2004). Individuals classified as slow metabolizers typically have blood concentrations in the 10–20 ng/mL range after the administration of one 20 mg dose (Hou et al., 1996). Repeated use of DXM in slow metabolizers will lead to the occurrence of much higher plasma concentrations than those seen in normal metabolizers.

6.8.6.3 Excretion

After a single dose, less than 2.5% of DXM is excreted unchanged in urine in 24 h; most is excreted as conjugated 3-hydroxymorphinan and conjugated dextrorphan (Willner, 1963).

6.8.7 Toxicity

Agitation is the most common presentation of DXM intoxication, progressing eventually to ataxia, tremors, hyperreflexia, nystagmus, and hypertension. Pupillary size is variable (Roberge et al., 1999; Hendrickson and Cloutier, 2007). None of the standard antidotes appears to be effective. Serotonin syndrome has been reported in DXM abusers (Schwartz et al., 2008), and sometimes during anesthesia (Bowdle, 1998; Karunatilake and Buckley, 2006). It also occurs in geriatric patients receiving OTC medications (Kinoshita et al., 2011).

When coadministered with chlorpheniramine (a sedating antihistamine with serotonin reuptake inhibitory properties), DXM can cause serotonin toxicity (Monte et al., 2010). The danger is that DXM can precipitate a potentially fatal outcome when combined with another serotoninergic drug such as a monoamine oxidase inhibitor, a selective serotonin reuptake inhibitor or serotonin–norepinephrine reuptake inhibitor, tramadol, or even MDMA (Chyka et al., 2007). However, deaths have also occurred after pure DXM overdose. Because deaths involving DXM are rare and produce only nonspecific findings, such as pulmonary and cerebral edema, it is hard to say what sort of mechanism would be involved. If full-blown serotonin syndrome had developed, hyperthermia and rhabdomyolysis should be evident, but having said that, there is no easy way to distinguish the symptoms and signs of DMX poisoning from those of neuroleptic malignant syndrome (NMS).

In the absence of hyperthermia, the diagnosis of DXM poisoning is hard to support. One way might be to closely examine the Purkinje cells. The selective loss of Purkinje cells has previously been described both in NMS and heat stroke. In case of either NMS or heat stroke, when the survival time is short, partial loss of cerebellar granule cells has been observed, and with longer survival times, extensive loss of granule cells should be evident. Cells in other areas of the brain known to be sensitive to hypoxic injury are not affected (Slettedal et al., 2011).

6.8.8 Postmortem Toxicology

Concentration ranges in adult decedents where the presence of DXM is only an incidental finding (e.g., a murder victim who also happened to be taking a DXM-containing OTC remedy) have never been systematically studied, and there are only a handful of case reports in the medical literature.

In one series of five decedents, postmortem blood concentrations ranged from 0.9 to 3.2 mg/L (Logan et al., 2009) but since the metabolizer status of the individuals was not known for any of the decedents, and since some of the reported measurements were made in heart blood, while others were made in iliac blood or collected from unstated sites, the findings cannot be interpreted. Even if all the measurements were made in blood collected from the same site, the metabolizer status of the decedents was not known in any of the reported cases, so quantitative DXM measurement would not allow one to conclude if misuse, or poor metabolizer status, was responsible for the results. Commercial DNA sequencing for CYP2D6 is now available, and in some situations, forensic considerations would justify the added expense of profiling.

DXM has occasionally been used to commit suicide. One report described the finding of two empty bottles of DXM next to a body, together with a suicide note. In another DXM

overdose, the diagnosis of suicide was based on high concentrations in the stomach contents. Femoral blood concentrations of DXM and dextrorphan were 9.2 and 2.9 mg/L in the first case and 3.3 mg/L and 1.5 mg/L in the second, respectively (Rammer et al., 1988). In Korea, DXM has been abused with zipeprol, a related antitussive, and that is known to have caused several deaths (Yoo et al., 1996; Chung et al., 1998).

Pediatric cases are even more problematic. When no other cause is immediately apparent, sudden death in infants is sometimes attributed to the use of DXM present in OTC medications (Rimsza et al., 2004; Marinetti et al., 2005b; Wingert et al., 2007; Ryan et al., 2008). Whether or not such a diagnosis would, in the absence of DNA profiling, be defensible in court, is difficult to say. Rarely, if ever, is DXM the sole compound detected in these cases, which is why genotyping (both hepatic and cardiac conduction channels) in pediatric decedents is warranted. Without knowing the phenotype of the decedent, it is impossible to rule out alternative causes. In such situations, a diagnosis "undetermined" might be more appropriate. It would certainly be easier to defend before a jury.

DXM concentrations in infants appear to be substantially lower than in adults (30–550 ng/mL) (Marinetti et al., 2005b) but the significance of these measurements is, again, impossible to ascertain. It is likely that the drug undergoes redistribution since it is relatively lipophilic. This means that great care needs to be exercised in any interpretation of postmortem toxicology data from pediatric patients.

6.8.8.1 Preanalytic Considerations

The drug and its key metabolite appear to be relatively stable (Vengurlekar et al., 2002). The main issue in any analysis is whether dextrorphan needs to be measured in addition to DXM, and if so, whether the conjugated form needs to be hydrolyzed back to dextrorphan or measured directly by LC–MS/MS.

6.8.8.2 Preferred Analytic Methods

DXM is not difficult to detect in biological specimens. Immunoassays can be used to screen for the drug (Freche et al., 1990; Rodrigues et al., 2008), although in forensic laboratories, chromatographic techniques are often preferred. A number of GC or GC–MS methods have been published that rely on a straightforward extraction followed by underivatized analyses (Rodrigues et al., 2008; Spanakis et al., 2009). LC–MS/MS methods are also available, and they enable detection of relevant metabolites, including dextrorphan, 3-methoxymorphinan, and 3-hydroxymorphinan. The use of chiral analysis to separate (and identify) DXM from levorphanol (the *l*-isomer of DXM) has been described (Eichhold et al., 2007; Kikura-Hanajiri et al., 2011).

6.8.8.3 Interpretation

A number of issues complicate the interpretation of DXM tissue concentrations and the meaning of toxicology data in any particular forensic case, and these have largely been discussed earlier. Slow metabolizers will have higher concentrations of the drug and also an increased risk of toxicity. While misuse can lead to hospitalization, deaths generally occur when DXM is used in conjunction with similar substances, particularly those with serotonin activity.

6.9 Propofol (Table 6.7)

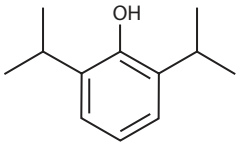
Propofol was introduced in the 1980s, but its addiction potential and illicit use (mostly by members of the medical community) have only come to public attention in the last few years. Similarly, the dangers of long-term use in the intensive care setting are only now becoming clear. Homicides have been committed by propofol administration, and, perhaps most importantly, previously unrecognized medical side effects are now being reported. Addiction is one of the previously unreported side effects, and it may rarely prove fatal. Death by medical misadventure (over sedation) comes under the medical examiner's jurisdiction, and death investigators must be aware that the presence of propofol may not just be an incidental finding.

6.9.1 Introduction

Propofol follows roughly the same pharmacokinetic pattern as all intravenous anesthetics. Because the molecule is small and hydrophobic, those parts of the body with the greatest blood supply, most notably the brain and spinal cord, preferentially take it up. It is effective on the first pass through the circulation. Propofol's mode of action differs from some of the other intravenous anesthetics in that it is an agonist at GABA-A receptors, which means that the major inhibitory pathways in the CNS become disrupted (Gatch and Forster, 2011). Interference with the GABA pathway leads to sedation, and

Table 6.7 Physiochemical Properties and Pharmacokinetics of Propofol

Chemical Name	2,6-Bis(1–methylethyl)phenol
Physiochemical properties, structure, and form	CAS 2078-54-8. MW, 178.3. V_d, 2–12. pK_a, 11.0. Protein binding, 95%–99%.
Brand and other names	Ansiven, Diprivan, Disoprivan, Rapinovet, Disoprofol; fospropofol is a prodrug.
Blood concentrations	3–10 mg/L during induction anesthesia. 2–4 mg/L maintenance anesthesia. Emergence from anesthesia about 1 mg/L.
Blood half-life	Propofol pharmacokinetics is best described by a three-compartment model: the initial half-life is 8 min, the redistribution half-life is between 30 and 70 min, and the terminal elimination half-life is up to 23 h.
Metabolism	1-Glucuronide, and 1 and 4 glucuronide conjugates of 1,4-quinol,1-sulfate conjugate.
Urinary excretion	Mostly excreted as conjugates, almost no free drug excreted; 2% in feces.
Postmortem artifacts	Little studied but likely to show significant increases in blood due to high tissue concentrations.
Interactions	Likely with CNS depressant drugs.
Key papers	Servin et al. (1990); Abad-Santos et al. (2003); Marik (2004); Kansaku et al. (2011); Han et al. (2013).



propofol given in sufficiently large doses causes sleep. Propofol binds to benzodiazepine receptors as well as binding to D2 dopamine receptors and also acts as an NMDA antagonist (Schulte et al., 2000), though whether these actions have anything to do with its ability to cause sedation is not known.

6.9.2 Incidence and Epidemiology

Nothing has ever been published about the prevalence or epidemiology of propofol abuse or propofol-related deaths. According to a review paper published in 2009, there had been 38 reported deaths, most of which were the result of accidental overdoses (Kirby et al., 2009). Prevalence of abuse is highest among medical professional and allied staff (e.g., nurses and radiologists) as they have the most ready access.

6.9.3 Illicit Production

No clandestine propofol laboratory has ever been seized. Virtually all the reported deaths have been the result of diverted drug use (or, as in the famous case of Michael Jackson, propofol that had been inappropriately administered). Propofol is not a scheduled drug, though the DEA has proposed a rule change (21 CFR Part 1308) that would place propofol in schedule 4 of the CSA.

6.9.4 Metabolism and Excretion

Propofol is conjugated substantially to the glucuronide and the 1-sulfate and 1- and 4-glucuronide conjugates of the 1,4-quinol. Almost no propofol is excreted into urine unchanged (0.3%) (Simons et al., 1988). Hydrolysis of urine (acid) liberates the propofol conjugated to glucuronic acid (Drummer, 1992).

Like other lipid-soluble intravenous anesthetics, the drug is removed only slowly from deep tissue sites. In the first 5 days after administration, only 60% of the dose is excreted into urine and very little is excreted in feces.

6.9.5 Clinical Syndromes

6.9.5.1 Cardiovascular

In hypovolemic patients and those with limited cardiac reserve, even small induction doses of propofol (0.75–1.5 mg/kg IV) can produce profound hypotension (Short and Bufalari, 1999). Generally, this is not an issue in routine surgery, especially if propofol is used in conjunction with other agents, such as ketamine, which causes an initial catecholamine release (Takki et al., 1972). Hypotension can also occur in patients with established myocardial ischemia (Maekawa et al., 2005). Prolonged exposure to high propofol concentrations may result in heart failure, particularly in children (Bray, 2000).

It has recently been discovered that propofol prevents myocardial injury secondary to free radical production and may have a role as a cardioprotective agent, especially during bypass surgery. Substantial evidence suggests that it may prevent the occurrence of arrhythmias secondary to ischemia (which lead to free radical formation) (Sayin et al., 2002). High doses of propofol, or prolonged treatment over several days,

can cause a Brugada-like pattern on the EKG (incomplete right bundle-branch block and ST elevation in the anterior precordial leads). Whether this occurs as a result of a genetic defect (as do most cases of Brugada syndrome), or some action of propofol itself, is not known. The latter seems more likely, as the Brugada-like changes in patients treated with propofol are associated only with long-term, high-dose treatment and are just one of a constellation of symptoms referred to collectively as the *propofol infusion syndrome* (PRIS).

The PRIS consists of unexplained lactic acidosis, hyperlipidemia, rhabdomyolysis, pancreatitis, and cardiovascular collapse (Vernooy et al., 2006; Junttila et al., 2008; Robinson et al., 2008; Riera et al., 2010), along with the aforementioned Brugada changes on the EKG. If the latter occurs, death almost always supervenes. PRIS is an uncommon but not rare disorder. Twenty-four cases had been described in children and another 14 in adults through 2003 (Okamoto et al., 2003; Vasile et al., 2003). Fourteen additional pediatric cases and five additional adult cases were reported between 2003 and 2009 (Fudickar and Bein, 2009). Since then, new case reports have appeared fairly regularly (Annecke et al., 2012; Mijzen et al., 2012).

The pathology of PRIS in humans has never been studied, but in animal experiments, a multiplicity of lesions has been described after long-term, high-dose propofol infusion. These include apparently unrelated lesions such as myocarditis, pulmonary edema, interstitial pneumonia, steatosis, cholangitis, and focal liver necrosis (Ypsilantis et al., 2006). Although there have been no autopsy studies, a recent case report describes the multiple physiologic changes observed in a 37-year-old woman with a ruptured cerebral aneurysm. The study was somewhat unique in that the patient had had a cerebral microdialysis catheter inserted, making possible more or less continuous measurement of numerous physiologic and biochemical variables. It was observed that a temporal association existed between propofol exposure and the cerebral lactate-to-pyruvate ratio (LPR). The LPR increased linearly after propofol was restarted following an off period, and the LPR decreased linearly after propofol was discontinued. Serum lactate correlated with clinical worsening after the onset of PRIS, whereas cerebral LPR correlated with propofol exposure (Pisapia et al., 2011).

On the other hand, no study has ever found evidence of any biochemical markers that predict or accompany propofol syndrome (Ozturk et al., 2013), so the etiology of this syndrome remains in dispute. Two possibilities have been proposed: (1) propofol may interfere with mitochondrial function by impairing fatty acid oxidation and interrupting oxidative phosphorylation via a nitric oxide–mediated mechanism (Fudickar et al., 2006), or (2) symptoms may be the result of hyperlipidemia-induced pancreatitis. Propofol contains 0.1 g of fat per milliliter. Concentrations of that magnitude can result in hyperlipidemia when infused for >72 h. Hyperlipidemia, in turn, can cause an acute episode of pancreatitis.

6.9.5.2 Psychiatric Abnormalities

Mild euphoria, sometimes associated with hallucinations, and sexual disinhibition may occur. This type of behavior is modulated by the dopaminergic system in the nucleus accumbens and by the glutamatergic system in the neocortex and limbic region (Balasubramaniam and Park, 2003; Grasshoff et al., 2005; Marchaisseau et al., 2008). Whether any distinctive neuropathologic lesion (besides those associated with anoxia) occurs is not known.

6.9.5.3 Addiction

Addiction is a consequence of abuse, mainly by medical staff (Roussin et al., 2006; Bonnet et al., 2008; Wilson et al., 2010), or inappropriate medical treatment (Brazzalotto, 1989). In a prospective study, it was observed that 40% of 542 patients described feelings of pleasure after awaking from propofol anesthesia, probably the result of rapid activation of GABA-A within mesocorticolimbic pathways. Propofol has increasingly become a drug of abuse largely because it is easily accessible (which will change once it is scheduled), because its onset of action is rapid following injection, and because the effects of the drug are very brief without significant long-term side effects (Kirby et al., 2009). As with almost all of the other abused drugs, medical complications seem to be confined to long-term abusers.

6.9.6 Blood and Tissue Concentrations

Typical anesthetic doses range from 1 to 3 mg/kg. A 2 mg/kg dose produces a plasma concentration of about 2 mg/L at 2 min post bolus dose (Abad-Santos et al., 2003). In general, the higher the plasma level achieved, the longer the duration of anesthesia. Induction anesthesia tends to occur at blood concentrations of about 6–10 mg/L, whereas maintenance occurs at about 2–4 mg/L. Patients awaken when blood concentration falls to approximately 1 mg/L (Kanto and Gepts, 1989). Individuals with the CP2B6 G516T polymorphism will have higher plasma concentrations than normal individuals given equal amounts of the drug (Kansaku et al., 2011).

Propofol is detectable in most alternative matrices. In addition to blood, propofol can be detected in hair (Cirimele et al., 2002), breath (Grossherr et al., 2009), and breast milk (Nitsun et al., 2006). However, very little is detected in urine in the free, nonconjugated form (Simons et al., 1988; Drummer 1992; Favetta et al., 2002).

6.9.7 Toxicological Testing

6.9.7.1 Preanalytic Considerations

Substantial amounts of propofol are lost when it is stored in infusion bags (Barann et al., 2000). Substantial losses have also been observed over 24 days when plasma was anticoagulated with citrate or EDTA and stored at 4°C. However, degradation does not occur when plasma is obtained from heparinized blood (Dawidowicz et al., 2000). Propofol stability appears to be highest in frozen plasma.

6.9.7.2 Preferred Analytic Methods

Propofol is readily extracted with conventional solvents and analyzed by high-performance liquid chromatography (Drummer, 1992; Dawidowicz et al., 2001) and GC–MS (Favetta et al., 2000). For the measurement of urine concentrations, prior acid hydrolysis is required to liberate propofol from the 1-glucuronide (Drummer, 1992). Higher sensitivity can be obtained by forming the methylpyridinium derivatives and using LC–MS/MS (Thieme et al., 2009).

6.9.7.3 Postmortem Blood Concentrations

Blood levels of propofol in the majority of propofol-related deaths fall within, or below, the concentrations seen with anesthetic use. This suggests that, barring the identification of significant pathologic findings, death in most of these cases may be the result of airway

obstruction. Patients who have died following a period of coma will have blood concentrations much lower than those that originally induced the coma and delayed death (Drummer, 1992). Propofol has a substantial and somewhat unpredictable volume of distribution, dependent on age and state of hydration, although measured blood concentrations are not noticeably dependent when there is underlying renal or liver disease. As a consequence, blood concentrations measured at autopsy will almost certainly be higher than plasma concentrations during life. Consequently, there is no concentration postmortem that will indicate the likely contribution to death without a thorough review of the circumstances and associated medical and pathologic information (Kranioti et al., 2007; Kirby et al., 2009).

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Anabolic–Androgenic Steroids 7

7.1 Prevalence and Epidemiology

Anabolic steroid use is said to be increasing, but neither the 2014 edition of the United Nations *World Drug Report,* the U.S. Government's National Drug Threat Assessment for 2010, nor the 2010 report of the European Monitoring Centre for Drugs and Drug Addiction even mentioned the anabolic steroids. A publication sponsored by the U.S. government, the *Monitoring the Future Survey* (MTF) (Johnston et al., 2011), does discuss steroid abuse, and it found, contrary to media claims, that the rate of anabolic–androgenic steroid (AAS) abuse is now very nearly equal to the rate reported three decades ago.

The most recent MTF survey (University of Michigan, 2013) showed that steroid use fell from 1.9% overall in 1989 to 1.1% in 1992—the low point since the survey began. From 1992 to 1999, a gradual increase occurred with rates reaching 1.7% in 2000. In 2001, AAS use rose significantly among 12th graders reaching 2.4% (possibly reflecting a cohort effect with the younger, heavier-using cohorts getting older) (Johnston et al., 2011).

Males are the predominant users of anabolic steroids so that MTF data based on all respondents can mask the higher rates and larger fluctuations that occur among males. For example, in 2011, annual prevalence rates were 1.0%, 1.4%, and 1.8% for boys in grades 8, 10, and 12, compared with 0.4%, 0.4%, and 0.5% for girls. Between 1991 and 1998, the overall annual prevalence rate was fairly stable among 8th and 10th graders, ranging between 0.9% and 1.2%. In 1999, however, use jumped from 1.2% to 1.7% in both 8th and 10th grades. Almost all of that increase occurred among boys, from 1.6% in 1998 to 2.5% in 1999 in 8th grade and from 1.9% to 2.8% in 10th grade. If the figures are accurate, rates among boys increased by about 50% in a single year (Figure 7.1).

With data going back to 1989, it is apparent that steroid use first fell from 1.9% overall in 1989 to 1.1% in 1992—the low point. From 1992 to 1999, there was a more gradual increase in use, reaching 1.7% in 2000. In 2001, use rose significantly among 12th graders to 2.4%. Use decreased significantly in 2005 to 1.5%, where it remained in 2010, before falling slightly more to 1.2% in 2011. Use is now down from recent peak levels by 56%, 59%, and 52% among 8th, 10th, and 12th graders, respectively. The use of androstenedione—a steroid precursor—has also declined sharply since 2001 (NIDA, 2012).

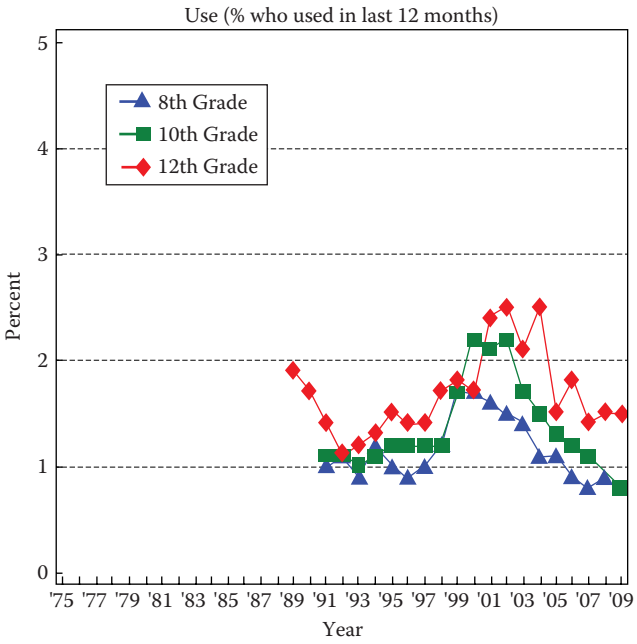


Figure 7.1 Percentage of U.S. students who had abused anabolic–androgenic steroids in the last year. (From: Substance Abuse and Mental Health Services Agency [SAMHSA].)

7.2 History

Anabolic steroids are synthetic compounds structurally related to testosterone, the male sex hormone. Testosterone has two different effects on the body: it promotes the development of secondary male sexual characteristics (androgenic effects), and it accelerates muscle growth (anabolic effects). The result depends on which androgenic receptors are activated (Saudan et al., 2006). The hormonal basis for male sexual characteristics was discovered in 1849, when it was observed that the male characteristics of roosters disappeared after they were castrated. These characteristics reappeared when the testes were implanted into the rooster's abdomen. It was correctly deduced that the testes were secreting something into the blood that controlled the development of male sexual characteristics.

In 1930, another scientist working at the same medical school in Göttingen where the original studies with roosters had been performed succeeded in isolating 15 mg of an anabolic compound from 25,000 L of urine collected from police department urinals. The compound was named androsterone for three reasons: it was virilizing (thus, *andro*, the Greek word for male), the nucleus of the molecule was like that of cholesterol (*ster*), and it contained a ketone group (*one*). A few years later, testosterone was crystallized from bull testes, and its chemical structure was characterized (Kochakian, 1990).

When testosterone was finally synthesized in the 1940s, it became apparent that the positive effects of testosterone on nitrogen balance and muscle growth could partially be separated from its androgenic effects. Substitutions at position 17 of the testosterone molecule proved vital in the process of separating the androgenic from the anabolic effects of testosterone. The androgenic agents, it transpired, were absorbed when taken orally, but with only a fraction of the androgenic effects exerted by testosterone. Further manipulations of

Figure 7.2 Anabolic steroids. When these agents first became available, they were often used for indications that are no longer considered acceptable today. This advertisement is from a 1961 issue of *JAMA*.

the testosterone molecule at position 17 have led to the production of a series of *anabolic* steroids that are active when taken orally (Figure 7.2).

Ethical considerations prevent physicians from participating in *megadose* steroid studies, which is why the effects of these drugs are not better understood. However, that was not always the case. During the Cold War, steroid abuse by East German athletes was common. No one had any idea how widespread the practice actually was until Werner Franke, a cell biologist at the German Cancer Research Center in Heidelberg, obtained copies of Stasi (state secret police) files and brought them to the West. His wife, a former Olympic competitor, assisted in this venture (Franke and Berendonk, 1997).

According to the documentation supplied by Franke and Berendonk, the extent of the problem was far greater than anyone had ever believed. Stasi records show that hundreds of doctors, scientists, and coaches were involved, all participating in a classified, state-sponsored program known as State Plan 14–25. Athletes were treated with steroids from 1974 to 1989, often without their knowledge, and the responses to treatment were measured. Much of the testing was carried out in East Germany's State Anti-Doping Laboratories, one of only a handful of such laboratories approved by the International Olympic Committee (IOC) to test

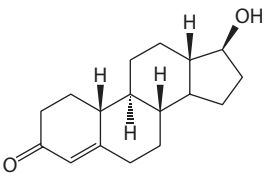
athletes for illegal drug use! In fact, the laboratory functioned as a *doping* laboratory. Excretion times were plotted for each athlete so that their coaches would know how long before a competition they would have to stop administering steroids. Since the original discovery became public, several successful suits have been brought against the state and the trainers.

7.3 Pharmacology (Table 7.1)

The effect produced by any specific steroid molecule depends on which position of the testosterone skeleton (Figure 7.3) has been manipulated and the type of receptor with which it interacts. Alkylation of the 17α-position allows for oral absorption. Substitutions at the 17β-position make the molecule more lipid soluble, allowing slow release into the general circulation. Anabolic activity is increased when substitutions are made on the A ring of the

Table 7.1 Physiochemical Properties and Pharmacokinetics of Testosterone

Chemical Name	17-beta-Hydroxyandrost-4-en-3-one
Physiochemical properties, structure, and form	Testosterone enanthate IM, patch, skin gel, and oral tablets; CAS 58–22–0 (free form); MW 288.43; and V_d 10–30 L/kg.
Synonyms	Testosteroid; testosterone; *trans*-testosterone.
Brand names	Androderm; Andropatch; Histerone; Malogen Aqueous (FM);Sterile Testosterone Suspension USP 23; Tesamone; Testandro; Testoderm; Testopel; Testosterone Implants; Testosterone Implants BP 1993; Testotop; and Homosten (FM).
Pharmacokinetics (cocaine)	C_{max} testosterone enanthate 200 mg IM.
Normal blood concentrations	Males, up to about 10 μg/L (diurnal variation and age-related changes). Females, up to about 1 μg/L.
Blood terminal elimination half-life	IV, 10–100 min; IM, 2 h; patch, 2 h; gel, 2 h.
Metabolism	Hepatic, metabolized to estradiol and DHT.
Urinary excretion	Little unchanged testosterone, mainly androsterone, epiandrosterone, and smaller amounts of etiocholanolone, estradiol, DHT, and others.
Postmortem artifacts	Unknown.
Interactions	Multiple interactions are theoretically possible: injected testosterone (1) causes increased clearance of propranolol, (2) causes decreased blood sugar with increased glucose requirements, and (3) may cause artifactual depression of total T_4.
Published major papers and reviews	Schurmeyer and Nieschlag (1984); White et al. (1998); Mazer and Shifren (2003); Araujo et al. (2011); and Carson and Rosano (2012)



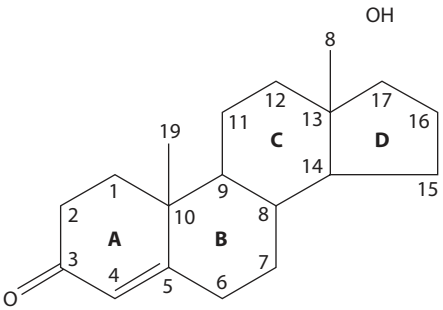


Figure 7.3 Testosterone. Testosterone is rapidly degraded by the liver when it is given orally. Modifications at position 17, such as esterification of the β-hydroxyl group, prevent hepatic breakdown and allow the drug to be given orally.

molecule, whereas removal of the C-19 methyl group all but eliminates androgenic activity. The enzyme 5α-reductase catalyzes the conversion from testosterone to dihydrotestosterone (DHT). Large quantities of this enzyme are found in the testicles, skin, prostate, intestine, and brain (Mottram and George, 2000). DHT causes only anabolic effects and is a more potent anabolic agent than testosterone, which exerts its anabolic effects only on muscle, bones, heart, and kidney. The reason for this specificity is that the other organs do not possess 5α-reductase. Anabolic steroids also displace glucocorticoids from their receptors. The net effect is the prevention of further muscle breakdown (Kuhn, 2002). Not surprisingly, testosterone was once used as treatment for wasting disorders.

Alterations of the testosterone molecule cause changes in androgenic receptor expression and metabolism. Alterations occur either as a consequence of changes in intracellular metabolism or because of changes in the structure of the androgen receptors (ARs) themselves. The final result depends on the interplay of these two factors and the tissue in which they occur (Bahrke and Yesalis, 2004). Once an androgenic receptor is activated, ligand-mediated transcription factors are produced, and depending on which molecule has initiated the process, a single copy member of the nuclear receptor superfamily will act upon the genome. The process is mediated by a complex series of proteins that target gene expression, either enhancing (coactivator) or restraining (corepressor) transcription (Liu et al., 2003). In eugonadal men, at least 95% of testosterone is produced in the testes, with a small amount being derived from peripheral metabolism of weaker androgens, that is, androstenedione (Luke and Coffey, 1994); the remainder is produced by the adrenals.

After testosterone is produced within the cytoplasm of testicular Leydig cells, it easily crosses the nuclear membrane and binds to a protein receptor in the nucleus, either as testosterone or DHT. Once the testosterone–protein complex is formed, it exerts additional effects on gene expression, ultimately resulting in the formation of the readily recognized male characteristics.

One-half of the testosterone circulating in the bloodstream is tightly bound to a liver protein called sex hormone–binding globulin (SHBG). The other half is loosely bound to albumin, leaving only 2% unbound or *free*. It had been thought that all of testosterone's effects were exerted by the 2% that was unbound, but now it is widely held that the fraction bound to albumin is available for tissue uptake and can exert testosterone's traditional effects. For this reason, the nomenclature has been changed; both *free* testosterone and testosterone complexed with albumin are now referred to as *bioavailable testosterone*.

Eugonadal men produce 3–7 mg of testosterone per day (Wang et al., 2004), resulting in a serum concentration of 3–10 ng/mg. Healthy women produce 0.1–0.4 mg/day (Burger, 2002), resulting in serum concentrations of less than 1 ng/mL. The main site of androgen metabolism is in the liver, which is particularly rich in steroid catabolic enzymes (Toscano, 1986). High hepatic concentrations secondary to AAS abuse may have some relationship to the fact that liver disease is seen so often in abusers.

Various andrology societies have set different values for normal plasma concentrations of total testosterone. Internationally, any value less than 3.4 ng/mL (12 nmol/mL) is considered evidence of *mild deficiency*. Total plasma concentrations below 2 ng/mL (7 nmol/L) reflect severe hypogonadism (Buvat, 2010).

7.4 Testosterone and Aging

Longitudinal and cross-sectional studies show that testosterone levels decrease as men age. The decrease is often greater than it appears because, at the same time that testosterone concentrations are decreasing, levels of SHBG are increasing. The net effect is that even less free testosterone is available. The rate of decline appears to be quite variable and difficult to estimate because while there are generally accepted ranges that define hypogonadism, there is no accepted definition of what constitutes a *normal* testosterone concentration. For the purposes of replacement therapy, which is becoming a frequent *antiaging* practice, most investigators define a *normal* testosterone in an aging man as being equivalent to whatever is considered the lower level of normal for a healthy 40-year-old man (Tenover, 1998; Bhasin et al., 2007). This assumption may, or may not, be correct.

Many health and lifestyle changes are associated with an accelerated rate of testosterone decline (Table 7.2). A 4–5 kg/m^2 increase in body mass index, or loss of spouse, is associated with declines in total serum testosterone comparable to those associated with approximately 10 years of aging. The same declines are seen in free testosterone. In fact, comorbidities and lifestyle practices may be as strongly associated with declining testosterone levels as age itself, at least in the short term (Travison et al., 2007).

Results of longer-term human clinical trials and epidemiologic studies are only now being published. Their results generally seem to offer justification for testosterone supplementation. There is convincing evidence that endogenous testosterone concentrations have

Table 7.2 Signs of Andropause

Loss of libido
Erectile dysfunction
Depression
Lethargy
Inability to concentrate
Sleep disorders
Irritability
Depression
Osteoporosis
Loss of muscle mass with fatty replacement

a major impact on known risk factors for the development and severity of coronary artery disease; anabolic steroids exert direct effects on blood vessel walls, at least in men, and prevent apoptosis in experimental models (Sanchez-Mas et al., 2010). In epidemiologic studies, they also improve the lipid profile (Webb and Collins, 2010). Recently, it was shown that while testosterone concentrations do not correlate with the degree of atherosclerosis present, they do correlate very well with concentrations of high-sensitivity C-reactive protein, and concentrations of the latter correlate very well with the severity of coronary atheroma (Wickramatilake et al., 2015).

The effects of AAS are not limited to the coronary arteries but seem to apply to other blood vessels as well. For example, lower concentrations of free testosterone and higher luteinizing hormone (LH) levels are independently associated with abdominal aortic aneurysm in older men, strongly suggesting that impaired gonadal function may be involved in both arterial dilation and occlusive vascular disease, especially in older men and very likely in AAS abusers as well (Yeap, 2010; Makrygiannis et al., 2014). In prospective epidemiologic studies, lower plasma testosterone concentrations are inversely related to the probability of developing carotid plaque (Dorr et al., 2009).

Testosterone clearly has an effect on both action potential duration and QT interval duration (Yang et al., 2010). The presence of testosterone very likely accounts for the fact that premenopausal women have longer QT intervals than normogonadal men (Yang and Clancy, 2010). This difference may account for the fact that torsades de pointes (especially when its occurrence is related to the use of hERG-blocking drugs) is so much more common in women than men (Zhang et al., 2011). Prospective studies have also shown that the lower the testosterone concentration, the longer the QT interval (and therefore, the higher the probability of developing ventricular tachycardia) (Zhang et al., 2011). There is an ongoing debate among those who test athletes whether a short QT interval may be a marker for testosterone abuse (Djordjevic et al., 2012).

7.5 Patterns of Steroid Abuse

Athletes use steroids because they believe that they will improve their performance. Specifically, it has been claimed that steroid use (1) increases lean body mass, (2) increases strength, (3) increases aggressiveness, and (4) leads to a shorter recovery time between workouts. Evidence supports all of these claims, particularly the increase in strength (Plymate and Friedl, 1992). However, steroid abusers routinely take doses of anabolic agents well in excess of those that any physician could ethically administer, and this may well explain why, in controlled clinical trials, modest doses of testosterone and nandrolone have not produced the dramatic changes seen in body builders (Isidori et al., 2005).

The various approaches to taking steroids are referred to as stacking, cycling, and pyramiding. Stacking is the practice of using several different steroid preparations at the same time, the hope being that maximal anabolic effects will be achieved, while at the same time, the androgenic effects are minimized. Cycling describes a pattern of usage where combinations of drugs are taken in alternating 6–12-week cycles; the rationale here is that the practice will prevent tolerance from occurring. “Pyramiders” start with low doses of the drug and gradually increase the amount of drug taken over several weeks, tapering off entirely before a competition. Not uncommonly, persistent steroid abusers combine all three approaches.

7.6 Testosterone and Nandrolone

Testosterone was at one time the drug of choice for body builders even though it was used mainly parentally (prior to the introduction of testosterone creams and gels). Injection was not a deterrent to persistent body builders, but testosterone abuse is easily detected, and many of the new *designer* steroids produce much more impressive results. Ease of oral administration explains why, for many years, nandrolone (19-nortestosterone, or 19NT; Figure 7.4) has remained the AAS of choice, at least among body builders and cattlemen attempting to produce more beef. Medically, nandrolone has been used with mixed results to treat a variety of hematologic disorders and also in wound care and as a treatment for cachexia. However, nandrolone detection is quite simple and, in any event nandrolone, like testosterone, is less potent than the new generation of designer steroids called selective androgen receptor molecules or selective androgen receptor modulators (SARMs) (Figure 7.5).

Exogenous nandrolone is commonly sold as its decanoate ester (Deca-Durabolin) and less commonly as a phenylpropionate ester (Durabolin). In the early 1990s, competitive athletes stopped using nandrolone because it was so easy to detect and because it remained in the body for so long. Current IOC regulations set a urine concentration limit of 2 ng/mL; however, the urine concentration in nonsteroid abusers never exceeds 0.6 ng/mL. A loophole in the regulations of most international sporting organizations allowed room for argument when urinary concentrations exceeded these limits. Nandrolone precursors, 19-norandrostenedione and 19-norandrostenediol, are openly sold in many health food stores and are perfectly legal. Once in the body, these compounds are, of course, rapidly converted into nandrolone (Lee et al., 1991). Under the World Anti-Doping Agency (WADA)'s doctrine of absolute liability, any athlete testing positive would still be disbarred.

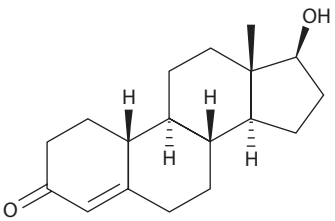


Figure 7.4 Nandrolone (19-nortestosterone) is an anabolic steroid that may be present naturally in the human body, but only in very small quantities. It is most commonly sold commercially as its decanoate ester (Deca-Durabolin) and less commonly as a phenylpropionate ester (Durabolin).

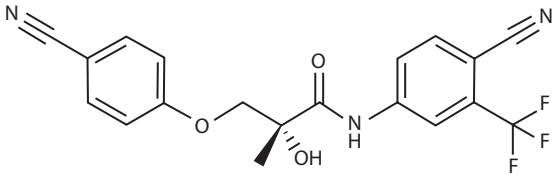


Figure 7.5 Molecular structure of a selective androgen receptor molecule, which is said to be as effective as testosterone but free of testosterone's undesirable side effects.

Other defenses for high urinary nandrolone can also be offered. While seemingly farfetched, innocent ingestion from dietary sources is possible. Meat from noncastrated male pigs normally contains 19-norandrosterone, and consumption of boar meat can cause positive urine tests in humans (De Wasch et al., 2001). Similar findings have been demonstrated after consumption of lamb (De Wasch et al., 2001). Trace contamination of the androstenedione sold in health food stores seems to be the most likely explanation for many of the positive tests. Contamination of androstenedione with 19-norandrostenedione is sufficient to cause positive urine test results for 19-norandrosterone, the standard marker for nandrolone abuse.

7.7 Other Abused Steroids

Tetrahydrogestrinone (popularly called *THG* or *The Clear*; Figure 7.6) binds to both the androgen and progesterone receptors (PRs), but not estrogen receptors (ERs) (Death et al., 2004). It is considered one of the *designer anabolics*, nonsteroidal compounds such as SARMs or clenbuterol. It is also closely related to other banned anabolic steroids such as gestrinone and trenbolone. On a weight for weight basis, THG is thought to be the most potent anabolic ever synthesized. This no doubt explains why so many Olympic competitors and professional athletes could not resist using it, forcing many to retire when their use of illicit supplements became known. The other reason for using THG is, of course, that it was difficult to detect—the molecule itself disintegrated when it was placed into the injection port of the gas chromatograph. It is now known that THC induces CYP1A1 and, more importantly, that this drug has dioxin-like effect (Moon et al., 2012).

When the U.S. Congress passed the Anabolic Steroids Control Act of 1990, it placed 27 anabolic steroids into schedule III of the Controlled Substances Act. The law increased penalties for steroid trafficking and imposed strict production and record-keeping requirements on pharmaceutical firms. Congress passed another Anabolic Steroid Control Act in 2004. It placed 36 additional steroids and over-the-counter prohormone dietary supplements under schedule III. This act also allows the Drug Enforcement Agency (DEA) to administratively classify new and novel steroids including THG as schedule III anabolic steroids. Androstenedione and its derivatives fall under this act, but they are readily available over the Internet. In fact, attempts at restriction amount to not much more than closing the barn door after the horse has escaped. Erythropoietin (EPO; Figure 7.7) and EPO-like drugs are now the performance-enhancing agents of choice. Even though doping with EPO and similar molecules is prohibited according to the World Anti-Doping Code, there has been ongoing development of new erythropoiesis-stimulating

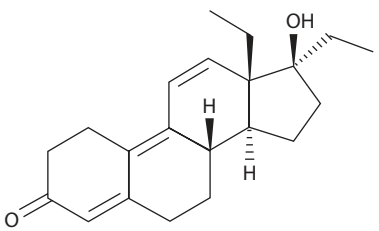


Figure 7.6 Tetrahydrogestrinone (popularly called *THG* or *The Clear*) binds to both the androgen and progesterone receptors, but not estrogen receptors.

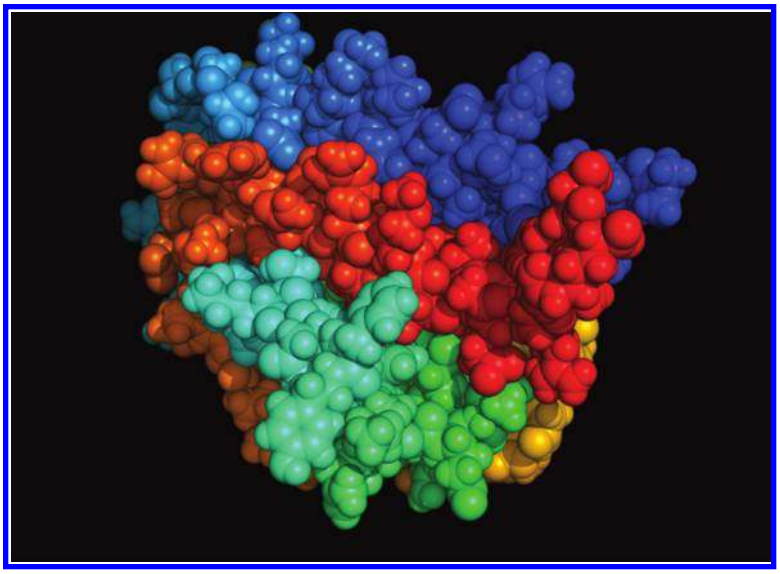


Figure 7.7 Erythropoietin (EPO) and EPO-like drugs are now the performance-enhancing drugs of choice. (From Wikipedia Commons.)

agents since the first introduction of recombinant human EPO for clinical use. This, of course, has also necessitated constant development of new methods for detecting the abuse of these substances (Reichel, 2012). As with many alleged performance-enhancing drugs, there is still substantial debate as to whether just increasing hemoglobin will have any positive effects on performance and taking too much may be countereffective (Hardeman et al., 2014).

7.8 Legitimate Indications for Dispensing Anabolic–Androgenic Steroids

The Food and Drug Administration (FDA) considers replacement therapy to be the only legitimate indication for testosterone supplementation. Multiple transcutaneous delivery systems are available and these are often prescribed off label for antiaging effects. The DEA classifies all testosterone-containing products as schedule III restricted drugs (Handelsman, 2006).

Androgens are occasionally used to treat women who have metastatic breast cancer involving bone, but higher blood concentrations of testosterone are associated with higher breast density and decreased apoptosis. Androgen use has largely given way to the prescribing of tibolone, which has androgenic and estrogenic effects (Hofling et al., 2005).

A testosterone derivative known as danazol (17-pregna-2,4-dien-20-yno[2,3-*d*]-isoxazol-17-ol), a synthetic analog of 17-ethinyl testosterone, binds with varying affinities to many different steroid hormone receptors (SHRs), including glucocorticoid, PR, ER, and AR. It can also displace other steroids binding to the same receptors (Tamaya et al., 1984). On occasion, it is still used to treat hereditary angioedema because, among other actions, it increases levels of C4 complement while at the same time increasing levels of deficient C1 esterase inhibitor. Danazol has also been used on occasion to treat patients with idiopathic thrombocytopenic purpura.

Compounds that exert both androgenic and anabolic effects are also indicated for the treatment of deficient red blood cell production (acquired aplastic anemia and myelofibrosis), although this indication has largely been abandoned because EPO is much more effective. Long-term treatment with testosterone leads to modest increases in hemoglobin and hematocrit that level off after a year to 15 months (Fernandez-Balsells et al., 2010; Haider et al., 2010). This self-limiting property probably does not occur in AAS abusers, and case reports of stroke and infarction in polycythemic AAS abusers have been published (Stergiopoulos et al., 2008).

7.9 Steroid-Related Disorders

7.9.1 Liver Disease

7.9.1.1 Peliosis Hepatis

This obscure disorder is characterized by the presence of small, scattered, cystic blood-filled cavities scattered throughout the liver (Figures 7.8 and 7.9). It has been recognized for more than 100 years. Some of the cysts may be lined with epithelium; others are not (Kalra et al., 1977). Collections of blood are often located adjacent to zones of hepatocellular necrosis. The lungs may also be involved in the same process, as may the entire reticuloendothelial system (Taxy, 1978).

Peliosis occurs as a complication of many apparently unrelated disorders. These include debilitating illnesses such as tuberculosis, hematologic malignancies, acquired immune deficiency syndrome, and posttransplant immunosuppression. Intravenous drug abusers, chronic alcoholics, and oral contraceptive and steroid users all are at risk (Tsokos and Erbersdobler, 2005). Peliosis only develops in organs that form part of the mononuclear

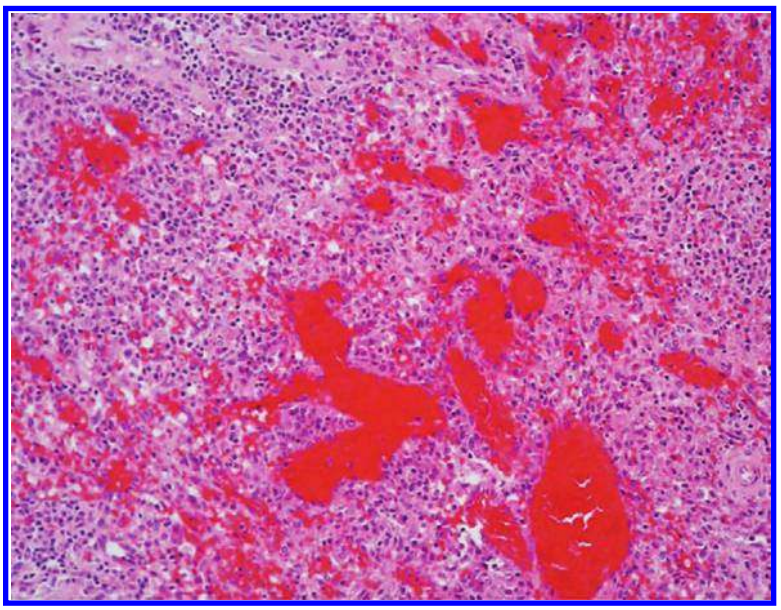


Figure 7.8 Peliosis hepatis. Whole liver demonstrating small lakes of blood located throughout the liver. (From Public Library of Science [PLOS].)

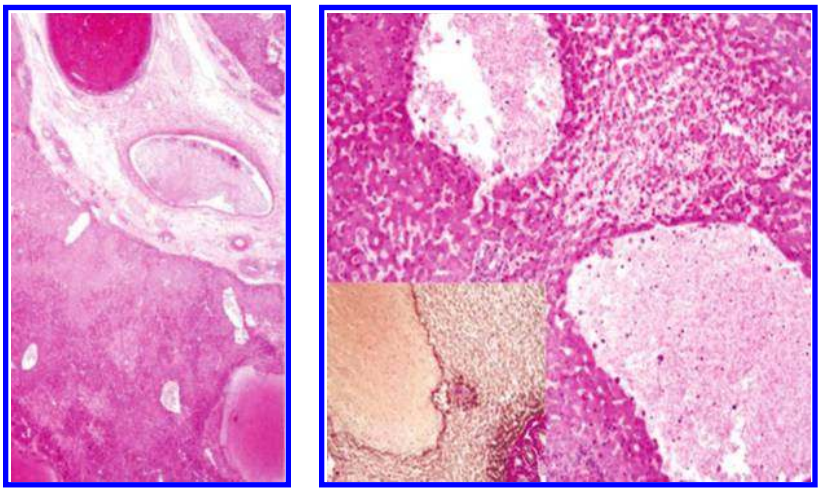


Figure 7.9 Peliosis hepatitis. This lesion is seen in many different disorders, including steroid abuse. It often goes undiagnosed during life. On cut surfaces, multiple, randomly distributed, and *blood*-filled cystic spaces can frequently be seen at gross inspection, giving the section a "Swiss cheese" appearance. The case shown is typical, and the appearance is even in the more striking gross specimen (Figure 7.8) than in the microscopic. (From Tsokos, M. and Erbersdobler, A., *Forensic. Sci. Int.,* 149(1), 25–33, 2005. With permission.)

phagocytic system (liver, spleen, bone marrow, and lymph nodes), though the occasional rare report has implicated other organs, including the lungs and the kidneys (Yamazaki et al., 2011). It has been suggested that the disorder is a congenital malformation of blood vessels, but there appear to be multiple possible etiologies, and the cause is still debated.

At autopsy the lesions have a "Swiss cheese" appearance. Microscopically, two forms of disease are recognized: parenchymal and phlebectatic. The parenchymal form consists of irregular unlined cavities that possess neither sinusoidal cells nor proliferating fibrous tissue. The second type, sometimes referred to *phlebectatic peliosis*, is characterized by the presence of regular, spherical cavities. These cavities are lined by *endothelium* and/or *fibrosis tissue*.

Of the possible differential diagnoses, the most obvious is secondary *hepatic congestion* due to veno-occlusive disease—*Budd–Chiari syndrome*. Peliotic lesions of the spleen may be clustered or disseminated, or they may follow some other uneven patterns of distribution. Histologically, the cavities usually have well-demarcated margins that may appear to be focally lined by sinusoidal *endothelium*. Alternatively, they may totally lack a clear cell lining.

Other diagnoses that must be considered besides *Budd–Chiari* syndrome include hemangioma and, if the spleen is involved, *hairy-cell leukemia*. *Spontaneous rupture* of the affected organ can occur, but more often rupture follows trivial trauma. There may be so much bleeding that the gross appearance of the abdomen mimics that of a violent death, particular if the liver is involved.

Peliosis is easily diagnosed by ultrasonography, conventional x-ray, and MRI scanning (Parmar et al., 2000; Iannaccone et al., 2006). Unfortunately, most individuals with peliosis are asymptomatic, so there is only a small probability they will be scanned before they hemorrhage. Patients have bled to death from these lesions (Nadell and Kosek, 1977), or they may die of hepatic coma. Most of those who have died of peliosis will have been

gravely ill with other disorders, and it may be difficult to determine what caused the fatal event, especially if a detailed histologic investigation is not undertaken. Multiple cases of peliosis in the HIV infected were reported in the early 1990s, making diagnosis even problematic, as it would be relatively easy to confuse Kaposi's sarcoma with peliosis (Hnatuk et al., 1994; Chang et al., 1996). Fortunately, since the introduction of highly active antiretroviral therapy, this complication has more or less disappeared.

While peliosis was once thought to be a congenital disease (Zak, 1950), cows with peliosis (known as St. George's disease) can be cured simply by a change of pasture (Graham and Kennedy, 1990). Most cases, particularly those occurring in the immunosuppressed, are of infectious origin. Bacillary angiomatosis and bacillary peliosis are both opportunistic infections caused by the bacteria *Bartonella henselae* and *B. quintana*. These organisms invade skin and bone as often as the liver. *B. quintana* and related organisms such as *B. henselae* (the organism responsible for cat scratch disease) can be identified in the lesions by DNA resequencing (Piemont and Heller, 1996). Peliosis due to *Bartonella* species is easily treated with antibiotics (Santos et al., 2000), but when the lesions are not related to infection, both the outcome and the treatment become less easily determined.

7.9.1.2 Cholestasis

The 17α-alkyl-substituted steroids can cause cholestatic jaundice. Bile accumulates in the canaliculi but without evidence of inflammation or necrosis (Foss and Simpson, 1959; Westaby et al., 1983). The frequency of cholestasis in testosterone abusers is unknown. Within the general population, different estimates suggest an incidence from less than 1% to at least 17% (Plymate and Friedl, 1992). The rate in AAS abusers is not known. Intrahepatic cholestasis can also occur as a consequence of pregnancy but as an acute, not chronic process. The hormone urocortin (UCN) exhibits a powerful concentration-dependent vasodilatation effect in the uteroplacental–fetal unit. However, UCN is downregulated during pregnancy and if the process is extreme (the mechanism is not known), the pregnancy can be adversely affected (Zhou et al., 2014).

Tissue culture studies show that 17α-alkylated steroids such as methyltestosterone, oxymetholone, and stanozolol are directly toxic to hepatocytes, but nonalkylated steroids such as testosterone cypionate, 19-nortestosterone, testosterone, and estradiol are not (Welder et al., 1995). With time, it has become clear that CYP3A4 genetic polymorphism plays a role in determining whether cholestasis occurs. CYP3A4 is responsible for metabolizing all types of AAS. Under normal circumstances, CYP3A production is upregulated in the presence of AAS and many xenobiotics, but that may not be the case in polymorphs.

Genetic polymorphism becomes an issue because production of CYP3A4 is mainly mediated by the pregnane X receptor (PXR), a nuclear receptor found in most cells. Its primary function is to sense the presence of foreign toxic substances and cause more CYP3A4 enzyme to be produced in order to speed the clearance of xenobiotics from the body. If PXR regulation is defective or an ineffective polymorphic form of CYP3A4 is present, then the xenobiotics can accumulate, which is another possible mechanism by which commonly abused AAS causes cholestasis (Wagner et al., 2010; Wallace et al., 2010).

Bile acids have other functions besides fat absorption and cholesterol metabolism. Bile acids also interact with plasma membrane G-protein-coupled proteins and nuclear receptors located in various tissues, including the heart (Vallim and Edwards, 2009). Elevated levels of bile acids alter vascular dynamics; bile acids reduce heart rate by regulating channel conductance and calcium dynamics in sinoatrial and ventricular cardiomyocytes.

Bile acids also regulate vascular tone via endothelium-dependent and endothelium-independent mechanisms. Changes in vascular dynamics are known to play a role in end-stage liver disease, obstructive jaundice, and even some types of cholestasis (particularly that of pregnancy) (Khurana et al., 2011). Whether supranormal concentrations of anabolic steroids trigger bile salt-related responses in the heart is not known, though the association with AAS and cholestasis is well established.

7.9.1.3 Hepatic Tumors

There is a causal relationship between the use of C17-alkylated androgens and the occurrence of certain hepatic tumors. Hepatocellular adenomas, similar in many ways to the adenomas that occur in the livers of women taking oral contraceptives, are not uncommon, even in men who are not steroid abusers. Judging from the number of reports, the incidence is 1%–3% among users of 17-alkylated androgens (Friedl, 1990; Buvat et al., 2010). Like peliosis, hepatocellular adenomas are usually silent; patients with adenomas only come to medical attention when an adenoma ruptures causing hemoperitoneum or when the adenoma is seen as an incidental finding at autopsy (Creagh et al., 1988). Liver function tests are likely to be normal in asymptomatic cases (Westaby et al., 1983).

In both sexes, the adenoma is composed of sheets of cells that look like normal hepatocytes. There are, however, some differences. One important difference is that androgen-related adenomas tend to be larger. Adenomas in steroid users range in size from a few millimeters to several centimeters in diameter. Androgen-related adenomas often form bile-containing acini and, absent a history of androgen abuse, acini formation is usually considered to be histologic evidence of malignancy.

Adenomas in steroid users may also display other features that are suspect for malignancy, such as bizarre nuclei and even rare mitoses (Creagh et al., 1988). The benign nature of most of these lesions is confirmed by their sharply demarcated margins, their failure to metastasize, the absence of demonstrable alpha-fetoprotein, and the absence of associated cirrhosis (the most frequent setting for hepatocellular carcinoma). The fact that adenomas regress when androgens are discontinued also argues against their malignant nature (Friedl, 1990). Nonetheless, hepatocellular carcinoma has been diagnosed in individuals taking C17-substituted androgens (Overly et al., 1984; Goldman, 1985; Gorayski et al., 2008), but these cases remain rare. Even so, the possibility for conversion from adenoma to carcinoma cannot be ruled out, particularly if, as described earlier, there is some derangement of PXR or other nuclear receptors (Boyd and Mark, 1977; Wagner et al., 2011). Rarely, nodular hyperplasia may result in portal hypertension, even in the face of a normal biopsy (Stromeyer et al., 1979). As a consequence, bleeding esophageal varices may occur (Winwood et al., 1990).

7.9.2 Cardiovascular Disease

Data regarding the consequences of AAS use on cardiovascular health are limited to case studies and a modest number of small cohort studies, making it virtually impossible to directly link specific cases of heart disease to abuse of a particular AAS. Regardless, anabolic androgens exert direct effects on cardiomyocyte growth, metabolism, and programmed cell death. Possible harms include direct effects on myocytes and endothelial cells, reduced intracellular Ca^{2+} levels, increased release of apoptogenic factors, and increased collagen

cross-links between myocytes (Angell et al., 2012). Whether the outcome is good or bad seems to depend largely on the amount of AAS consumed. In fact, a mounting body of medical evidence suggests that, on balance, the effects of AAS are more positive than negative (Hackett, 2012).

Evidence of toxic effects in AAS abusers has been recognized for more than a quarter century (Luke et al., 1990; Hausmann et al., 1998; Di Paolo et al., 2007). Cardiovascular complications including hypertension, cardiomyopathy, stroke, pulmonary embolism, arrhythmias (fatal and nonfatal), and myocardial infarction have all been reported in AAS abusers (Shimada et al., 2012), but not in any large epidemiologic study. A recently described case series is typical. It included three cases of sudden cardiac death (SCD) and one of death due to congestive heart failure in previously healthy athletes, all of whom were *AAS* and polydrug abusers. Concentric cardiac hypertrophy with focal fibrosis (one case), dilated cardiomyopathy with patchy myocyte death (two cases), and eosinophilic myocarditis (one case) were observed. Molecular investigation for viral genomes was positive in one case (Epstein virus) (Montisci et al., 2012).

Accelerated atherogenesis has been proposed as the etiology because supraphysiologic doses of AAS alter lipid metabolism, increasing LDL and decreasing HDL (Melchert and Welder, 1995; Feller et al., 2002). There is, however, no evidence that pharmacologic doses of testosterone used for replacement therapy exert any effect on lipid profiles, except, perhaps, to improve them.

Case reports describing myocardial infarction in steroid abusers are not uncommon (Winwood et al., 1990; Ferenchick and Adelman, 1992; Kennedy et al., 1993; Hourigan et al., 1998; Fineschi et al., 2001, 2007; Wysoczanski et al., 2008), but the etiology is problematic, as is evidence concerning the dose-related effects of AAS on the vasculature. Published research often yields conflicting results (probably because different AAS and different animal models are used), and testosterone and other anabolics all cause hematocrit to increase. As discussed earlier, it may well be that polycythemia played a role in some of the reported deaths (Stergiopoulos et al., 2008).

Development of an atheromatous plaque would perpetuate endothelial dysfunction, promote platelet aggregation, and make intracoronary thrombus formation more likely (Ajayi et al., 1995; Nieminen et al., 1996). AAS may cause a hypercoagulable state increasing production of thromboxane A_2 while, at the same time, increasing platelet thromboxane A_2 receptor density. Together these actions favor platelet aggregation.

Endothelial dysfunction has been proposed as a mechanism and it may contribute to abnormal vessel reactivity, but in human studies this particular injury has been hard to substantiate (Sader et al., 2001). Still, mounting evidence strongly suggests a relationship between disease and abnormal hepatic endothelial function. For example, it has been shown that in patients with kidney disease, low levels of testosterone are associated with diminished flow-mediated dilation (FMD), while normal levels of testosterone are associated with greater degrees of FMD (Yilmaz et al., 2011).

Myocardial necrosis can be demonstrated in the hearts of steroid abusers who die suddenly (Fineschi et al., 2001), as can areas of myocardial disarray (Figure 7.10) and other anatomic abnormalities that are normally seen in the hearts of polydrug abusers. These changes are easily produced in the rat model of steroid toxicity (Tseng et al., 1994), and all of these changes have been observed in steroid-abusing athletes (Dickerman et al., 1998; Simopoulos et al., 2002). Taken together, the changes are referred to as myocardial remodeling.

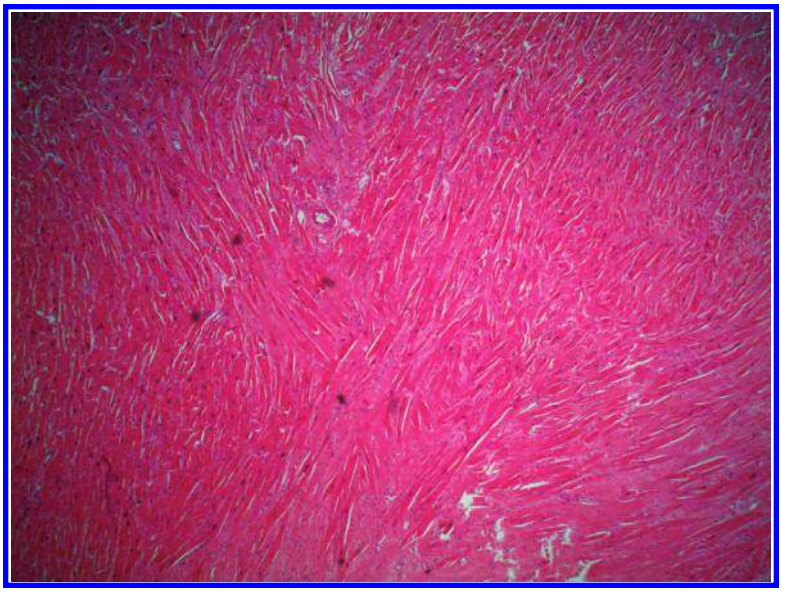


Figure 7.10 Myocardial disarray in the heart of a chronic steroid abuser with sudden cardiac death. (Courtesy of John Rutherford, Wellington, NZ.)

Whatever the upstream event that initiates the signaling chain, calmodulin kinase II activation seems to be the final common pathway for remodeling. Hypertension, cocaine abuse, and AAS are all associated with the same kind of myocardial remodeling, including small vessel disease, myocyte hypertrophy, and interstitial fibrosis (John et al., 2004; Henning and Cuevas, 2006). Any one of these structural alterations is a sufficient substrate to generate lethal arrhythmias and SCD (John et al., 2004; Shah et al., 2005).

Finally, cardiomyopathic changes have been described in the hearts of steroid abusers, but since all those affected were taking supraphysiologic doses of AAS, there is no credible evidence of cause and effect. There have been no systematic studies, so it is impossible to say whether the change is due to the drugs or underlying disease. Given that AAS abusers are, by definition, polydrug abusers, they likely have been exposed to many other agents that might also have produced cardiomyopathy. Furthermore, ongoing clinical trials suggest that supplemental treatment with testosterone may actually improve symptoms of heart failure and cardiomyopathy (Borst et al., 2010; Bell et al., 2011), although the first controlled clinical trial has yet to be reported.

7.9.3 Neurologic Disorders

Episodes of cerebral, coronary artery, intracardiac, and peripheral thrombosis have all been linked to steroid abuse (Frankle and Borrelli, 1990; Akhter et al., 1994; Fisher et al., 1996; Hartgens and Kuipers, 2004; Kindermann and Urhausen, 2004; Urhausen et al., 2004; Kindermann, 2006), but reports are still relatively rare. Anabolic steroid abusers have higher hematocrit and homocysteine plasma concentrations than the general population, suggesting users are at increased risk for stroke and thrombotic episodes (Graham et al., 2006), but there are so few cases that any connection must remain speculative. This caveat applies not just to testosterone but also to abuse of cortisol, growth hormone, prolactin, cocaine, and platelet-derived preparations (Lippi and Banfi, 2011).

Psychiatric disturbances are, on the other hand, more common. There is some evidence that, in susceptible individuals, particularly the aging (Lunenfeld, 2006), suicidal ideation is more common in steroid abusers (Thiblin et al., 1999). Otherwise, the behavioral effects of AAS are known to include hypomanic or manic behavior, sometimes accompanied by aggression or violence, which usually occurs while taking AAS. Users are also at risk for developing depressive symptoms occurring during AAS withdrawal. However, these symptoms are idiosyncratic and afflict only a minority of illicit users; the mechanism of these idiosyncratic responses remains unclear. AAS abusers are likely to have ingested a range of other illicit drugs, including both *body image* drugs to enhance physical appearance or performance and classical drugs of abuse (Kanayama et al., 2010). Thus, what some might classify as an episode of *roid rage* might, in fact, be an episode of methamphetamine-induced psychosis (Klotz et al., 2007).

Even though corticosteroid-induced behavioral and mood abnormalities are fairly common, especially in the young being treated for lymphoblastic leukemia, a legal defense based on AAS-induced psychosis (*roid rage*) has never been employed successfully in the United States, probably because of the absence of clinically proven behavioral effects in humans.

7.9.4 Musculoskeletal Disease (Figure 7.11)

AAS abuse promotes both long- and short-term changes in the integument. The first double-blind controlled study to demonstrate increased muscle size and strength after exogenous administration of supraphysiologic doses of testosterone (600 mg/week for 10 weeks), even in the absence of a strength training program, was published in 1996 (Bhasin et al., 1996). Results of the study leave no doubt that supraphysiologic doses of

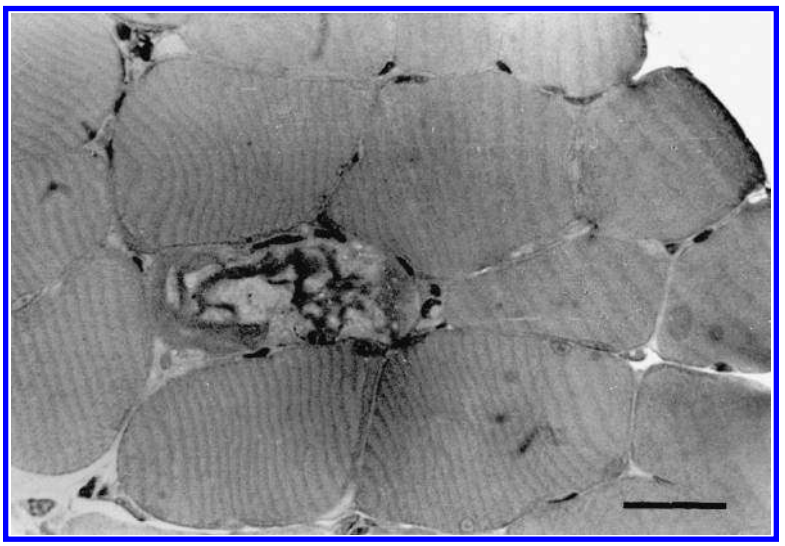


Figure 7.11 Degenerating muscle in steroid-treated rat. The peripheral muscle of rat treated with nandrolone decanoate and forced to exercise. Focal necrosis occurs, with degenerating fibers intermingled with normal-appearing fibers (scale bar = 50 μm). Morphometric analysis of these same fibers shows decreased numbers of capillaries when compared to controls. (Courtesy of Dr. J. M. Soares, Faculty of Sport Sciences, University of Porto, Porto, Portugal.)

testosterone, especially when combined with strength training, increase fat-free mass, muscle size, and strength in normal men, regardless of their age (Bhasin et al., 2005). A series of studies showing dose-dependent increases in muscle mass has been published. Strength gains of about 5%–20% of the initial strength and increments in body weight of 2–5 kg occur after short-term (<10 weeks) AAS use. Most of the weight gain may be attributed to an increase of lean body mass (Hervey et al., 1976; Kadi et al., 1999a,b; Hartgens et al., 2001).

ARs are located in the nuclei of muscle fibers and in capillaries coursing through the muscle. More ARs are located in the neck and shoulders than in the arms or legs (Hartgens et al., 1996). When supraphysiologic doses of AAS are consumed, the number of ARs located in other muscle groups in the body increases, especially those muscles that form the shoulder girdle (Kadi et al., 1999a), including the vastus lateralis (Sinha-Hikim et al., 2002), which explains the pattern of muscular hypertrophy usually seen in AAS users. When muscle hypertrophy is induced by testosterone (as opposed to resistance training), the number of so-called satellite cells increases.

Satellite cells are small mononuclear progenitor cells found in muscles. They contain hardly any cytoplasm and lie between the basement membrane and the cell membrane of individual muscle fibers. They may be almost impossible to distinguish from the nuclei of the muscle fibers. The satellite cells attach themselves to existing muscle fibers to form new, larger, fibers. The satellite cell is, in fact, the oldest known type of adult stem cell. Satellite cells are involved in the normal growth of muscle, but in adults, they are usually dormant and only undergo growth and cell division when there is disease or injury or when AASs are consumed (Sinha-Hikim et al., 2003, 2006).

AAS abuse, in connection with exercise, is associated with tendon rupture, in both humans and experimental animals. Ingestion of nandrolone and other AAS leads to dose-related ultrastructural changes of collagen fibrils within tendons (Michna, 1987). The net result of these changes is to favor tendon rupture. Tendon rupture, while not exceedingly common, is not rare either, and there are a number of reports in the literature (Hill et al., 1983; Kramhoft and Solgaard, 1986; Bach et al., 1987; Herrick and Herrick, 1987), though the majority of reports describe rupture after direct injection of the tendon for therapeutic purposes (Nguyen and Jones, 2012; Zhang et al., 2012).

The precise mechanism of tendon rupture is not entirely clear (Marqueti et al., 2006). One common explanation is that as muscle groups grow larger and larger, they exert more force on tendons than they were ever intended to support. This is an oversimplification. The dynamic interaction between muscle and tendon influences force transmission between muscle and bone (Reeves et al., 2003; Benjamin et al., 2008), but the mechanical properties of tendons differ depending on their level of usage (Reeves et al., 2003), and tendon morphology varies from muscle group to muscle group depending on the tensile and recoil properties appropriate to the function of each particular muscle group. In short, the tendons are regionally specialized.

The result of regional specialization is that tendons do not all react in the same way when AASs are administered. In general, ingestion of all AAS can be counted upon to lower tendon elasticity and the capacity to resist load. When AASs are consumed during training, the tendon's ability to adapt to complex exercises is compromised and rupture may occur. As each tendon is adapted to a specific kind of exercise, but AASs affect all tendons in exactly the same way, some tendons are at greater risk for rupture than others (Marqueti et al., 2010).

7.9.5 Other Musculoskeletal Effects

Occasional reports of rhabdomyolysis, both localized (Farkash et al., 2009) and generalized (Braseth et al., 2001; Adamson et al., 2005), have been published, but it is hard to know what to make of them. Given that rhabdomyolysis occurs in conjunction with intense physical exertion, especially in long-distance runners, football players, and even weight lifters who are not using AAS, it seems almost impossible to draw any conclusions about causation (Braseth et al., 2001; Adamson et al., 2005; Nikolopoulos et al., 2010). Much less controversial is the simple observation that androgens are important in the control of periosteal bone formation that contributes to the greater width of bone cortex, particularly in the long bones. Thus, while AAS use may make tendon rupture more likely, the chances of a long bone fracture are decreased.

7.10 Selective Androgen Receptor Modulators

The AR is just one type of nuclear receptor; other receptors exist for ER, PR, glucocorticoids, and mineralocorticoids. Activation of the AR by testosterone or its metabolite, DHT, is essential for the differentiation and growth of male reproductive organs, as well as the formation of masculine characteristics. Once an AR is activated, it combines with other coregulators and transcription factors. The activated AR forms a homodimer to exert its effects. Within the last decade, synthetic steroidal AR ligands have been developed for the treatment of male hypogonadism, muscle wasting, anemia, and even prostate cancer, but the use of these agents is limited because bioavailability is poor when they are taken orally and because of concerns for toxicity. Recently, the process has become much more sophisticated and it has been possible to develop a number of AR agonists that are not only potent but highly specific. As a group, these compounds are referred to as SARMs. Although they are not exactly comparable to testosterone or DHT, there are many points of overlap. These compounds first became available in 1999. As a group, SARMs are unique in that they are not substrates for aromatase and 5α-reductase (Chen et al., 2005). The evidence suggests that they are potent androgens, increasing bone strength and muscle mass, but that they do not exert the adverse effects on lipids, prostate, and cardiovascular system that are seen in association with AAS.

Studies have been performed to address specifically the relationship between SARMs, the prostate, and prostate cancer. Studies of in vitro prostate cancer cells have not revealed any prooncogenic features, but there is no question that SARMs retard the growth of prostate tumor cells (Tesei and Leonetti, 2013). At the same time, enobosarm, a nonsteroidal SARM and the best characterized clinically, when used in small clinical studies, has consistently demonstrated increases in lean body mass and better physical function across several populations along with a lower hazard ratio for survival in cancer patients in general. *Enobosarm* (GTx-024, S-22) is a recently developed SARM, developed by GTx Inc. (TN, United States), which has been tested in phase I, II, and III trials with promising results in terms of improving lean body mass and measurements of physical function and power in cachectic patients. *Enobosarm* has received fast-track designation by the U.S. FDA and seems likely to be on the market in the near future (Srinath and Dobs, 2014).

It may be that more women will use agents in this class, rather than resort to AAS, because SARMs do not cause virilization (Schmidt et al., 2009). These agents, it would seem, have the

potential to maximize positive androgenic effects and minimize negative side effects (Segal et al., 2006). Since SARMs retain all the advantages of performance-enhancing AAS but have few side effects, avoiding undesirable side effects, it seems likely they are already being used as doping substances. In anticipation of this trend, not surprisingly, WADA prohibited SARMs on January 1, 2008, and antidoping labs are already trying to identify potential targets for routine doping control (Segal et al., 2006; Nagata et al., 2011; Thevis et al., 2011).

7.11 Detecting Steroid Abuse in the Living

Anabolic steroid abusers fit a clinical profile, and reference to it is often helpful, especially if the history of the individual is unclear. Typical findings are shown in Table 7.3. Testosterone blood concentrations vary too widely to be used for detection purposes (Jensen et al., 1991). Postmortem blood testosterone concentrations have still not been studied. A number of papers have been published on postmortem testosterone concentrations in the brain and testes, but reliance on these measurements would be unreliable and indefensible.

Because blood measurement is an unpredictable indicator for testosterone administration, it cannot be used for doping control. As an alternative, the IOC has chosen to base its determinations on the ratio of testosterone to epitestosterone (T/E) excreted in the urine. Epitestosterone is a natural component of human biological fluid, but its biosynthetic pathway and the site of its formation in man have not been unequivocally characterized. Epitestosterone (E) production apparently parallels the formation of testosterone (T), but its concentration is not influenced by exogenous administration of testosterone, which is why the T/E ratio was originally selected as a screening tool for sports doping (Starka, 2003).

In 1984, the IOC declared that a urine T/E ratio greater than 6 was proof that supplemental testosterone was being used. But, in 2005, WADA, the agency charged with IOC testing, dropped the acceptable T/E ratio to 4. While this is a much more realistic approach than the old one, it still allows careful athletes to cheat because, in reality, most of the population has a T/E ratio much closer to 2, or even lower among Asians. It is possible to take supplemental testosterone and still not exceed the 2:1 ratio, so even at the new lower level, false negatives continued to appear.

One reason why use of the T/E ratio remains problematic is racial and ethnic variation. Different ethnic groups are polymorphic for a gene called *UGT2B17,* which influences how quickly testosterone is metabolized and excreted. In an important study published in 2009, the steroid profiles, along with the genotypes, of 57 Africans, 32 Asians, 50 Caucasians, and 32 Hispanics, all competitive weight lifters, were compiled and compared. Highly significant differences were found between all ethnic groups. A *UGT2B17* deletion/deletion genotype was present in 22% of Africans, 81% of Asians, 10% of Caucasians, and 7% of Hispanics. What this means is that, in any given individual, genetic variation determines the absolute amount of testosterone excreted and found in the urine, so that the T/E ratio may be abnormal even in those who do not use exogenous testosterone. There is a good case for abandoning the T/E ratio altogether (Strahm et al., 2009).

Other methods have been proposed to diagnose steroid abuse. One is to quantitate LH and measure the ratio of LH to testosterone (Kicman et al., 1990). However, this approach can be manipulated to give a false-negative result and is far from foolproof. The ^{13}C to ^{12}C ratio has also been proposed as an indicator for abuse, since the ^{13}C content

Table 7.3 Profile of a Steroid Abuser

Social
Recent changes in friends and acquaintances
Obsession with health, exercise, and weight lifting
Spending most of the time in gyms or health clubs
Taking large amounts of vitamins and food supplements
Very high calorie intake
Physical
Rapid weight gain and muscle development
Increased body hair, deepening of voice
Acne (both sexes)
Hair loss (both sexes)
Breast enlargement (males)
Testicular atrophy
Difficulty in urinating
Elevated blood pressure
Complaints of stomach upset
Jaundice
Edema of extremities
Mental changes
Increased aggression
Hyperactivity, irritability
Auditory hallucinations
Paranoid delusions
Manic episodes
Depression and anxiety
Panic disorders
Suicidal thoughts
Laboratory findings
Decreased HDL cholesterol
Decreased LH
Decreased follicle-stimulating hormone
Decreased thyroid-stimulating hormone
Decreased thyroid hormones
Elevated liver enzymes
Increased hematocrit
Increased LDL cholesterol
Increased triglycerides
Increased glucose

Source: Adapted from Narducci, W. A. et al., *J. Toxicol. Clin. Toxicol.,* 28(3), 287–310, 1990.

of pharmaceutical testosterone preparations is different from that of endogenously produced steroid (Shackleton et al., 1997; Saudan et al., 2009). Given the metabolic variabilities between persons, including genetic makeup, repeat *baseline* testing is now used to indicate aberrant results that may suggest abuse (Kicman and Cowan, 2009).

Testosterone and nandrolone-like drugs can reliably be detected in hair, and the evidence suggests that this approach is much more likely to detect abusers than urine testing. In one study, the effectiveness of urine and hair testing was compared in samples obtained simultaneously

from a group of professional bicyclists; 12% of the hair tests, but none of the urine tests, disclosed the presence of anabolic agents (Gaillard et al., 2000). Hair concentrations of AAS are low (in the picogram range), but currently available analytic techniques allow for reliable detection, particularly now that liquid chromatography–tandem mass spectrometry (LC–MS/MS) testing has become much more widely available (Bresson et al., 2006). Unfortunately, hair testing for anabolic steroids is not yet accepted by most sports regulatory bodies.

New designer steroids and SARMs are synthesized with such rapidity that the IOC and WADA continually find themselves in the difficult position of fighting the war with outdated weapons. Methods already exist to allow for the use of epigenetic changes as gene markers, no matter the type of doping agent used (Schwarzenbach, 2011).

7.12 Postmortem Analysis

7.12.1 Postmortem Considerations

There are a few scenarios where it would be reasonable to classify steroid abuse as a cause of death, but diagnosis will not be made by blood or urine testing, although hair testing may very well play a role. For example, should a body builder bleed to death from peliosis and AAS use be confirmed by hair testing, causation could be reasonably inferred. The same could be said for anyone with myocardial remodeling. Remodeling itself is a sufficient cause of death, and if the use of AAS could be established, the situation would be not much different than in a long-term cocaine abuser with a markedly enlarged heart who dies an arrhythmic sudden death when he is not using the cocaine. Drug use was ultimately responsible for the heart disease and it would not be unreasonable in that situation to call cocaine the cause of death. Other scenarios are much less obvious. If an AAS abuser develops hepatic adenocarcinoma, is there enough science to support a ruling that AAS were probably responsible? Probably not. Toxicology testing, at least as it is commonly practiced, adds very little to the diagnostic process except to prove or disprove a pattern of abuse (Abushareeda et al., 2014).

7.12.2 Analysis

Most forensic postmortem laboratories do not have the expertise to measure testosterone or indeed any of the other related steroids and anabolic agents. A specialist laboratory such as those accredited by the IOC usually conducts testosterone measurements, though as demand for testosterone testing increases, measurement may be available from some local clinical laboratories.

Such laboratories are able to screen for a large number of related anabolic agents, usually in urine; hence, at least 10 mL of urine is preferred. These laboratories hydrolyze the conjugates in urine and extract the molecule prior to tandem MS analyses. The use of LC–MS/MS avoids the need for derivatization and has the advantage of also detecting compounds that are not easily derivatized by standard techniques, for example, THG (Pereira et al., 2009).

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Solvents

8

Volatile solvents, liquids that vaporize at room temperature, are commonly abused, most often in the form of pressurized gases (liquefied petroleum gas, butane lighter fluid, nitrous oxide, etc.). The practice of solvent inhalation is referred to as "huffing" or "chroming." The abuse of inorganic volatile solvents is confined largely to less affluent teenagers and young adults, but it is also popular among disadvantaged adults as well. The particular agent abused seems to be largely a function of availability.

Inhalants can be conveniently divided into five categories:

1. Volatile (industrial and household solvents such as paint thinners, degreasers, dry-cleaning fluid, and gasoline)
2. Art or office supply solvents (correction fluids, felt-tip marker fluid, and electronic contact cleaners)
3. Aerosols (household aerosol propellants found in spray paints, hair spray, deodorants, and fabric protector sprays)
4. Gases (including helium, butane lighters, and propane tanks)
5. Whipping cream aerosols or dispensers and refrigerant gases

If an accelerant has been used to start a fire, it may very well be present in the bodies of decedents found at the scene. Persons caught up in these situations will inhale the volatile compounds, and these can be detected in their blood (or lung fluid) at low concentrations and even after fairly long periods of time.

Toluene is the major active component in many of the agents listed above and the agent most likely to be responsible for cases of fatal intoxication (Figure 8.1). It seems likely that much "abuse" occurs accidentally, with intoxication being a result of occupational exposure (Bowen et al., 2005). Toluene is a lipophilic compound, and it binds strongly to myelin and other lipid-containing organs. This property explains why toluene seems to concentrate in the brain (Chan et al., 2004).

The more exotic agents, such as gases used for medical anesthesia, are much less commonly encountered. The nitrites should probably be considered as a unique category; volatile organic nitrites such as cyclohexyl, butyl, and amyl nitrite, commonly known as "poppers," are closely linked with sexual practices and not drug abuse per se. These agents are often sold, both in the United States and in Europe, in small brown bottles labeled as "video head cleaner," "room odorizer," "leather cleaner," or "liquid aroma."

8.1 Incidence and Epidemiology

Solvent inhalation abuse (*huffing*) is more widespread than was originally supposed. According to results of the National Survey on Drug Use and Health, which is performed yearly by the U.S. National Institute on Drug Abuse, in 2008, 729,000 persons aged 12 years

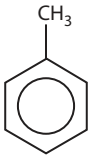

Figure 8.1 Molecular structure of toluene, the major active component in many cases of solvent abuse.

or older had used inhalants for the first time within the past 12 months; 70.4% of these were under the age of 18 years. There was no significant difference in the number of inhalant initiates between 2007 and 2008, but the 2008 number was significantly below that in 2003 (871,000), 2004 (857,000), and 2005 (877,000). At the same time, however, there was a significant decrease in the average age at time of first use among recent initiates aged 12–49 years, from 17.1 years in 2007 to 15.9 years in 2008. Unlike nearly all other classes of drugs, inhalant use is most common among younger adolescents, and the incidence of use seems to decline with increasing age. Use of inhalants may reflect the fact that many inhalants are cheap, readily available (often in the home), and legal to buy and possess (U.S. Department of Justice, 2010).

The U.S. National Poison Data System (NPDS) tracks real-time caller requests for help/information made to 60 U.S. poison centers. A 2010 NPDS study analyzed demographic, geographic, product, outcome, and treatment site data for the 35,453 inhalant cases that had been reported to the system between 1993 and 2008. The prevalence of reported inhalant cases decreased 33% between 1993 and 2008, with prevalence highest among children aged 12–17 years and peaking among in 14-year-olds. In contrast to national survey data showing nearly equal use of inhalants by both genders, 73.5% of the NPDS inhalant abusers were boys, which may reflect the fact that boys generally pursue riskier behaviors. Most cases (67.8%) were managed in health-care facilities.

More than 3400 different products are known to contain dangerous solvents. Propellants, gasoline, and paint were found to be the most frequently abused product categories, but only the abuse of propellants has substantially increased over time. Abuse of butane, propane, and air fresheners was found to have the highest fatality rates. Prevalence for all inhalants was highest in western mountain states and West Virginia, but geographic distribution varied according to product type. Gasoline was a proportionately greater problem for younger children; propellants were an issue for older children (Marsolek et al., 2010).

A 20-year retrospective study of autopsied deaths in South Australia between January 1983 and December 2002 identified 39 inhalant deaths in an autopsy pool of 18,880 individuals, with a male-to-female ratio of 12:1. Sixty-four percent of the victims ($n = 25$) died from voluntary inhalation of volatile substances and 28% ($n = 11$) committed suicide utilizing a volatile substance or gas. One case was the result of a workplace accident, and there were two cases of autoerotic death in which inhalants had been used to augment solitary sexual activity. The mean age of the 28 victims of accidental inhalant death was 21 years (range, 13–45 years), considerably younger than that of the 11 suicide victims of 31.5 years (range, 17–48 years). No homicides were identified. Approximately one-quarter of the victims in this study were aboriginal ($n = 11$) and 10 of whom had died as a result of gasoline inhalation (*petrol sniffing*). Other common substances of abuse were aliphatic hydrocarbons such as butane. The study showed that those most at risk for accidental or suicidal

inhalant deaths were young males; 92% of victims overall were male and 77% were under 31 years of age. Gasoline inhalation remains a significant problem in aboriginal communities in South Australia (Wick et al., 2007).

8.2 General Considerations: Availability and Types of Products

Hundreds, perhaps thousands, of commercial and household products contain solvents that can be abused. The medical complications of acute solvent toxicity remain poorly characterized, but much more is now known about the consequences of chronic exposure. Solvent abuse is not a new problem. Recreational solvent abuse was recognized before World War I, and the abuse of ether was popular in England during the 1890s. Deaths from recreational chloroform abuse were first reported at an even earlier date, but the current high levels of solvent exposure—unintentional or intentional—are a modern phenomenon.

Most abused solvent products are readily available at either retail stores (e.g., grocery, hardware and paint stores, and stores selling car parts) or gas stations (petroleum products and cleaning agents). Table 8.1 lists some of the more commonly abused agents by patterns of toxicity. Table 8.2 lists some of the other solvents and gases involved in fatal poisoning.

Table 8.1 Partial List of Agents That May Be Responsible for Inhalant Abuse Toxicity, Grouped by Pattern of Toxicity

A. Aerosol propellants (air fresheners, deodorant spray, hair spray)
Bromochlorodifluoromethane (from fire extinguishers)
Butane
Diethyl ether
Carbon tetrachloride
Ethyl chloride
Halogenated fluorocarbons
Perchloroethylene
Trichloroethylene
B. Gas fuels (disposable cigarette lighters)
Liquid petroleum gas
Propane
Butane
C. Chlorinated solvents (commercial dry cleaning/degreasing agents)
Carbon tetrachloride
Dichloromethane
Methanol
Tetrachloroethylene
Toluene
D. Solvents from adhesives (also paints, nail polish, varnish remover)
Acetone
Butane
Cyclohexanone
Toluene
Xylene

Note: Agents from group A are more likely to be associated with traumatic injuries and death.
Agents from the other three groups are more likely to manifest direct toxicity.

Table 8.2 Other Key Solvents and Gases Known to Cause Harm, Including Death

Agent	Source
Ethylene glycol	Antifreeze
Hydrogen sulfide	Sewers, tanks, and mines
Helium	Party balloons, laboratory gas
Carbon monoxide	Exhaust gases, fires
Hydrogen cyanide	Fires, industrial uses as a salt

8.3 Disposition: Absorption and Tissue Concentrations

Not all solvents are abused. In order to have abuse potential, a compound must be sufficiently volatile to be inhaled. This explains the generally low abuse potential of petroleum distillates, such as kerosene and ethylene glycol. Industrial workers exposed to ethylene glycol fumes can develop an assortment of chronic disorders, including testicular degeneration (Lee and Kennedy, 1991), but reports of toxicity in chronic abusers are rare.

It is not practical to discuss the specifics of all volatile compounds likely to be of interest to forensic investigators. It should suffice to say that, effectively, all such compounds are absorbed and distributed to all key organs and tissues within minutes. No matter which drug, the effects are usually almost immediate, although the response may be delayed where metabolic activation is required to produce toxicity (Figure 8.2). For example, several

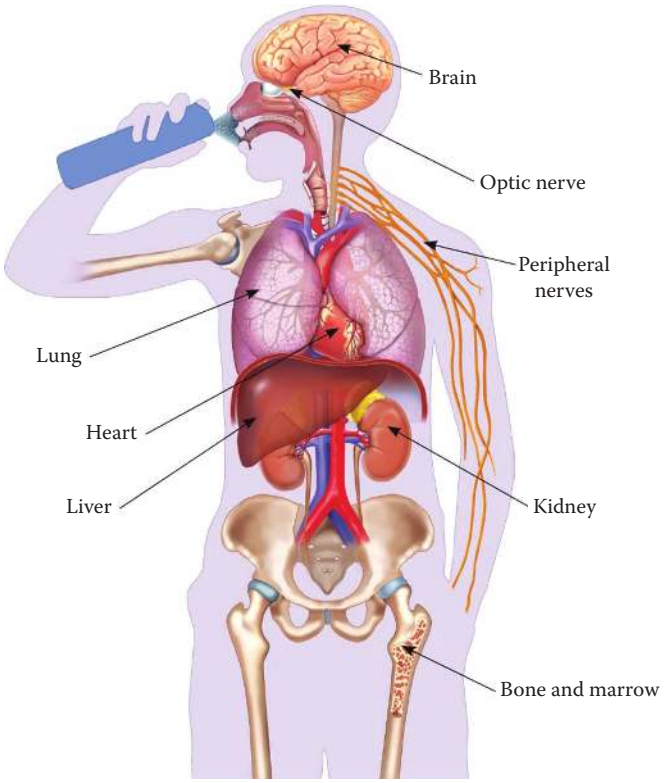

Figure 8.2 Organs affected by solvent inhalation. (From Howard, M.O. et al., *Addict. Sci. Clin. Pract.*, 6, 24, 2011. With permission.)

hours elapse following methanol inhalation before the acidosis from formic acid production becomes severe (Wallace and Green, 2009). The same applies following ethylene glycol inhalation; oxalic acid is metabolically formed and crystallized, and it is these crystals, not the parent compound, that damage the kidneys (Brent, 2001).

Toluene, the solvent most often used in contact adhesives, is highly volatile and frequently abused. The popularity of individual solvents depends on their boiling range. The range for toluene is quite low, allowing abusers to pour the solvent into a plastic bag, gather the ends of the bag together, hold the top of the bag over the mouth and nose, and then inhale the vapors. Plastic bags can also be used to collect propellants from aerosol cans. Volatile agents such as gasoline are simply sniffed from soaked rags (Flanagan and Ives, 1994). When toluene is inhaled, it is rapidly taken up by the brain and by fat stores in the body. It is then slowly released over the course of many hours. Once released back into the circulation, hepatic conjugation is followed by renal excretion as hippuric acid. The coingestion of ethanol increases plasma concentrations of most solvents (Dossing et al., 1984); concentrations of toluene may be nearly double those seen when the solvent is inhaled by itself.

Toluene toxicity can be largely explained by its very low solubility in water. The methyl group of toluene is more easily oxidized by cytochrome P450 2E1 (CYP2E1) (James et al., 2008) than its benzene ring. With chronic exposure, toluene induces the production of more CYP2E1, and 95% of toluene is oxidized to become benzyl alcohol and then excreted (Kim et al., 1997). The remaining 5% is oxidized to form benzaldehyde and various cresols (Chapman et al., 1990). Most of the reactive products are detoxified by conjugation with glutathione, but the remainder can cause cell damage, particularly if cells are exposed to high concentrations or for prolonged periods. One case report described a man who ingested a large quantity of toluene and died about 30 min later; the tissue distribution of toluene in the liver was 434 μg/g, pancreas 88 μg/g, brain 85 μg/g, heart 63 μg/g, blood 28 μg/mL, fat 12 μg/g, and urine 2.1 μg/mL (Ameno et al., 1989).

Deaths associated with inhaling lighter fluid, which mainly contains low molecular weight hydrocarbons (largely propane, butane, and isobutane), are relatively common (Esmail et al., 1993; Rohrig, 1997; Sugie et al., 2004; Pfeiffer et al., 2006). Isobutane is present in brain at much higher concentrations than in blood, and the metabolite 2-methyl-2-propanol has also been detected (Pfeiffer et al., 2006).

Deaths due to occupational exposure to hydrogen sulfide are surprisingly common. The gas is a respiratory poison found in closed environments such as sewers, tanks and pits, mines, and in volcanic emissions. The degree of exposure can be measured from the detection of the metabolite thiosulfate in the blood, kidney, and lungs (Fuller and Suruda, 2000; Hooser et al., 2000; Hendrickson et al., 2004; Smith and Cummins, 2004; Ago et al., 2008; Cantrell and Young, 2009; Poli et al., 2010).

8.4 Pharmacology and Toxicology

Solvents share some characteristics with other depressants such as barbiturates, benzodiazepines, and even alcohol (Evans and Balster, 1991). Chemicals such as toluene, 1,1,1-trichloroethane, and trichloroethylene (TCY) affect ligand-gated ion channel activity. In vitro studies have shown that solvents may cause reversible enhancement of γ-aminobutyric acid A ($GABA_A$) receptor-mediated synaptic currents in hippocampal

brain slices and increase the expression of α-1-glycine receptors (Beckstead et al., 2000). These are essentially the same as the effects produced by sedative hypnotic drugs such as barbiturates and benzodiazepines.

The ability of solvents to produce a state of true dependence remains a matter of some dispute (Miller and Gold, 1991), but the results of animal studies certainly suggest that solvent abusers can become physically dependent (Evans and Balster, 1991). Other animal studies clearly demonstrate that exposure to toluene and related compounds at concentrations of 2000–6000 ppm produces anxiolytic-like effects (Paez-Martinez et al., 2003).

Solvents are highly soluble in lipids, and they rapidly enter the central nervous system, where they act as depressants. Animal studies have disclosed a host of different molecular targets. Many of the solvents appear to share a common mechanism; they inhibit *N*-methyl-D-aspartate (NMDA) receptors. Prolonged exposure can modulate the expression of these glutamatergic receptor subtypes, but the final outcome depends on which animal model is being studied. Many other receptors appear to be involved. $GABA_A$, glycine, and type 3 serotonin receptors are all disrupted by solvent exposure (Bowen et al., 2006).

Toluene also reversibly and noncompetitively inhibits neuronal acetylcholine receptors, at least in animals (Bale et al., 2002).

Some toluene deaths may be attributed to its ability to reversibly inhibit cardiac voltage-activated sodium channels, which are required for the initial phase of the cardiac action potential (Cruz-Nunez et al., 2003).

Psychiatric, neurologic, renal, and hepatic disorders have been reported as complications of solvent abuse, as has teratogenesis, but the primary risk is sudden death (see Section 8.5).

A U.K. study (Flanagan et al., 1990) analyzed the patterns and mechanisms of death in a series of 1237 solvent abusers over a 20-year period. Deaths were divided into four different groups according to the type of solvent involved: aerosol propellants, gas fuels, chlorinated and other types of solvents, and solvents from adhesives (mostly toluene). The proportion of deaths due to direct toxicity, aspiration, and asphyxia was remarkably similar in all the groups, except for the adhesive solvent group. Among individuals abusing adhesives, trauma was the most frequent cause of death, suggesting that impairment of judgment is more likely with the use of adhesives than with other types of solvents.

In 51 individuals admitted to hospital for suspected toluene toxicity, the average age of the 34 males was 21.4 years and of the 17 females was 16.2 years. Toluene concentrations in the blood collected on admission, widely from 0.3 to 232.8 μg/g (~0.3–23 mg/L). Blood concentration measurements were poor predictors of toxicity. Nine of the patients with concentrations of more than 3.0 μg/g (~3 mg/L) were quite ill, but twice as many of the subjects (n = 18) with blood toluene concentration greater than 3.0 μg/g (~3 mg/L) had no physical signs whatsoever. In three semicomatose individuals, the blood toluene concentrations exceeded 10.0 μg/g (~10 mg/L). Still, in the majority of cases (24), concentrations were below 3.0 μg/g, and these individuals had no physical signs (Miyazaki et al., 1990).

Toluene abuse has led to arrest for driving under the influence (DUI). In Norway, 114 drivers arrested for DUI had blood toluene concentrations greater than 10 μM (1.1 mg/L), with a mean of 109 μM (12 mg/L). There was no simple relation between blood toluene concentration and degree of impairment, although significant loss of toluene would have occurred during transport and storage (Gjerde et al., 1990).

Fires release a diverse assortment of volatile poisons, including carbon monoxide and hydrogen cyanide, and both should be measured in any decedent exposed to fumes from fires (Alarie, 2002). Benzene is among the most common volatile compounds to be released; others are propene, xylenes, 1-butene/2-methylpropene, toluene, propane, 1,2-butadiene, 2-methylbutane, ethylbenzene, naphthalene, styrene, cyclopentene, 1-methylcyclopentene, and isopropylbenzene (Austin et al., 2001). In cases of arson, other hydrocarbons can be targeted to prove use of accelerant as distinct from exposure to burning materials (Morinaga et al., 1996).

Death (usually intentional) from exhaust gases channeled into the cabin of a motorized vehicle is well recognized. Death is caused by the rapid buildup of carbon monoxide (CO) in the closed environment, leading to blood saturations well in excess of 60%. Occasionally, investigators may be surprised to find decedents with no measurable CO in blood. This occurs because modern catalytic converters do not liberate sufficient CO once the converter has warmed up, and in these cases, death is due to deprivation of oxygen in conjunction with excessive levels of carbon dioxide (Schmunk and Kaplan, 2002; deRoux, 2006; Vevelstad and Morild, 2009). Analysis of blood specimens for the presence of fuel-derived hydrocarbons can prove exposure to exhaust gases (Morinaga et al., 1996).

8.5 Clinical Syndromes

8.5.1 Neurologic Disorders

An acute syndrome consisting of toluene-induced nausea, abdominal pain, impaired judgment, altered consciousness, and seizures is well recognized (Watson, 1982). Transient neurologic symptoms, but no neurologic sequelae, can also occur after the use of amyl nitrite and related compounds. At autopsy, the presence of paint around the nares should suggest the diagnosis (Byard et al., 2000). Chronic abuse leads to irreversible brain damage.

The first reports describing a toluene-related neurologic disorder were published more than 40 years ago (Grabski, 1961; King et al., 1981). Cerebellar signs predominate, and patients present with ataxia, tremor, and nystagmus (Rosenberg et al., 2002). A variety of other neurologic disorders may occur, ranging from relatively minor degrees of cognitive dysfunction and poor performance in school (Fornazzari et al., 1983; Hormes et al., 1986) to much more serious disorders, including some with evidence of pyramidal tract damage. Cerebral and cerebellar atrophy have been described (Hormes et al., 1986). Cranial nerve injury has also been reported. Some users, particularly adults, may present with a disorder mimicking Guillain–Barré syndrome (Streicher et al., 1981), and, in extreme cases, even central pontine myelinolysis (Hong et al., 1996).

Acute intoxication results in bizarre behavior. Nearly all of the inhalants produce effects much like those any other anesthetic agent but shorter acting. The most common symptoms are euphoria and headache. Ataxia is seen after chronic use and seizures after concentrated exposure. Given that most of these agents can induce seizure activity, clinical diagnosis can be problematic. Toluene use presents a special problem, because toluene reacts with other drugs, such as aminophylline, which also can cause seizures, making seizure more likely and more severe (Weiss, 1983; Evans and Balster, 1991; Chan and Chen, 2003).

It is the norm for most of these symptoms to disappear, or at least improve, when exposure to solvent ceases. In some cases, however, symptoms persist. Seizure disorders and evidence of cognitive impairment may be permanent (Byrne and Kirby, 1989). Chronic

toluene abuse also causes paranoid psychosis with schizophrenic symptoms that may be atypical, including visual (rather than auditory) hallucinations. Only a limited number of neuropathologic studies have been published (Escobar and Aruffo, 1980; Rosenberg et al., 1988), and the available evidence suggests that when symptoms persist, it is usually because widespread demyelination has occurred. If these observations can be further substantiated, they would suggest that methamphetamine and toluene share a common mechanism for psychosis production, as they seem to be causing damage to the same structures. The demyelination changes are easily visualized with MRI. Preliminary studies suggest that the same considerations may apply to other solvent abusers. In rats, exposure to inhalants that contain toluene during adolescence and early adulthood appears to differentially affect white matter maturation and behavioral outcomes, although recovery can occur following abstinence. However, the results of studies in toluene abusers have not been reported.

The cerebellum is particularly vulnerable to intoxication and poisoning, especially the cerebellar cortex and Purkinje neurons. In humans, the most common cause of cerebellar toxicity is alcohol related, but the cerebellum is also a main target for drug exposure (such as anticonvulsants, antineoplastics, lithium salts, and calcineurin inhibitors), drug abuse, drug addiction (such as cocaine, heroin, and phencyclidine), and even environmental toxins (such as mercury, lead, manganese, and especially toluene/benzene derivatives). The prevalence and incidence of cerebellar lesions as a result of toxin exposure is still largely unknown, but toxin-induced cerebellar ataxias are not rare and the possibility of solvent exposure should always be considered (Manto, 2012).

In autopsy studies of three paint sniffers described by Kornfeld et al. (1994), the essential feature was severe but generalized loss of myelin, with only relatively mild axonal loss and gliosis. Macrophages filled with PAS-positive granules were a constant feature. The histologic appearance was no different from the picture seen in adrenoleukodystrophy. Biochemically, the lesions were characterized by an increase of very long chain fatty acids in the white matter (Kornfeld et al., 1994). There is no question that chronic toluene use might lead to frank leukoencephalopathy. Many of toluene's behavioral effects are a consequence of NMDA blockade combined with the disruption of normal dopaminergic transmission (Paggiaro et al., 1984).

White matter changes in solvent abusers seem to begin in the deep periventricular white matter and then extend peripherally. With continued toluene abuse, there is loss of the normal perceived gray–white matter boundary. As lesions become more severe, there is deposition of iron due to demyelination and axonal loss. This is the most probable mechanism for the thalamic hypointensity commonly observed in MRI scans of solvent abusers. One important consequence of demyelination and axonal loss is a decrease in presynaptic dopamine reuptake (Aydin et al., 2002; Papageorgiou et al., 2009).

As MRI shows that toluene preferentially affects white matter structures and periventricular/subcortical zones relative to cortical regions (Yucel et al., 2008), histologic examination should focus on these areas. Neuropil vacuolization without inflammatory change is another notable feature of toluene intoxication. This pattern seems to be unique to toluene poisoning. Damage to white matter usually precedes any clinical evidence of cerebellar dysfunction or psychiatric disease. Disruption of normal dopaminergic transmission may explain the occasional episodes of Parkinsonism, observed in toluene abusers (Papageorgiou et al., 2009).

Whatever the underlying mechanism responsible, spasticity and cognitive changes occur in chronic abusers. Children born to mothers who use toluene during pregnancy develop a

distinctive fetal solvent syndrome, including growth retardation, craniofacial dysmorphism, hearing loss, cleft palate, developmental delay, cerebellar dysfunction, and hyperactivity (Bowen et al., 2005, 2006; Hougaard et al., 2005; Grandjean and Landrigan, 2006).

MRI scanning confirms the loss of white matter, with cerebral atrophy most evident in the corpus callosum and cerebellar vermis (Kamran and Bakshi, 1998). Bilateral abnormalities of the basal ganglia, red nucleus, and thalamus have also been described (Yamanouchi et al., 1995; Caldemeyer et al., 1996; Miyagi et al., 1999). MRI studies of relatively naïve users are likely to appear normal, even though decreased perfusion of the thalamus and basal ganglia can be demonstrated with single photon emission computed tomography scanning (Ryu et al., 1998).

More recent data suggest that chronic solvent abuse can produce central hypothyroidism, even when MRI of the pituitary shows no significant changes. Toluene's extreme lipophilicity means it easily enters the brain and, like other organic solvents, alters dopaminergic and adrenergic transmission within various parts of the brain. The result may well be abnormal secretion of pituitary hormones, resulting in transient central hypothyroidism and abnormal gonadotropin levels (Chen et al., 2003).

Other inhalants, in particular butane, can result in asphyxia, while still others may cause encephalopathy. Unfortunately, the neuropathology of butane-related deaths has not been investigated.

8.5.2 Renal Disease

Toluene inhalation should be included in the differential diagnosis of any young patient who presents with unexplained hypokalemia and normal anion gap metabolic acidosis (Tang et al., 2005). Occasionally, acute respiratory failure with hypokalemia can also occur and may lead to secondary rhabdomyolysis with acute renal failure (Kao et al., 2000). Hematuria is common (Crowe et al., 2000), and glomerulonephritis has been documented (Streicher et al., 1981), but the actual incidence of these complications is, in fact, quite low. Disorders of the renal tubules are more frequent than disease of the glomerulus (Taher et al., 1974; Fischman and Oster, 1979; Moss et al., 1980; Voigts and Kaufman, 1983).

Rats chronically exposed to toluene vapors displayed histologic evidence of interstitial cell infiltration and interstitial nephritis; the greater the exposure, the greater the degree of damage (Cobanoglu et al., 2007). The mechanism by which toluene damages the renal parenchyma is not known.

8.5.3 Gastrointestinal Disease

The liver is the main organ responsible for the metabolism of drugs and toxic chemicals, and so is the primary target organ for many organic solvents. Many people work with these compounds and suffer significant occupational exposure without ever really knowing it. There is no question that some of the organic solvents used in industrial processes may be associated with hepatotoxicity.

Several factors contribute to liver toxicity; among these are species differences, nutritional condition, genetic factors, interaction with medications already in use, alcohol abuse and interaction, and age. The main pathogenic mechanisms responsible for functional and organic liver damage caused by solvents are inflammation (Figure 8.3), dysfunction of

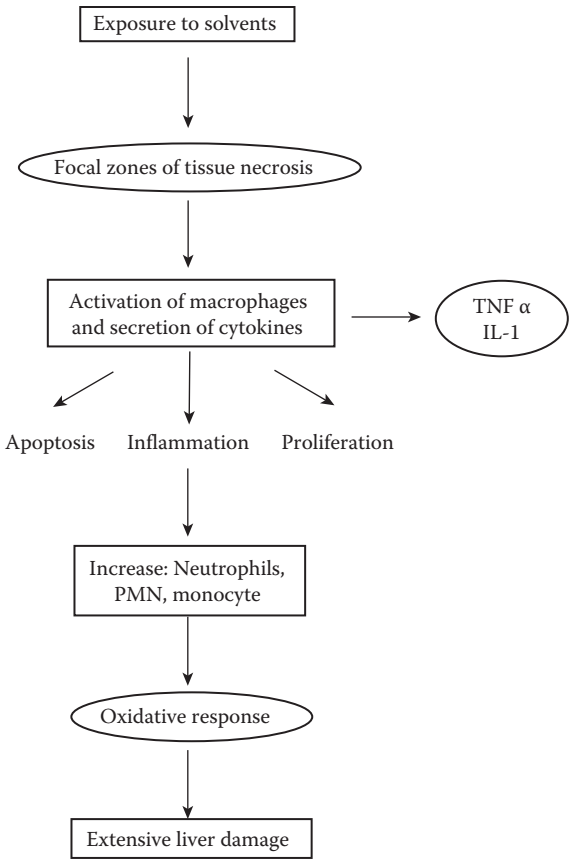

Figure 8.3 Hypothetical role of inflammation in chemical-induced hepatotoxicity. TNF, tumor necrosis factor; IL, interleukin; PMN, prime neutrophils. (From Malaguarnera, G. et al., *World J. Gastroenterol.*, 18, 2756, 2766, 2012.)

cytochrome P450, mitochondrial dysfunction, and oxidative stress (Malaguarnera et al., 2012), as discussed elsewhere in Chapter 1.

Although histologic evidence of gastrointestinal disease is uncommon among solvent abusers, symptoms are frequent. Solvent-related centrilobular necrosis was first reported more than 40 years ago (Baerg and Kimberg, 1970), but only one case of fulminant hepatic failure has ever been linked to human solvent abuse (McIntyre and Long, 1992). Surveillance studies of workers with long-term solvent exposure have not found evidence for subclinical alterations in liver or kidney function (Brogren et al., 1986). In animal experiments, the simultaneous administration of methamphetamine enhances carbon tetrachloride hepatotoxicity (Roberts et al., 1994). In theory, a solvent abuser taking amphetamines might be at increased risk. In practice, except for ethanol, solvent abusers rarely abuse other drugs at the same time.

8.5.4 Cardiovascular and Pulmonary Disease

Sudden cardiac death is the most common cause of death in solvent abusers, although the mechanism seems to depend upon the type of solvent being abused. Some toluene deaths may be attributed to that compound's ability to reversibly inhibit cardiac voltage-activated

sodium channels, required for the initial phase of the cardiac action potential. Whether or not inhibition occurs depends on the toluene concentration, which may explain the phenomenon of sudden cardiac death in "huffers" or "chromers" (Cruz et al., 2003). Earlier theories simply postulated that exposure to toluene and other organic solvents, via some unknown mechanism, sensitized the myocardium to the effects of catecholamines (Cunningham et al., 1987). It has now been demonstrated that toluene inhalation is associated with QT interval prolongation and QT dispersion, favoring the occurrence of torsades de pointes (Alper et al., 2008), though atrioventricular block has also been reported in some patients (Tsao et al., 2011; Pan and Lin, 2012).

Toluene-induced arrhythmias may involve decreased intracytosolic calcium concentrations. TCY (and probably other halogenated solvents as well) reduces calcium levels within cardiomyocytes (Hoffmann et al., 1992). The force of myocyte contraction depends on intracytosolic calcium. In order to initiate a contraction, intracytosolic calcium must increase to a certain critical set point. Decreased availability of calcium within the cardiac myocytes translates into decreased force of contraction. Whether or not solvent abuse results in myocardial depression sufficient to decrease coronary artery perfusion, causing ischemia and sudden death, has not been established. At the time this mechanism was first proposed, the importance of the ryanodine receptor had not been recognized. It is now known that mast cells release a proinflammatory mediator leukotriene D(4), which induces an increase in intracellular free Ca^{2+} in at least some human muscles, and that the increase is ryanodine mediated (Bouchelouche et al., 2003).

Studies with isolated membrane fractions have shown that calmodulin (CaM) inhibits the ability of cardiac muscle cells to release Ca^{2+} from the ryanodine receptor channel 2 (RyR2), the principal ryanodine channel. Taken together, the data suggest that impaired CaM inhibition of RyR1 is associated with defective sarcoplasmic reticulum Ca^{2+} release and altered gene expression in such a way as to induce cardiac hypertrophy and early death (Yamaguchi et al., 2007). When it is sought, mutation of the *RyR2* gene can be detected in nearly a third of young sudden death victims. It may be that toluene-related deaths only occur in individuals who are polymorphic for *RyR2*, but this hypothesis has yet to be tested (Tester et al., 2005).

Amyl nitrite inhalation causes methemoglobin formation, and fatal levels can be produced if too much amyl nitrite is inhaled (Guss et al., 1985; Sarvesvaran et al., 1992). Alternatively, amyl nitrite–induced vasodilation and intense vagal stimulation could also lead to arrhythmias and sudden death. Still another possibility is fatal respiratory depression; if solvent concentrations in the brain reach sufficiently high levels, fatal respiratory depression could occur. Asphyxial death from vomiting with aspiration is common. Flanagan and Ives (1994) reported that aspiration was the cause of death in 20%–30% of the solvent-related sudden deaths they reviewed. Nonetheless, respiratory depression seems to be a real possibility because of the high brain toluene concentrations that are often detected at autopsy (Yajima et al., 2005).

The mechanism responsible for ventricular arrhythmias and sudden death secondary to butane inhalation, though uncommon (Rohrig, 1997; Bland and Taylor, 1998; Ago et al., 2002; Sugie et al., 2004; Pfeiffer et al., 2006), seems to be asphyxial. Standard hematoxylin–eosin staining of the myocardium reveals characteristics of chronic and acute myocardial hypoxia, but no atherosclerotic heart disease. The asphyxial nature of the deaths can be confirmed by immunohistochemical staining (for myoglobin, desmin, fibronectin, fibrinogen, and CC9) (Novosel et al., 2011). Pulmonary edema

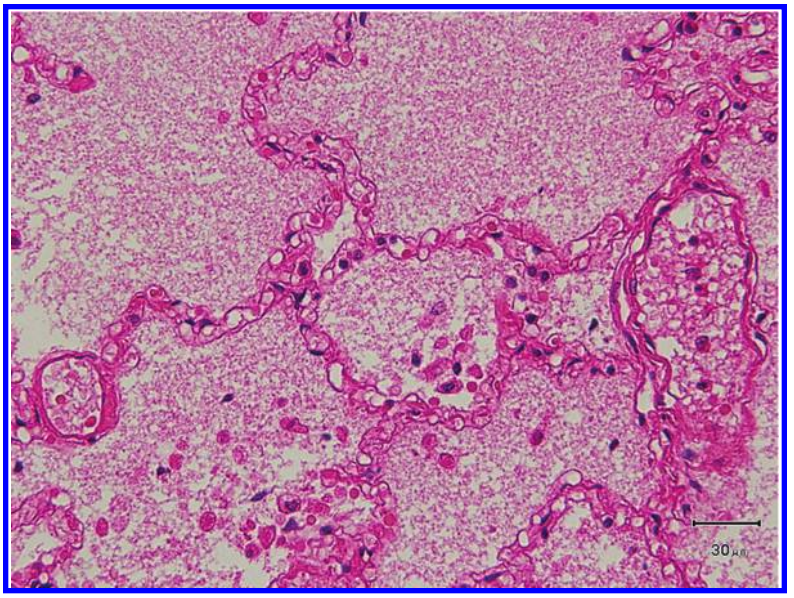

Figure 8.4 Intra-alveolar edema with macrophages and vesicularization of the capillary endothelia in the alveolar walls (hematoxylin–eosin). This abnormality appears distinctive for solvent abuse. (From Sakai, K. et al., *Forensic Sci. Int.*, 206 (1–3), e58, 2011. With permission.)

is likely to be present, and the alveolar septa are likely to be vacuolated and possibly infiltrated (Figure 8.4) (Sakai et al., 2011).

8.5.5 Maternal/Fetal Considerations

Clinical cases of toluene-related embryopathy and malformations have been reported after toluene abuse by pregnant women (Bowen et al., 2006). Very high levels of maternal solvent exposure, typical of abuse, can lead to perinatal death, and there are reports that surviving neonates show evidence of teratogenicity. At birth, affected infants are typically premature with growth retardation and microcephaly, severe facial dysmorphology (e.g., deep-set eyes, small face, low-set ears, and micrognathia), and spatulate fingertips with small fingernails. The term "fetal solvent syndrome" has been adopted to describe this constellation of morphologic and behavioral effects, following the model of fetal alcohol spectrum disorders and comparing the phenotype of toluene embryopathy to the effects of prenatal alcohol exposure (Jones and Balster, 1998). Data suggest that the high levels of toluene exposure typical with inhalant abuse are more detrimental to fetal development than typical occupational exposure (Hannigan and Bowen, 2010; Hougaard et al., 2003).

8.6 Toxicology

8.6.1 Preanalytic Considerations

The chemical properties and volatile nature of volatile substances make proper sample collection, storage, and handling especially important in their analytic investigation. However, useful qualitative results can still be obtained, even if the conditions for sample

storage and handling are less than ideal. In fact, the greatest forensic difficulties posed by these solvents arise from establishing their presence.

Loss of the volatile substances by evaporation makes quantitative toxicology in these cases very difficult, although in most instances, this will not be necessary. It is advisable that analysis of the sample should start as soon as possible in order to avoid missing the presence of these agents. Volatile substances diffuse from the sample into the atmosphere until equilibrium is reached. Every time the sample container is opened, solvent loss occurs due to displacement of its vapor by air. Every time fresh air enters the container, diffusion of the solvent will create a new equilibrium. Samples should therefore, be stored in gas-tight, well-sealed containers with minimal headspace.

Sample should be protected against contamination from environmental and laboratory sources of volatiles. Storage, transport, and handling of the sample should always be at approximately −5°C to 4°C. Lower temperatures should be avoided for long-term blood sample storage because they will lead to the formation of *n*-hexanol from degradation of fatty acids. The presence of *n*-hexanol often interferes with the analysis of low toluene concentrations; however, in true cases of solvent intoxication, this interference is limited. Samples should only come into contact with inert materials such as glass, Teflon®, or aluminum foil. Soft rubber stoppers should be avoided due to their high affinity for and permeability to toluene.

If the blood sample to be analyzed has been obtained from the living, an anticoagulant (lithium heparin or fluoride/oxalate) must be added. Tubes containing EDTA and gel separators should not be used to collect blood at autopsy because they may well yield false-positive results for xylenes (methylbenzenes), ethylbenzene, toluene, and 1-butanol; all of these compounds have been reported in the separator gel and, if present, will contaminate the sample. Other agents, such as 1-butanol and 2-methyl-2-propanol, have also been detected in tubes coated with EDTA (Francis et al., 1982). Addition of sulfuric acid or sodium fluoride (Flanagan et al., 1990; Streete et al., 1992) is advised when esters such as ethyl acetate are present in the sample; adding sulfuric acid abolishes esterase activity, allowing preservation of the sample.

The common fluoride/oxalate blood alcohol tube is sufficient for most purposes. Ensure it is sealed as tightly as it can be and then kept cold, and the sample analyzed as soon as practicable. Sodium azide should also be added to the tube to prevent growth of microorganisms (Flanagan et al., 1990).

When the goal is to monitor for occupational and environmental exposure, urinalysis is probably the method of choice. Analysis of metabolites in urine allows an extended window for detection. In postmortem cases, metabolites seem to be of less importance, although trichloroethanol and trichloroacetic acid are routinely analyzed in cases of chloral hydrate and TCY poisoning. For most volatile substances, the level of parent substance in urine is sufficient to be measurable, even if the solvents have low water solubility (Takeuchi et al., 2002).

8.6.2 Preferred Analytic Methods

Gas chromatography is the analytic technique used almost exclusively for the detection of volatile substances. It is ideally suited to separate the analyte from other low molecular weight compounds that are also volatile.

Headspace analysis is the most convenient method; a small sample is placed into a vial along with an aqueous buffer or water containing an internal standard. The vial is then sealed, heated slightly, and a headspace sample injected into a column. A temperature

program is used to increase the precision of the separation of solvents (and/or gases) and to allow less volatile substance to elute from the column.

There are two ways to increase specificity and improve separation: using columns of differing polarity or two detectors (flame ionization and electron capture) (Streete et al., 1992). Gas chromatography–mass spectroscopy (GC-MS) is recommended as it provides spectral data to allow identification of the compound(s) beyond that achieved with retention times (Morinaga et al., 1996; Bouche et al., 2002). Solid-phase microextraction allows an even more convenient method of absorbing volatile compounds onto a microfilter that has been applied directly to the sample (Tranthim-Fryer et al., 2001; Gottzein et al., 2010).

More exotic solvents and gases require more specific methods. For example, thiosulfate measurement is used to test for exposure to hydrogen sulfide (Poli et al., 2010), helium (Grassberger and Krauskopf, 2007), hydrogen cyanide (Gambaro et al., 2007), and CO by gas chromatography (Lewis et al., 2004).

8.6.3 Data Interpretation

Exposure to volatile substances can be determined by analysis of any tissue, although predominately blood, urine, and fluid taken from the lung (in decedents) are used. Quantitative analysis of volatile compounds in acute poisonings is rarely useful because, by its very nature, the volatility of the compounds being measured means it can never be accurate. At best, semiquantitative analysis may give some idea of the degree of exposure. The problem is well illustrated by the wide range of blood toluene levels found in a now 2-decade old study by Miyazaki et al. (1990) of patients with suspected toluene toxicity (see Section 8.4).

The type of case and clues about the circumstances (i.e., type and source of solvent) is needed so that the toxicologist can perform their analyses appropriately. Even using highly sensitive techniques like GC-MS and capillary columns does not guarantee every volatile compound is detected. For example, the most volatile gases, such as helium, methane, and nitrous oxide, are unlikely to be detected without the use of specific techniques for these gases. Ethylene glycol is also more difficult to detect since it is very polar and normally requires either the use of derivatization or a specially treated column to facilitate chromatography.

When exposure to complex mixtures, such as petroleum products (distillates used in motor vehicles, diesel, kerosene, aviation fuel, etc.) is believed to have occurred, profiles of hydrocarbons can be used to determine the type of distillate or mixture. Clinical diagnosis is very important; telltale signs observed at the time of clinical presentation or at autopsy can provide clues as to the type and nature of exposure to volatile poisons.

Only a few reports describing postmortem toluene concentrations have ever been published. One report described the toxicological findings in a known solvent abuser who died suddenly. Using gas chromatography, the peaks of toluene, xylene, and ethylbenzene were detected in the blood and gastric contents. The brain is especially useful for purposes of postmortem analysis, though it cannot necessarily be used to determine the cause of death, because no specific level has ever been shown to absolutely cause toxicity. In one reported case, the concentration of toluene in the brain was 20 μmol/g, but the value was considered nonlethal because the decedent had obviously died of head trauma (Yajima et al., 2005).

There is also the problem of decomposition. Numerous volatile substances can be detected from decaying bodies, including toluene. In one case report, the toluene concentration was 10.11 nmol/L (Statheropoulos et al., 2005), and similar findings have been observed in the pig model (Dekeirsschieter et al., 2009).

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Marijuana

9

Marijuana is the most widely used drug in the world. The United Nations (UN) estimates that between 125 and 203 million individuals consume marijuana annually (UNODC, 2011).

By 1980, 423 different natural compounds had been identified in *Cannabis sativa L.* (Turner et al., 1980). By 1995, the number had risen to 483, and more recently, six new compounds, four cannabinoids and two flavonoids, have been described (Ross et al., 2005). Some of these components (e.g., cannabinoids such as cannabidiol [CBD]) appear to be neuroprotective, in spite of numerous reports describing psychosis in cannabis users (Howes and Perry, 2011). The major psychoactive constituent of marijuana is Δ9-tetrahydrocannabinol (THC). THC may be synthesized using citral and olivetol in boron trifluoride and methylene chloride (Lander et al., 1976).

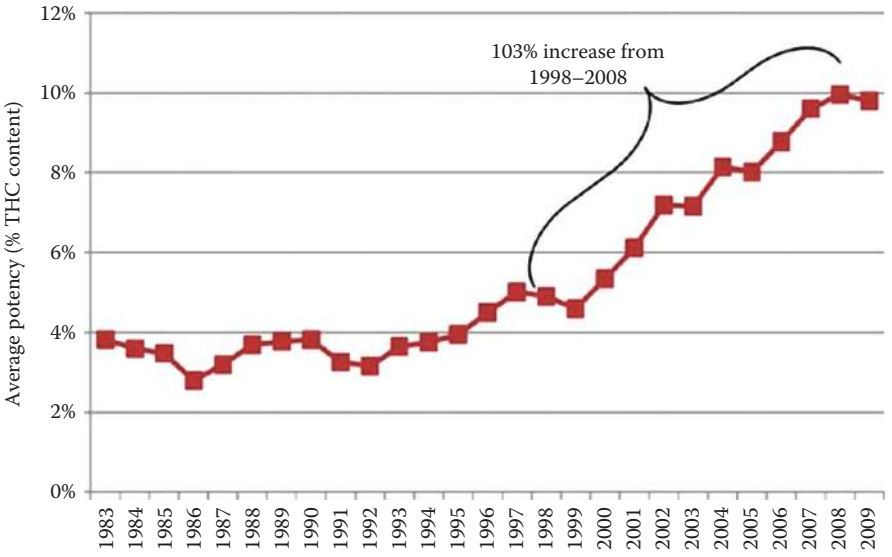
From 1993 to 2003, the price of hashish remained relatively constant; however, the mean yearly potency varied from 2.5% to 9.2% and from 12% to 29% later in the decade (2004–2008). Hash oil potencies also varied considerably during this period (16.8% ± 16.3%). The increase in the potency of cannabis preparations is mainly due to the increasing availability of the more potent nondomestic samples. Figure 9.1 shows the mean potency of marijuana and sinsemilla in domestic and nondomestic samples from 1985 to 2009 (Mehmedic et al., 2010).

9.1 Botany

C. sativa L. is an annual plant that grows in all parts of the world, generally reaching a height of 16–18 ft. It is widely accepted that *C. sativa L.* belongs to the family Cannabaceae, which has only one genus (*Cannabis*) and one species (*sativa*) (Ross et al., 2005).

"Marijuana" is the general common name for most parts of the plant *C. sativa L.*, including the seeds, the resin extracted from any part of the plant, and every compound, salt, derivative, or mixture derived from the plant, but it does not include the mature stalks, fiber produced from the stalks, or oil or cake prepared from the seeds (Farnsworth, 1969).

Cannabis is also grown commercially to produce hemp. The bulk of the commercial plant consists of stalks with very little foliage, except at the apex. In contrast, wild plants, and those cultivated illegally, possess many branches and the psychoactive ingredient is concentrated in the leaves (Figure 9.2) and flowering tops. There may be significant differences in the gross appearance of marijuana plants due to climatic and soil conditions, the closeness of the plants to other plants during growth, and the origin of the seed stock grown. Marijuana is the nonchemical name given to the crude drug derived from the plant.



Source: University of Mississippi, National Center for Natural Products Research, *Potency Monitoring Project Quarterly Report* 107 (*January* 2010).

Figure 9.1 Potency of marijuana seized in the United States. Potency of seized specimens has increased by 103% from 1998 to 2008. (From the Office of Drug Control Policy [ONDCP]. https://www.whitehouse.gov/sites/default/files/ondcp/Fact_Sheets/marijuana_fact_sheet_jw_10-5-10.pdf, last accessed May 18, 2012.)

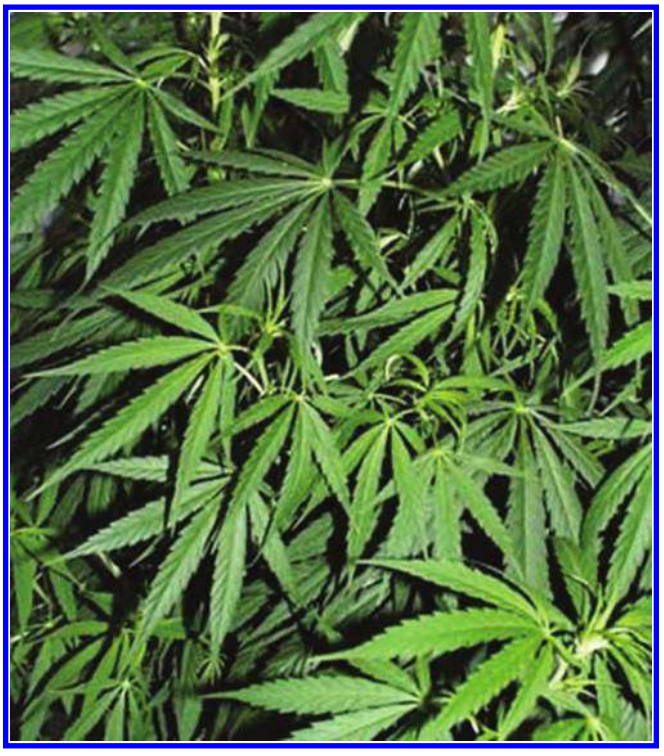


Figure 9.2 Marijuana leaves.

Figure 9.3 Dried marijuana buds (implying higher potency). (From the DEA website.)

Figure 9.4 Hashish is a cannabis preparation composed of compressed and/or purified preparations of stalked resin glands, called trichomes, collected from the unfertilized buds of the cannabis plant. It contains the same active ingredients—such as THC and other cannabinoids—but in higher concentrations than unsifted buds or leaves. (From the DEA website.)

Different parts of the plant contain varying concentrations of THC. The leaves contain <1%–10% THC by weight, while hashish resin prepared from the flowering tops (Figures 9.3 and 9.4) of the plant contains approximately 15% THC. The highest concentration of THC found in a natural cannabis (marijuana) sample was 37% as analyzed by the Southwestern Regional DEA Laboratory.

9.2 Epidemiology

Use is a function of availability, and the amount of marijuana available is simply not known. The U.S. Department of Justice (DOJ, 2010) states

> No reliable estimates are available regarding the amount of domestically cultivated or processed marijuana. The amount of marijuana available in the United States—including marijuana produced both domestically and internationally—is unknown. Moreover, estimates as to the extent of domestic cannabis cultivation are not feasible because of significant variability in, or nonexistence of, data regarding the number of cannabis plants not eradicated during eradication seasons, cannabis eradication effectiveness, and plant-yield estimates.

The DOJ added that no estimates are available for the amount of marijuana produced by Asian, Caucasian, Mexican, and Cuban traffickers in the United States. Currently, no national-level eradication statistics are compiled or recorded by the producing group. The lack of such estimates precludes a precise determination of the extent to which each group is involved in marijuana production within the United States (U.S. Department of Justice, 2010). The Department of Enforcement Affairs (DEA) protests aside, rough estimates can be based on the size of seizures; multiton seizures are now frequently reported.

Although no estimates are available, marijuana is known to be widely available in the United States, in part because of increasing production in Mexico coupled with decreased enforcement activity. The amount of marijuana produced in Mexico has increased by an estimated 59% since 2003, with plants from both indoor and outdoor sites more than doubling since 2004 (Figure 9.5).

The UN estimates that 29.5 million people in North America used cannabis at least once in 2008, a decrease from the estimated 31.2 million in 2007. Cannabis use in the United States and Canada has been declining or stabilizing over the past decade. In the

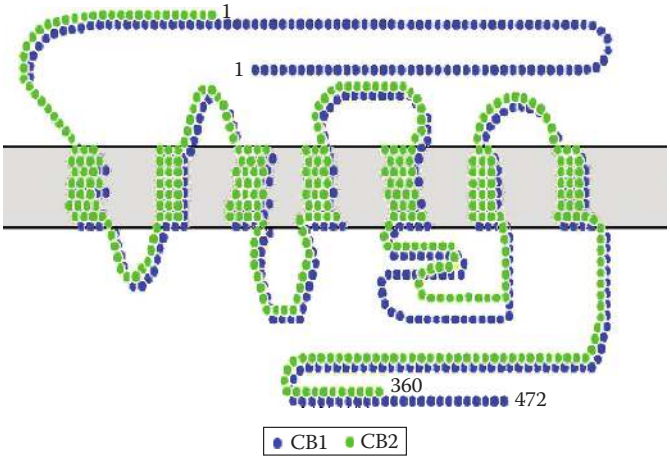


Figure 9.5 Structures of cannabinoid receptors CB1 and CB2. G-coupled proteins attach to each structure. (From Wikipedia Commons. With permission.)

United States, between 2002 and 2007, there was a significant decrease in the annual prevalence of cannabis use within the population aged 12 years and older, from 11% to 10%; however, in 2008, this increased, reaching levels observed in 2006 (10% of this population). A similar trend has been observed among younger secondary school students. Despite the large decline in use since 2002, cannabis remains the preferred illicit drug in the United States. In 2008, cannabis was used by 76% of all current illicit drug users and was the only drug used by 57% of them.

Increases in cannabis use among school students occurred in a number of European countries between 1995 and 2003, but in most Western European countries, it stabilized or decreased in 2007. It is hard to generalize because different patterns of use can be observed across Europe.

In the Oceania region, between 2.1 and 3.4 million people are estimated to have used cannabis in the past year (9.3%–14.8% of the general population aged 15–64 years). The only recent reliable estimates for cannabis use in this region are for Australia, Fiji, and New Zealand. In Australia, the annual prevalence of cannabis use has been declining since 1998, with close to a 20% decline between 2004 and 2007. The major decline in the younger population (aged 14–19 years) from 35% in 1998 to 13% in 2007. In New Zealand, the annual prevalence of cannabis use fell from 20% in 2003 to 13% in 2006, only to increase again in 2008 to 15%. Prevalence of use was highest for men in the 18–24 year age group and for women in the 16–17 and 18–24 year age groups (UNODC, 2010).

9.3 Pharmacology

Marijuana is usually smoked or taken orally. In the past, typical doses were 5–20 mg (Kiplinger and Manno, 1971) but probably are double that due to the prevalence of higher-strength THC-containing cannabis. The pharmacological effects include sedation, euphoria, hallucinations, and temporal distortion.

It was believed that the effects of THC were nonspecific, but in the late 1980s, a cannabinoid receptor was identified in rat brains. Two types of receptors have been discovered: CB1 and CB2 (Figure 9.6). These receptors are the primary targets for endogenous cannabinoids (endocannabinoids), with THC binding to both receptors. Specific antagonists are now available for the CB1 and CB2 receptors. In addition, THC acts as an agonist at benzodiazepine, opioid, and cannabinoid receptors, in addition to exerting effects on the synthesis of prostaglandins, DNA, RNA, and even protein metabolism (Bhattacharya et al., 1980; Cabral and Staab, 2005).

CBD significantly reduces the extent of liver inflammation, oxidative/nitrative stress, and cell death and attenuates both bacterial endotoxin-triggered necrosis factor (NF)-κB activation and tumor necrosis factor (TNF)-α production in the isolated Kupffer cells of experimental animals. In humans (where liver changes have not been studied except in situ), adhesion molecule expression in primary human liver sinusoidal endothelial cells is stimulated by TNF-α and the attachment of human neutrophils to the activated endothelium as well. These protective effects appear to be the result of CB1 agonism (Mukhopadhyay et al., 2011). Cannabinoid receptors are coupled to G proteins and are involved in the control of many processes, including metabolic regulation, food craving, pain, anxiety, bone growth, and immune function. The exogenous

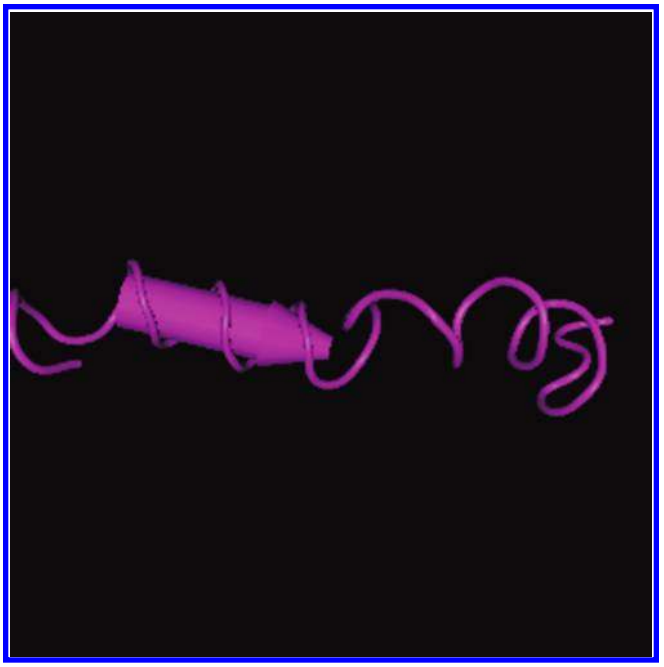


Figure 9.6 Computer model of the cannabinoid receptor, type CB1. The short tail on the left, trailing from the body of the protein, represents a G-coupled receptor that can turn CB1 and CB2 off and on. (From Medj et al., PubMed Data Base.)

cannabinoids found in marijuana plants can also exert effects via G proteins, which negatively modulate cyclic AMP levels and activate the inward rectifying K^+ channels (Mormina et al., 2006). Manipulation at either receptor site may have important clinical consequences, and therapies based upon cannabinoid receptor interactions are under development.

The highest concentrations of the CB1 receptor are found in the brain and nerve tissues. Stimulation of presynaptic CB1 receptors inhibits neurotransmitter release by stimulating K^+ channels and inhibiting calcium channels (Howlett, 2002). The highest levels of CB2 receptors are found in the immune tissues such as the spleen, thymus, and tonsils; their stimulation seems to have immunosuppressive effects, including inhibition of proinflammatory cytokine production (Maresz et al., 2007).

In the periphery, CB receptors are expressed in the heart, kidney, spleen, small intestine, and testis or ovary, with minimal expression in the liver (Pertwee, 1997). CB receptor stimulation leads to enhanced expression of acetyl-CoA carboxylase-1 and fatty acid synthetase and thereby increases lipogenesis (Osei-Hyiaman et al., 2005). In the normal heart, both CB1 and CB2 receptors are found in roughly equal proportions, though in patients with heart failure, the concentration of CB2 receptors is greatly elevated (Weis et al., 2010). Whether this is a cause or an effect is not known. Only CB1 receptors are found in coronary arteries and their number is increased in coronary artery disease. Blood concentrations of endocannabinoids are increased at the same time (Sugamura et al., 2009).

Animal studies have shown that cannabinoid CB1 and CB2 receptors regulate Ca^{2+} levels and/or K^+ currents in a variety of cell types, including oligodendroglial and heart cells. In oligodendroglial tissue cultures, cannabinoid compounds promote the Ca^{2+} influx elicited by transient membrane depolarization due to elevated extracellular K^+

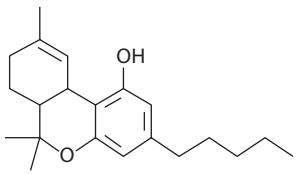
(Mato et al., 2010). If cannabinoids exert the same effect on cardiomyocytes, this could explain the tachycardia seen in marijuana smokers (Karschner et al., 2011a).

In mammals, the cannabinoid receptors for THC, particularly CB1, as well as its endogenous ligands, and the endocannabinoids anandamide and 2-arachidonoylglycerol have an effect on the control of energy balance in mammals. Cannabinoids are known to enhance energy storage in adipose tissue and reduce energy expenditure by influencing both lipid and glucose metabolism. Although lipid and glucose metabolism are normally well controlled by the complex interactions between hormones and neuropeptides, marijuana can disrupt both central and peripheral aspects of the endocannabinoid regulation of energy balance, possibly contributing to obesity and dyslipidemia (Di Marzo et al., 2011). All of these observations suggest that cannabinoid agonists and antagonists may have a role in the treatment of many different diseases, although their forensic significance remains questionable.

9.4 Metabolism and Disposition

A summary of marijuana's physiochemical properties and pharmacokinetics is given in Table 9.1.

Table 9.1 Physiochemical Properties and Pharmacokinetics of Δ9-tetrahydrocannabinol

Chemical Name	(−)-*trans*-Δ9-tetrahydrocannabinol
Physiochemical properties, structure, and form	Plant material, resin, or in an oil base CAS 1972-08-3 MW 314.5 
Synonyms and common names	Bhang, blunts, dagga, dope, ganja, grass, hashish, marijuana, kif, spliff, dope, pot, weed, sinsemilla
Brand names	Dronabinol (as Marinol®)
Pharmacokinetic parameters	Bioavailability 6%–20% (oral), 10%–35% (inhalation) C_{max} (oral) usually <10 ng/mL C_{max} (inhalation) up to about 200 ng/mL V_d 9–11 L/kg Protein binding 95%–99%
Common blood concentrations in drug users	Up to ~200 ng/mL after smoking, reducing rapidly post smoking; usually <10 ng/mL when taken orally Plasma-to-blood concentration ratio ~1.6
Blood half-life	Apparent half-life (0–8 h) 1–2 h Terminal elimination half-life 2–5 days
Metabolism	Hydroxylation and oxidation with conjugation to 11-OH and carboxy metabolites, numerous known metabolites. 11-OH is active but only formed in significant amounts when THC is taken orally. CYP2C9 is the main oxidative enzyme
Excretion	30% in urine; 40% in feces over several days
Postmortem artifacts	Likely to both increase and decrease after death by approximately twofold
Key papers	Hunt and Jones (1980), Ohlsson et al. (1980), Huestis et al. (1992a,b), McGilveray (2005), and Ramaekers et al. (2006)

9.4.1 Absorption

THC, the main psychoactive substance, is produced on heating cannabis leaf matter from the carboxylated precursor Δ9-tetrahydrocannabinolic acid A (THCA A), itself an end product of THC biosynthesis in the cannabis plant. Marijuana is almost always self-administered by smoking. Smoking marijuana results in rapid transit of the drug across the lungs to the brain. However, loss of drug occurs during the smoking process due to pyrolysis and in sidestream smoke. In an in vitro experiment specifically designed to minimize loss due to sidestream smoke, Perez-Reyes et al. (1983) documented a 30% loss of THC due to pyrolysis alone. Once THC reaches the lungs, it is rapidly absorbed. Peak plasma THC concentrations of 100–200 ng/mL occur after 3–8 min. THC is present in blood after the first puff from a marijuana cigarette. In controlled studies, mean ± SD THC concentrations of 7.0 ± 8.1 ng/mL and 18.1 ± 12.0 ng/mL were observed after the first inhalation of low-dose (1.75%) or high-dose (3.55%) marijuana cigarettes, respectively. A different study found peak concentrations 9 min after the first puff (Lemberger et al., 1970). Cone and Huestis (1993) demonstrated that physiological and subjective measures of drug effect occurred simultaneously with the rise in blood THC concentrations.

Reported values for the bioavailability of THC after smoking range from 18% to 50%. This wide range reflects the large inter- and intrasubject variability that occurs with smoking (McGilveray, 2005). Altering the number, duration, and spacing of puffs, the length of time the inhalation is held, and the inhalation volume or depth of puff all may vary the amount of drug delivered (Grotenhermen, 2003), although most people tend to titrate the frequency of inhalations to their desired physiological response.

Measures can be taken to minimize the loss of side and mainstream smoke, as well as to optimize the temperature for drug volatilization. Such measures increase the amount of drug available for delivery to the lungs. One facet of smoking that cannot be controlled by the smoker is drug deposition on nonabsorbent, or poorly absorbing, surfaces within the body (e.g., the nasopharyngeal region or the upper bronchial tree). Deposition outside of the lungs is usually a function of drug particle or vapor size. This reduces the amount of drug reaching the lung alveoli where rapid absorption into the blood and subsequent transport to the brain occurs.

THC is also well absorbed after oral administration, but bioavailability is low, with a much lower peak plasma concentration than after smoking, which takes much longer to achieve. Perez-Reyes et al. (1983) reported a mean peak plasma THC concentration of 6 ng/mL after oral ingestion of 20 mg, whereas Wall and Perez-Reyes (1981) found that peak plasma THC concentrations occurred 30 min after intravenous administration. Substantial amounts of the 11-hydroxy metabolite (11-OH-THC) are produced following oral administration, resulting in concentrations similar to those of THC.

9.4.2 Distribution

THC is 95%–99% bound to plasma protein with very little present in red blood cells. Highly perfused organs, such as the brain, accumulate THC rapidly after administration, whereas THC distributes more slowly into, and is released more slowly from poorly perfused tissues such as fat. The distribution of THC into various tissues and organs, such as brain, liver, heart, kidney, salivary glands, breast milk, fat, and lung is a result of its extremely large volume of distribution (~10 L/kg) (Ohlsson et al., 1980; Johansson and Halldin, 1989).

Hunt and Jones (1980) proposed a four-compartment model to describe THC distribution after intravenous injection. In their studies, they observed average half-lives of 1 min, 4 min, 1 h, and 19 h to distribute into each of the compartments (Hunt and Jones, 1980). They concluded that "pseudoequilibrium" is achieved between plasma and tissues 6 h after an intravenous dose. Thereafter, THC is slowly eliminated as it diffuses from tissue to the blood. The terminal elimination half-life is at least 1 day but has been reported to be as long as 3–13 days in frequent users, although at this point the blood THC concentration is usually well below 5 ng/mL (Huestis et al., 1992a,b).

9.4.3 Metabolism and Excretion

The liver metabolizes almost all THC. Hepatic metabolism consists primarily of hydroxylation by CYP2C9 to form the 11-OH- form of THC, which is also psychoactive. Hydroxylation is followed by oxidization to the carboxy metabolite (THC-COOH), probably by alcohol dehydrogenase, but possibly by a microsomal enzyme known as aldehyde oxygenase, another member of the CYP2 subfamily. This acid metabolite may be conjugated with glucuronic acid and substantial amounts may be excreted in the urine.

Multiple other compounds are produced by the actions of CYP isozymes. In addition to catalyzing the formation of significant amounts of 7-OH-THC, it has also been shown that hepatic microsomes form 6β-OH-THC at approximately the same rate. In addition, 1α, 2α-epoxyhexahydrocannabinol is also produced, but at only one-third the rate of 7-OH-THC (Bornheim et al., 1992). Several other minor oxidation products are also produced. THC is eventually excreted as THC-COOH in the urine after first forming a glucuronide at the carboxyl group.

Over 20 metabolites have been identified in the urine and feces of human marijuana users (Widman et al., 1975). Metabolism in humans involves allylic oxidation, epoxidation, aliphatic oxidation, decarboxylation, and conjugation. The two monohydroxy metabolites, 11-OH-THC and 8β-OH-THC, are active, with the former exhibiting similar activity and disposition to THC, while the latter is less potent.

The average plasma clearance for THC is 600–980 mL/min with a blood clearance of 1.0–1.6 L/min, which is very close to hepatic blood flow, making the rate of THC metabolism dependent on hepatic blood flow. Approximately 70% of a dose of THC is excreted in the urine (30%) and feces (40%) within 72 h. Because significant quantities of the metabolites are excreted in the feces, enterohepatic recirculation of THC metabolites is likely. This might explain the slow elimination, and hence the long plasma half-life, of THC. Unchanged THC is present only in very low amounts in the urine (around 2% of a given dose) but can be found in the urine for at least 24 h (Lowe et al., 2009).

The other urinary metabolites consist of conjugates of THC-COOH and unidentified acidic products. Following a single smoked 10 mg dose of THC, urinary THC-COOH concentrations peak within 16 h of smoking at levels of 6–129 ng/mL (McBurney et al., 1986). Huestis and Cone (1998) reported a mean (± SEM) urinary excretion half-life for THC-COOH of 31.5 ± 1 h and 28.6 ± 1.5 h in six healthy volunteers after administration of a single marijuana cigarette containing 1.75% or 3.55% THC, respectively.

Passive exposure to marijuana smoke may also produce detectable urinary metabolite concentrations, but the concentrations are so small they could never account for a workplace positive drug test unless persons are exposed to sidestream smoke in a

small, underventilated, room. In 2010, Dutch researchers analyzed the findings in eight healthy volunteers exposed to cannabis smoke for 3 h while sitting in a well-attended "coffee shop" (such shops contain high ambient levels of THC smoke). Blood samples were taken at baseline and again at 1.5, 3.5, 6, and 14 h after the start of the study. All the volunteers absorbed some THC, but the concentrations detected were very low. None of the urine samples produced immunoassay results above the cutoff concentration of 25 ng/mL. THC-COOH concentrations of up to 5.0 and 7.8 ng/mL before and after hydrolysis, respectively, were found in the urine. THC could be detected in trace amounts, close to the levels of detection, in the first two blood samples after initial exposure (1.5 and 3.5 h), but not after 6 h. THC-COOH could be detected after 1.5 h and was still detectable in three of the eight participants after 14 h in concentrations between 0.5 and 1.0 ng/mL (Rohrich et al., 2010).

THC may be ingested orally by consuming food products containing the seeds or oil of the hemp plant. Ingestion of 0.6 mg/day (equivalent to 125 mL of hemp oil containing 5 μg/g of THC or 300 g of hulled seeds containing 2 μg/g) for 10 days resulted in urine THC-COOH concentrations of < 6 ng/mL (Leson et al., 2001). In another study, the maximum urinary concentration of THC-COOH after ingestion of hemp oil containing 0.39–0.47 mg of THC/day for 5 days was 5.4–38.2 ng/mL ($n = 7$). In still another study, oral administration of a higher dose (7.5 or 14.8 mg of THC/day), peak urinary concentrations of THC-COOH ranged from 19.0 to 436 ng/mL.

Controlled studies have shown that at the federally mandated U.S. and European Union cannabinoid cutoffs, it is possible, but unlikely, for a urine specimen to test positive after ingestion of manufacturer-recommended doses of low-THC hemp oils (Gustafson et al., 2003a). On the other hand, a patient taking Marinol, the synthetic form of THC approved by the U.S. Food and Drug Administration for the control of nausea and vomiting in cancer patients, will almost certainly test positive. Dronabinol or synthetic THC is present in Marinol capsules. Elsohly and Slade (2005) found that within 24 h of administering a single 15 mg dose of dronabinol to four subjects, peak urine THC-COOH concentrations were between 189 and 362 ng/mL.

Since synthetic THC (dronabinol, Marinol) and naturally occurring THC are identical, this presents difficulties in determining if the source of urinary THC metabolites is the antiemetic or signifies that the person being tested is a marijuana smoker. One way to make the distinction is to test for the C3 homolog of THC, known as Δ9-tetrahydrocannabivarin (THCV). THCV is a natural component of most cannabis products and is found, along with THC, in the marijuana plant. Marinol is a synthetic product and contains no THCV. THCV is metabolized by human hepatocytes to 11-nor-Δ9-THCV-9-carboxylic acid (THCV-COOH), and its presence in a urine sample should be taken as proof of marijuana smoking. This theory has been confirmed in a controlled study in human volunteers (Leson et al., 2001).

9.4.4 Blood and Tissue Levels

THC blood levels increase very quickly after smoking, peaking before the smoker has finished the cigarette; concentrations then rapidly decline. At any given time, mean peak blood concentrations of 11-OH-THC are much lower than those of THC, with peak levels occurring just as smoking is complete. Levels of the inactive THC-COOH rise very gradually, plateau, and then persist for some hours. Peak THC-COOH levels occur approximately

2 h after the end of smoking. Blood concentrations of the conjugated THC-COOH–glucuronides are always higher than concentrations of THC. THC-COOH–glucuronides are labile and can convert back to free THC-COOH, hence complicating any interpretation, particularly when there is a long storage period or putrefaction has occurred.

Plasma concentrations of 11-OH-THC after marijuana smoking typically amount to <10% of those of THC but are similar to THC concentrations if marijuana is consumed orally. Two additional hydroxy compounds have been identified, namely, 8α-OH-THC and 8,11-dihydroxy-THC, but they are believed to be devoid of THC-like activity.

Daily smokers accumulate cannabinoids in their blood, as was demonstrated when clearance time was measured in 28 daily marijuana smokers. At the time of admission, 15 had no cannabinoids detectable in whole blood samples. In the remainder, the mean concentrations of THC, 11-OH-THC, and THC-COOH were 1.1 ± 1.7 (range 0.03–7.0), 1.1 ± 1.7 (range 0.3–6.3), and 19.2 ± 20.4 ng/mL, respectively. After 7 days of witnessed abstinence, the values were 1.3 ± 1.0 (range 0.4–4.0), 0.6 ± 0.3 (range 0.3–0.9), and 6.0 ± 8.4 (range 0.4–36.5) ng/mL, respectively; a few of the participants actually had a modest increase in whole blood THC concentration (Schwilke et al., 2007). This confirms that THC remains in deep fat stores and is slowly released back into the blood over an as yet to be defined interval. Chronic heavy marijuana smokers may have whole blood concentrations in excess of 5 ng/mL even when they have not been actively smoking for some days after last use.

Gronewold and Skopp (2011) measured THC and its major metabolites, including 11-OH-THC, THC-COOH, and its glucuronide (THCCOOglu), as well as the cannabinoids CBD and cannabinol (CBN), in order to estimate redistribution in five autopsy cases (4 men and 1 woman, ranging in age from 24 to 31 years). In no case was the anatomic cause of death immediately apparent. All five cadavers were autopsied within 24 h of death and stored at 4°C. Samples of cerebrospinal fluid, bile, gastric contents, and small pieces of tissue taken from the brain, liver, lungs, muscle (psoas), and kidneys were collected and analyzed. Except for femoral blood, which was stored at 8°C, all of the other specimens were stored frozen (−22°C) and thawed just prior to analysis. The highest concentrations of all the metabolites, except THC and CBD, were found in bile. The highest concentrations of THC were found in muscle tissue, as were the highest concentrations of CBD. Conversely, metabolites of THC were nearly undetectable in muscle tissue, even though their concentrations were very high in bile. THC was also measurable in the lungs, but levels in the liver were low or undetectable. Essentially none of the metabolites could be detected in brain, but high concentrations of all the metabolites were found in the kidney and liver, which is only to be expected since THC glucuronidation occurs in the liver and excretion occurs in kidneys. Also of note was that cannabinoids were hardly detectable within the vitreous humor.

Similar findings have been found in an experimental model (Brunet et al., 2010). In the animal studies, the site of collection had a profound effect on the concentration of THC detected. Postmortem blood THC concentrations ranged from 0.7 to 10.2 ng/mL (mean 3.2 ± 3.3 ng/mL) in the inferior vena cava, from 1.7 to 37.4 ng/mL (mean 10.9 ± 9.9 ng/mL) in the right cardiac ventricle, and from 3.2 to 48.8 ng/mL (mean 17.5 ± 15.5 ng/mL) in the left cardiac ventricle. Concentrations in both ventricles were higher than in blood from vena cava. The findings are particularly relevant because the experimenters found that peak THC concentration in pig blood samples was essentially the same as in human samples.

In one study, published nearly 20 years ago, THC concentrations in fat samples from heavy marijuana users obtained 1 week before and 4 weeks after smoking cessation were analyzed. The concentration of THC in these samples ranged between 0.4 and 193 ng/g wet tissue (Johansson and Halldin, 1989). Other studies have demonstrated that the ability of adipose tissue to store THC is almost limitless (Nahas, 2001). Nonetheless, fat THC concentrations are rarely measured, although it would be a perfectly reasonable matrix for the qualitative demonstration of THC in the postmortem setting.

Sativex, an oromucosal spray containing THC and CBD, is being evaluated as an adjunct to opioids for treatment of cancer pain and spasticity. A double-blind randomized study of 5 and 15 mg of synthetic oral THC, low-dose Sativex (5.4 mg of THC and 5.0 mg of CBD) and high-dose Sativex (16.2 mg of THC and 15.0 of mg CBD), in nine cannabis smokers showed no significant differences in maximum plasma concentrations and areas under the curve between similar oral THC and Sativex doses (Karschner et al., 2011b). This shows that CBD does not affect the pharmacokinetics of THC.

A novel formulation of Δ-9-THC (98% pure THC tablets) called Namisol has now successfully completed Stage II clinical trials. In a human study (double-blind, double-dummy, randomized, crossover), six men and six women were treated with sublingual (crushed tablet) and oral administration of 5 mg of THC. Sublingual administration showed a flat concentration profile compared with oral administration. The apparent half-life of oral THC was 72–80 min, T_{max} 39–56 min, and C_{max} 2.92–4.69 ng/mL. Heart rate increased (5.6 beats/min, 95% CI 2.7, 6.5), but otherwise no ill effects were observed (Klumpers et al., 2012). Positive therapeutic effects are yet to be established.

9.5 Detection Times

This is a key issue in workplace testing and situations such as testing for driving under the influence, where actual last use of cannabis needs to be reconciled with reported last use and the likelihood of impairment. The problem is that depending on the extent of use, the main metabolite (THC-COOH) may be detectable in urine from a few days up to weeks after last use, and there is no accurate way to predict the time since last use (Westin, 2011). To differentiate between new intake and residual drug excretion, urinary THC-COOH concentrations must be measured quantitatively, and the interpretation must take into account urine dilution (and urine creatinine) and the time span between the dates of specimen collection and concentration measurement (Westin, 2011). The problem is illustrated by a case study reported in 2008. Urine was collected from seven healthy volunteers (aged 20–35 years; four male), all of whom were chronic cannabis users, during enforced abstinence on a locked ward for periods as long as 29 days. Every urine specimen collected during the period of confinement was analyzed using a method that had a 2.5 ng/mL limit of quantification. The minimum time until the urine was cleared of 11-OH-THC ranged from 7.56 to 29.8 days, with concentrations ranging from 25 to 133 ng/mL. Maximum urinary concentrations of THC-COOH fell into the same time range as for 11-OH-THC. In federally regulated workplace testing, a 15 ng/mL cutoff for THC-COOH (after hydrolysis of conjugates) is considered positive, and these would have been considered active marijuana smokers even though they had not smoked for more than 1 week (Lowe et al., 2009).

Forensically robust conclusions also cannot be drawn from plasma concentrations. Twenty-eight self-reported daily marijuana smokers (aged 19 to 36 years, with roughly equal numbers of men and women, 84% of whom were African American) underwent enforced abstinence in a locked ward (Karschner et al., 2008). Plasma specimens were collected when the volunteers arrived on the locked ward and then daily. After not smoking marijuana for 16 h, 93% of the participants were still positive for the drug (THC >0.25 ng/mL—the minimum level of detection). On the seventh day of observed abstinence, half of the participants continued to test positive for THC, and four of these individuals had levels >2.0 ng/mL, the value that is usually considered proof of recent use by the EU and some U.S. states. The median THC-COOH concentration in this group was 11.5 ng/mL after 1 week's abstinence. This demonstrates that the detection of THC in plasma does not reliably differentiate between acute and chronic use when blood concentrations are less than about 5 ng/mL (about 8–10 ng/mL in plasma). This observation is almost certainly explained by the accumulation of THC in deep tissue compartments, with gradual release of THC from tissue stores into the bloodstream, particularly in persons who have accumulated large amounts of THC to the point of saturating the storage sites within their tissues. In the living, the detection of low THC concentrations (<5 ng/mL blood) does not reliably identify recent users (Karschner et al., 2008). The same is true in the postmortem setting, where the degree of tissue release can be greater.

Two more recent studies tend to support the variable and sometimes long excretion times in urine. In the first study, in an attempt to confirm the validity of now 30-year-old studies, Swiss researchers evaluated 12 male volunteers who were occasional marijuana smokers. Each smoked a cannabis cigarette standardized to contain 70 mg of THC. Plasma and urine were collected simultaneously. Total THC, THC-OH, and THC-COOH were all determined after hydrolysis followed by solid-phase extraction and gas chromatography–mass spectroscopy (GC–MS). Eight puffs delivered a mean THC dose of 45 mg, in line with previous results of bioavailability. Plasma levels of total THC, THC-OH, and THC-COOH ranged from 0.2 to 59, 0.1 to 3.9, and 0.4 to 16 ng/mL, respectively. Peak concentrations were observed at 5, 5–20, and 20–180 min, respectively. Urine levels ranged from 0.1–1.3, 0.1–14, and 0.5–38 ng/mL, peaking at 2, 2, and 6–24 h, respectively. The times of the last detectable levels were 2–8, 6–96, and 48–120 h, respectively. In addition, high to very high THC-COOH (245 ± 111 ng/mL), THC (3 ± 8 ng/mL), and THC-OH (51 ± 246 ng/mL) concentrations were found in 65% and 98% of cannabis-positive urine samples, respectively (Brenneisen et al. 2010).

Cannabinoid pharmacokinetics in occasional marijuana users differ from those in heavy chronic users (usually defined as marijuana use 4–25 times in the previous week). In the second study, a controlled study of 12 heavy cannabis smokers compared to light smokers, most of the heavy smokers had plasma THC concentrations in excess of 12 ng/mL even before the study, that is, before smoking either marijuana or placebo. During the 8 h after smoking, their distribution and elimination patterns were comparable to those of the occasional users and the concentrations returned to 68%–196% (median 110%) of the initial values. However, the maximal concentrations and the actual amount absorbed were significantly higher in the heavy users, but they varied markedly from person to person (Toennes et al., 2008). In short, the observed concentration ranges are too broad to allow accurate prediction of dose or time of use.

9.5.1 Alternative Matrices

9.5.1.1 Saliva Testing

THC is absorbed into the tissues of the oral cavity, largely from deposition during smoking, and is present in oral fluid. The oral fluid-to-plasma (or blood) ratio is quite variable within and between subjects and is also dependent on the type of collection device used. In one study, oral fluid-to-serum ratios showed a wide distribution of 0.3–425 (median 16) among volunteers who smoked a standard joint (average dose 33 mg) (Toennes et al., 2010). The maximum concentrations in occasional users (0.4–6.4 μg/g) were lower than those in chronic users (0.4–72 μg/g). THC tended to persist for longer in oral fluid than serum, probably due to the higher concentrations achieved. Earlier studies found much lower oral fluid-to-serum ratios (0.5–2.2), possibly due to use of collection devices that stimulated saliva production (Huestis and Cone, 2004).

Oral fluid-to-serum ratios were even higher in another study in volunteers smoking low (18 mg of smoked THC) and high doses (36 mg) of marijuana—46 and 36, respectively. THC concentrations in oral fluid peaked very early after the start of smoking (0.25 h) at 900 (SD 589) and 1041 (SD 652) ng/mL, respectively. At 6 h the concentration was 18 (SD 12) ng/mL (Kauert et al., 2007).

While there is a relationship between oral fluid and plasma THC concentrations, it is not strong enough to allow the concentration of one to be predicted from the other (Huestis and Cone, 2004; Ramaekers et al., 2006). The measurement of THC-COOH in oral fluid has been suggested as a way to distinguish personal use from inadvertent exposure (Milman et al., 2010). In Australia, random testing of drivers for THC in oral fluid showed an overall incidence of about 0.7% and a median oral fluid concentration of 81 ng/mL (Drummer et al., 2007).

9.5.1.2 Hair Testing

THC is present in hair after marijuana use, as is THC-COOH and other cannabinoids such as CBD and CBN.

Subjects using marijuana are found to have a wide range in hair concentrations. In one study, THC and THC-COOH concentrations ranged from 3.4 to >100 pg/mg of hair and from 0.10 to 7.3 pg/mg of hair, respectively. There was a weak correlation between THC and THC-COOH concentrations ($r = 0.38$) (Huestis et al., 2007). The sum total of cannabinoids had a better correlation with cumulative use of the drug, but the relationship was still weak (Skopp et al., 2007).

One of the more significant issues in hair analyses is differentiating inadvertent exposure from actual personal use. Sidestream smoke and other types of environmental contamination can cause hair samples to test positive for THC (Uhl and Sachs, 2004). Analysis of hair for THC-COOH is recommended to distinguish inadvertent from personal use. However, the acidic nature of this metabolite provides a much lower rate of incorporation into hair compared to THC. For example, concentrations of THC-COOH in the hair of users ranged from 0.002 to 0.39 ng/mg of hair, with an average of 0.12 ng/mg of hair (Kintz et al., 1995). The Society of Hair Testing recommends that to confirm marijuana use, THC-COOH should be detected in concentrations of >0.2 pg/mg of hair (see http://www.soht.org). This is much lower than the cutoff used for THC (0.1 ng/mg).

Δ9-THCA A is the preliminary end product of THC biosynthesis in the cannabis plant. Unlike THC, it is nonpsychoactive and is regarded as a THC precursor. When heated or

smoked, it is largely decarboxylated. This substance is present in blood and urine of cannabis users, as well as in hair (Auwarter et al., 2010). It also can be found in exhaled smoke and could be a cause of external hair contamination.

9.6 Cardiovascular Effects

In humans, the vascular response to THC is a largely dose-dependent increase in heart rate, usually accompanied by a mild increase in systolic pressure. Orthostatic hypotension is a recognized complication in occasional users but is generally not an issue because near complete tolerance quickly develops to THC's tachycardic and hypotensive effects. Electrocardiographic alterations produced by marijuana smoking are said to be minimal (Benowitz and Jones, 1975).

Smoking marijuana is believed by some to trigger myocardial infarction in individuals who already have preexisting coronary artery disease, and there is a good rationale for thinking so. If a preexisting coronary artery stenosis exists and CB1 stimulation causes the heart rate to increase, then the myocardium may be compromised further by the inability of the diseased arteries to increase oxygen supply in the face of the increased demand caused by an increased heart rate. This possibility is supported by the results of a clinical study involving nearly 4000 patients (1258 women) who had been hospitalized with acute myocardial infarction. When interviewed, 124 (3.2%) reported smoking marijuana in the prior year, 37 within the previous 24 h, and 9 within 1 h of the onset of symptoms. Typical patients were more likely to be men (94% vs. 67%, p <0.001), more likely to be current cigarette smokers (68% vs. 32%, p <0.001), and more likely to be obese (43% vs. 32%, p = 0.008). The risk of myocardial infarction onset in the marijuana smokers was elevated 4.8 times over baseline (95% confidence interval, 2.4–9.5) in 60 min after marijuana use, dropping to a relative risk of 1.7 in the second hour, after which no increased risk was apparent (Mittleman et al., 2001).

A number of case reports have described episodes of acute coronary syndrome in marijuana smokers who were later found to have normal coronary arteries (Dwivedi et al., 2008; Basnet et al., 2009; Kocabay et al., 2009; Bailly et al., 2010; Safaa et al., 2012), leading to the conclusion that coronary artery spasm, probably induced by stimulation of CB1 receptors in the coronary arteries, had occurred. However, this contention is impossible to prove in the case of fatality and incomplete autopsy; myocarditis can cause exactly the same symptoms as coronary spasm, including similar electrocardiography changes. Even if an autopsy were performed, myocarditis still could not be excluded without DNA resequencing or immunohistochemical testing. Thus, it cannot be said with any certainty whether a coronary event is a consequence of marijuana-induced coronary artery spasm or undiagnosed myocarditis (Pomara et al., 2006; Basic et al., 2010; Agewall et al., 2011).

Adding to the confusion is the proven ability of the activated CB2 receptor to protect against myocardial ischemia/reperfusion injury (Lamontagne et al., 2006; Pacher and Hasko, 2008) and the observation that both CB1 and CB2 receptors exist on platelets. Whether their presence in this location plays any role in thrombus formation is not known (Mendizabal and Adler-Graschinsky, 2007; Catani et al., 2010).

In summary, it is far too early to determine whether these cardiovascular effects are of clinical benefit or detriment.

9.7 Pulmonary Complications

Chronic marijuana smokers are generally found to have nondiagnostic lung damage (Barsky et al., 1998) (Figure 9.7). Changes consistent with acute and chronic bronchitis may be apparent, but there is no way to distinguish the changes caused by marijuana smoking from those caused by cigarette smoking or the inhalation of any other environmental pollutant. In the only major population-based study, involving nearly 1000 smokers, smoking both tobacco and marijuana synergistically increased the risk of respiratory symptoms and chronic obstructive pulmonary disease (COPD). Smoking only marijuana does not appear to be associated with an increased risk of respiratory symptoms or COPD (Tan et al., 2009).

Marijuana smoking generates a greater amount of particulate matter than does cigarette smoking, making damage to the respiratory tract more likely. In the only published autopsy series, the lungs were examined in 13 cases of sudden death in known marijuana smokers (aged 15–40 years). Accumulations of pigmented monocytes were present within the alveoli, and variable, spotty infiltrates of monocytes and lymphocytes were seen within the interstitium (Morris, 1985). Whether or not the changes are dose related is not known.

THC clearly alters human immune responses. Alveolar macrophages recovered from the lungs of marijuana smokers have a decreased ability to release proinflammatory cytokines and to produce nitric oxide (NO). This makes these macrophages less effective at killing bacteria. Lymphocytes of marijuana smokers contain increased amounts of mRNA encoding for both CB1 and CB2 receptors. THC suppresses T-cell proliferation, inhibits the release of interferon-γ, and alters the production of T-helper cytokines (Roth et al., 2002)

9.8 Synthetic Cannabinoids

This group of compounds has been available for more than 50 years but only recently have they begun to appear under different names on the black market. Three series in particular stand out: (1) the HU series developed by Hebrew University in the 1960s, (2) the cyclohexylphenol series developed by Pfizer in the 1970s, and (3) the JWH series developed by John

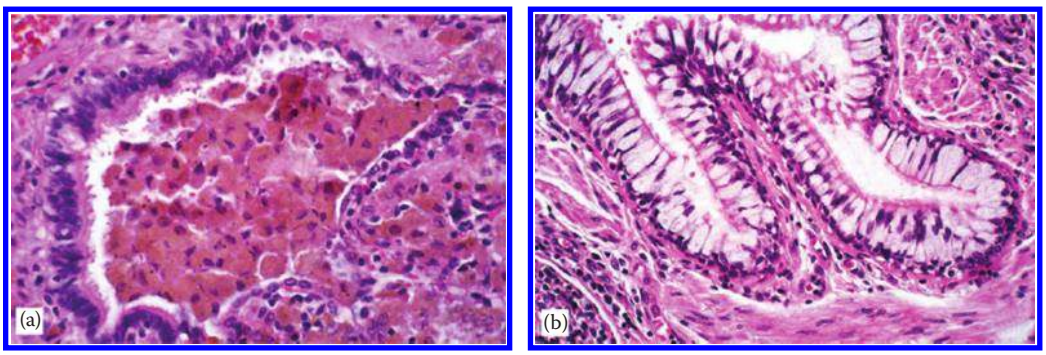


Figure 9.7 (a) Pigmented macrophages around the bronchiole in a 19-year-old marijuana smoker. (b) goblet cell hyperplasia. (From Tomashfeski et al., *Curr. Diagn. Pathol.*, 204, 413. With permission.)

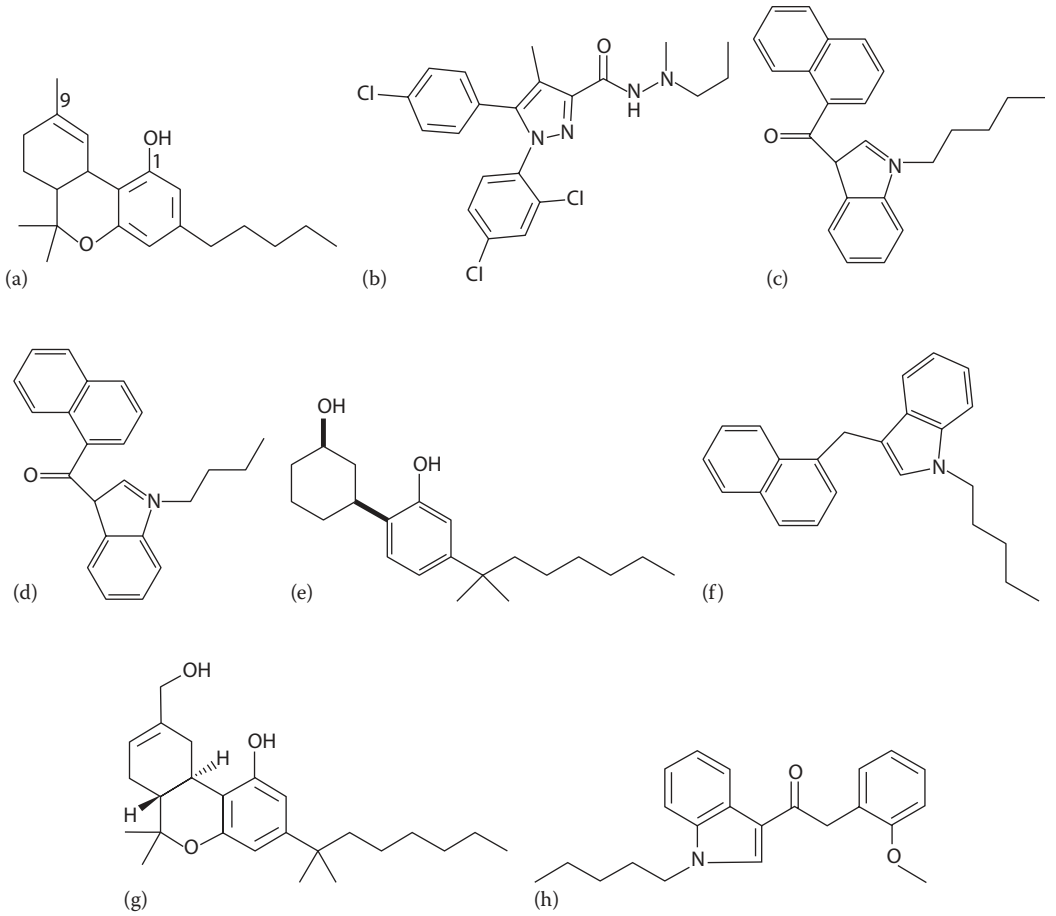


Figure 9.8 Structures of some THC analogs. (a) THC, (b) rimonabant, (c) JWH-018, (d) JWH-073, (e) CP-47, 497, (f) JWH-175, (g) HU-210, and (h) JWH-250.

W. Huffman in the 1990s (Figure 9.8). These drugs share strong similarities. They should not be confused with "bath salts," which are synthetic cathinones, completely unrelated to marijuana. Nonetheless, the JWH compounds are potent, nonselective agonists of G-protein-coupled cannabinoid receptors.

Various amounts and combinations of synthetic cannabinoids are sprayed onto a blend of herbs and then sold as herbal incense under many different names, including K2, K9, Spice, and Skunk (Vardakou et al., 2010) (Figure 9.9). However, they are also smoked and produce symptoms closely mimicking intoxication with marijuana, but these symptoms persist for much longer. These drugs are also much harder to detect on typical urine screens (Lindigkeit et al., 2009). In order to improve detection, the Cayman Chemical Company (Ann Arbor, MI, USA) has synthesized a number of JWH compounds, and its results suggest that the ability to effectively detect synthetic CBs in urine and serum is near at hand, at least for those that have been identified.

These herbal mixtures have emerged as popular legal alternatives to marijuana, especially among adolescents and young adults (Brook, 2011). On March 1, 2011, the Drug Enforcement Administration (DEA) finally issued an order temporarily banning five synthetic cannabinoids (JWH-018; JWH-073; JWH-200; CP-47,497; and CP-47,497 C8) that

Figure 9.9 Commercial versions of "Spice" or "K2" sold in head shops and over the Internet. They are generally labeled "not for human consumption."

had already been outlawed in 18 other countries (Brook, 2011). Similar legal bans have now been adopted in the European Union, Australia, and New Zealand.

German scientists were the first to detect the presence of JWH-018, or its homolog CP 47, 497, in one of these "herbal" mixtures. These same compounds can be found in several different herbal blends, including the original Spice product (Lindigkeit et al., 2009). When the chemists who produced these drugs tried them on themselves, they noted mood changes, alterations in perception, and deep conjunctival injection. Tachycardia and xerostomia also occurred. However, almost as soon as sale of the JWH compounds was restricted, a new synthetic cannabinoid receptor agonist (XLR-11), often sold under the name "blueberry spice," was identified in some apparent cases of driving under the influence (Lemos, 2014).

In an analysis of 46 differently named herbal products, Uchiyama et al. (2011) found varying ratios of JWH-018, JWH-073, and CP-47, 497 C8 in 44 of the confiscated samples (Administration, 1980). Many samples also contained CP-47,497, its *trans*-diastereomer, and the *trans*-diastereomer of CP-47,497 C8, as well as the endo CB oleamide. In addition, three other compounds related to CP-47,497 were observed, but not formally identified. Taken together, these studies demonstrated that many herbal blends are actually blends of natural and synthetic cannabinoids.

Little is known about the metabolism of synthetic cannabinoids, so most episodes of use are missed by drug testing laboratories. However, some recent analytic studies of urine samples following herbal product ingestion did detect them and some of their metabolites. These results supported an earlier analysis of the metabolism of JWH-015 performed in liver microsomes. Studies on the identification of JWH-018 in human serum following its

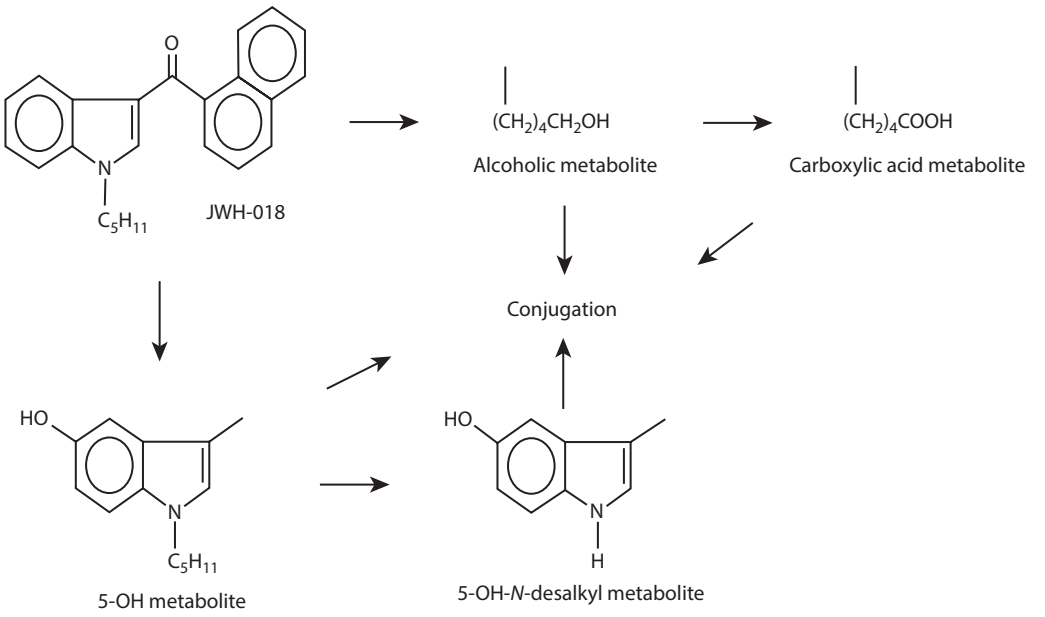


Figure 9.10 "Spice" metabolites.

consumption by smoking indicate that this drug remains in the circulation for a few hours. Measuring its metabolites can therefore, be a forensically valuable tool.

JWH-018 is biotransformed primarily via hydroxylation of its indole ring phenyl group, along with one or more of the pentyl chain carbons (Figure 9.10). Minor pathways include *N*-dealkylation, mono- and dihydroxylation of the naphthalene ring, and carboxylic acid formation from the pentyl alcohol product. At least 13 different metabolites have been identified, most having undergone glucuronidation. The major urinary metabolite is monohydroxylated on the alkyl side chain (Sobolevsky et al., 2010). The other major urinary metabolite is monohydroxylated on the alkyl side chain (Möller, 2011). Concentrations of this alcoholic metabolite, primarily in conjugated form, were found to range from 6 to 50 ng/mL in urine from suspected users (Beuck et al., 2011; Moran et al., 2011). When two healthy adults smoked a powdered form of JWH-018 (50 mg/70 kg dose), serum concentrations reached values of approximately 10 ng/mL and, within 3 h, dropped to <10% of the measured maximum concentrations (Teske et al., 2010).

What makes these compounds particularly interesting is that their metabolites, unlike those of most drugs, are hydroxylated compounds, as described earlier for JWH-018, but not the carboxylated metabolite; nonetheless, both retain in vitro and in vivo activity at the CB1 receptor. This receptor is located presynaptically on both glutamatergic and GABAergic synapses. This location almost guarantees that the drug will disrupt normal neural signaling. These observations, combined with the higher CB1 affinity and activity of JWH-018 relative to THC, may contribute to the greater prevalence of adverse effects observed with JWH-018-containing products relative to cannabis.

Acute renal damage has been reported as a rare complication of smoking synthetic cannabis, and it remains unclear whether this toxicity is a consequence of previously unrecognized nephrotoxicity or the presence of some unknown contaminant. An outbreak of renal disease associated with synthetic cannabis in 19 individuals occurred

in the United States. The more or less simultaneously reported cases occurred in six separate parts of the country. Acute tubular injury was documented in four of the six patients who underwent renal biopsy; all of the patients had evidence of acute interstitial nephritis and five required dialysis, although all recovered. Extensive toxicological testing failed to disclose any synthetic cannabinoid common to all the cases, suggesting that an adulterant was responsible (Anon., 2013). No further cases have been reported.

Recent online reviews by users suggest that sellers are offering different blends, some spiked with CBs and some not, with different blends being sent to different countries. Customers, as well as enforcement agencies, may be challenged when trying to work out what any particular blend contains as this may not match what is on the label. There are no autopsy reports and no tissue studies, except for the aforementioned renal biopsies.

By the end of 2011, at least two deaths and multiple hospitalizations attributable to K2 ingestion had been reported. One adolescent committed suicide after smoking K2; he had been experiencing extreme anxiety following use. A second adolescent died following a coronary ischemic event (Gay, 2010). Another report described a 48-year-old man who experienced a generalized seizure within 30 min of ingesting a mixture of white powder that he had purchased from the Internet and then dissolved in ethanol. In addition to the grand mal seizure, he also experienced refractory supraventricular tachycardia on day 1 of his hospitalization. His blood ethanol concentration was 3.8 mg/dL and creatinine phosphokinase 2649 U/L. Urine drug screening by EMIT was negative for common drugs of abuse, including THC, but later urinalysis demonstrated the presence of JWH-018 and one of its metabolites (Lapoint et al. 2011).

Marinetti and Antonides (2013) have published their findings from a large series of bath salt deaths. In 23 postmortem cases where results from multiple tissue matrices were available, the concentration range for blood methylenedioxypyrovalerone was 10–640 ng/mL, with an average value of 109 ng/mL. When peripheral and heart blood values were available, the average heart-to-peripheral blood ratio was 1.48, with a range of 1.3–1.67, suggesting that bile might be a good testing matrix. After review, the authors concluded that blood concentration does not appear to predict outcome regarding fatalities or impairment.

9.9 Selective THC Agents

Rimonabant acts as a selective reverse agonist at CB1 receptors and blocks the effects of THC (Huestis et al., 2001; Fong and Heymsfield, 2009). It was available as SR141716 (trade names Acomplia, Bethin, Monaslim, Remonabent, Riobant, Slimona, Rimoslim, Zimulti, and Riomont) for a short time to treat obesity but was withdrawn due to the risk of depression and suicidal behavior. It was also suggested that it could be used to treat nicotine dependence and improve short-term memory (Cahill and Ussher, 2007).

Ninety milligram doses are required to block the effects of standard doses of THC. Chronic dosing with 40 mg/day produces steady-state plasma concentrations within 13 days (C_{max} 196 ng/mL; C_{min} 92 ng/mL). The drug shows some instability with a 30% loss when stored at −20°C for 3 weeks (Grassin et al., 2008).

9.10 Postmortem Considerations

THC is subject to postmortem redistribution, complicating any inference of ingestion time or amount consumed. As already mentioned, large amounts of THC accumulate in fat and muscle, including the brain. In life, when a regular marijuana user stops smoking, THC is slowly released from deep body stores, so it is not uncommon to find THC-COOH in the urine weeks after use has been discontinued. There is usually little or no THC in blood if cutoffs of 5 ng/mL are used, even in heavy users.

After death, THC may continue to be released from tissues and contribute to the redistribution phenomenon. Recent studies at the Victorian Institute of Forensic Medicine have shown that blood THC concentrations often decrease in the day or two following receipt of nonputrefied cases to the mortuary, possibly due to continued uptake of THC into muscle and fat (O.H. Drummer and D. Gerostamoulos, unpublished observations). It is likely that as time since death increases, release of THC will occur from these tissues into pooled blood.

As discussed in Section 9.6, there is no way to rule out myocarditis at autopsy, short of DNA resequencing or immunohistochemical staining. However, before making the diagnosis of marijuana-related coronary spasm and myocardial ischemia, myocarditis must first be excluded. Failing that, there is insufficient evidence to support a civil or criminal charge. The incidence of a finding of myocarditis is not really known because, in many instances, no infiltrate is visible and the presence of a virus can only be demonstrated with studies not readily available to most medical examiners (Kuhl et al., 2003; Tschope et al., 2005).

9.11 Toxicology

9.11.1 Preanalytic Considerations

The use of mathematical models (Huestis et al., 1992b, 2005) to calculate time since last use, or to extrapolate to the concentration some hours before death, is to be avoided because of the unpredictable changes in blood concentration that are known to occur. Furthermore, autolysis occurs after death, making it is almost impossible to obtain serum postmortem. Therefore, it is not known whether blood or plasma (serum) concentrations are the more accurate (Giroud et al., 2001). In the case of blood samples taken after death, the use of these models to assess the time of cannabis use is not recommended.

Sample preservation is an important issue in forensic cases, and this requirement is no different for THC from any other drug. THC shows poor stability in blood when stored at ambient temperatures for extended periods (Johnson et al., 1984), and most THC is lost when samples are stored frozen at −20°C for over 1 year (Holmgren et al., 2004). Long-term stability studies in frozen urine showed losses of about 15% over 1 year (Moody et al., 1999). In serum collected from blood preserved with fluoride/oxalate and stored at −20°C for 15–32 months (average 19 months), the loss of THC, 11-OH-THC, and THC-COOH was 15%, 6.4%, and 5.2%, respectively, when compared to the original assay result (Rickert et al., 2011).

THC is an extremely lipophilic molecule that will bind to hydrophobic surfaces, leading to an apparent loss of concentration. It has been reported that THC binds to rubber stoppers and to some plastic containers (Christophersen, 1986; Levine and Smith, 1995).

9.11.2 Preferred Analytic Methods

There is no shortage of methods for the analysis of THC and its key metabolites, particularly 11-OH-THC and THC-COOH. Conventionally, laboratories have performed immunoassay screens on urine using a cutoff concentration of 50 ng/mL (total cannabinoids). Positive samples are then confirmed using GC–MS methods using a cutoff THC-COOH concentration of 15 ng/mL, although some applications have used different cutoffs (Korte et al., 1997). In forensic cases, particularly where death has occurred, there is no need to use cutoffs designed for workplace drug testing. The limits of detection and quantification usually are applied in these cases to prove prior exposure. ELISA-based kits for screening of blood/plasma, oral fluid, and hair are also available and are widely used (Pujol et al., 2007; Schwope et al., 2010).

A variety of GC–MS methods for the analysis of blood or plasma (serum) have been published (Huang et al., 2001; Steinmeyer et al., 2002; Gustafson et al., 2003b). These have used silylation (e.g., BSTFA containing 1% TMCS), methylation, or formation of perfluorinated acyl derivatives. There is generally little to distinguish the methods. More recently, liquid chromatography (LC)–MS/MS has been used as an alternative methodology for determination of these species in plasma (Maralikova and Weinmann, 2004; Teixeira et al., 2007). These methods are more easily adaptable to other fluids such as saliva and urine (Teixeira et al., 2007) and for the measurement of the glucuronide metabolite directly (Weinmann et al., 2000).

Prior hydrolysis is required if "total" THC-COOH is to be measured since most of these metabolites (and the 11-OH-THC) will be present as glucuronide conjugates (Abraham et al., 2007). THC can also be measured in hair using MS methods (Kim et al., 2005; Coulter et al., 2009). Some authors recommend measuring THC as well as CBN and CBD in order to better assess exposure to cannabis products (Kim et al., 2005; Nadulski and Pragst, 2007; Skopp et al., 2007). The measurement of THC-COOH in hair can prove personal use as distinct from external contamination; however, concentrations are much lower (in the pg/mg range, not ng/mg) than for other cannabinoids (Uhl and Sachs, 2004). Analysis of synthetic THC-like drugs is possible using either GC–MS or LC–MS/MS (Teske et al., 2010). These are not detected using conventional cannabinoid immunoassays designed for THC and/or THC-COOH, although some kits are being developed and are likely to be available soon for selected synthetic cannabinoids.

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Appendix A: Conversion Formulas

Toxicology reports are not standardized. Depending on the units of measure used, what looks like a very large number may in fact be a very small one. Forensic toxicology laboratories report results in µg/mL.

Converting Units of Measure

A blood cocaine concentration of 1200 ng/mL (approximately the plasma concentration after smoking one rock of cocaine) might be reported as

$$1200 \text{ ng/mL} = 1.2 \text{ µg/mL} = 1200 \text{ µg/L} = 1.2 \text{ mg/L}$$

The concentrations of hormones such as epinephrine are much lower than the concentrations of exogenous drugs and are usually expressed in picograms (pg). If the concentration of cocaine in the aforementioned example were expressed in picograms (which, as a practical matter, it never is), 1200 ng/mL would be equal to 1,200,000 pg/mL.

Converting Moles into Grams

Clinical laboratories express results in millimoles (mmol) or standard international (SI) units. To convert to standard concentration measurements, divide 1000 by the molecular weight and then divide that number into the concentration value expressed as µmol/L.

Example: In a research study of morphine pharmacokinetics, the maximum blood concentration after giving a 10 mg subcutaneous injection of morphine to a 70 kg man was reported as 262 ± 49 nmol/L. To convert that concentration into ng/mL,

1. Divide 1000 by the molecular weight of morphine:
   1000/285.34 (the molecular weight of morphine) = 3.50.
2. Convert nanomoles (nmol) into micromoles (µmol):
   262 nmol/L = 0.262 µmol/L.
3. Divide the number of mmol/L by 3.50:
   0.262/3.50 = 0.0748 µg/mL = 74.8 ng/mL.

Appendix B: Blood Ethanol Concentrations

Individuals who take abused drugs often ingest ethanol at the same time. Widmark's formula is the standard method used by forensic toxicologists to calculate blood ethanol concentrations (BECs) and is universally recognized by the legal system. However, an approach first suggested by Charles Winek, from Duquesne University, works equally well and is easier to remember (*Forensic Sciences,* C.W. Wecht, Ed., Matthew Binder Press, New York, chap. 31B, 1984). Winek's formula is based on the observation that a 150 lb man will have a BEC of 0.025% after drinking 1 oz of 100 proof (50%) ethanol. Given that assumption (which is accurate under almost all circumstances), then the formula for calculating the BEC is

$$\text{BEC} = (150/\text{body weight})(\%\ \text{ethanol}/50)(\text{ounces consumed})(0.025)$$

Example: A 200 lb man drinks five 12 oz cans of beer. The beer contained 4% ethanol. The BEC would be given by the following equation:

$$\text{BEC} = (150/200)(4/50)(60)(0.025)$$

$$\text{BEC} = (0.75)(0.08)(60)(0.025)$$

$$\text{BEC} = 0.090\%$$

Remember when using this calculation that it assumes, all the ethanol was ingested at one time.

Appendix C: Volume of Distribution Calculations

Some drugs, such as morphine, rapidly leave the blood and distribute widely throughout the body. Other drugs, such as morphine metabolites, stay mostly in the blood. The tendency for a molecule to remain in the blood or distribute into tissue can only be determined by actual measurement. The volume of distribution is the apparent volume needed to contain all of the drugs injected in the body at the same concentration as observed in the blood. If, for example, 10 g of food coloring were dissolved in a 10 L aquarium, the resultant concentration would be 1 g/L, and the volume of distribution, abbreviated as V_{ss}, would be 10 L. The V_{ss} for drugs that remain mostly in the bloodstream, such as the morphine glucuronides, will be much less than 1. The V_{ss} for drugs that penetrate widely into tissue, such as cocaine (V_{ss} = approximately 3), will be much greater than 1. V_{ss} calculations can be used to estimate the amount of drug administered:

$$\text{Dose} = (\text{body weight [kg]}) \times (\text{volume of distribution [L/kg]}) \times (\text{blood concentration [mg/L]})$$

V_{ss} calculations apply *only to the living*. Postmortem redistribution and other postmortem changes make V_{ss} calculations in the deceased extremely unreliable. An example taken from an actual court case appears as follows. An individual was charged with accidentally administering a lethal dose of diphenhydramine (Benadryl). Witnesses observed that the accused administered one injection with a 10 cc syringe. The decedent weighed 72.6 kg and at autopsy had a blood diphenhydramine concentration of 5.1 mg/L. Thus, the accused would have to have injected:

$$\text{Dose} = 72.6\ \text{kg} \times 4.5\ V_{ss} \times 5.1\ \text{mg/L} = 1666.2\ \text{mg}$$

The average 30 mL multidose vial of diphenhydramine contains only 500 mg. The accused had only a 10 cc syringe. If the V_{ss} calculation is to be believed, the accused would have to have injected the victim with more than three vials of diphenhydramine, a process that would have required at least 10 separate injections!

The main utility of V_{ss} calculations in postmortem investigations is as a quality assurance check of reported blood concentrations. If the V_{ss} calculation suggests that an implausible amount of drug has been ingested, an error in laboratory or sampling methods may be indicated (the blood analyzed may, for example, have been scooped from the chest cavity).

Appendix D: Methods for Determining Normal Heart Weight

Kitzman's Table

Normal Heart Weights

Predicted Normal Heart Weight (g) as a Function of Body Height in 392 Women and 373 Men

Body Height		Women[a]			Men[a]		
(cm)	(in)	L95	P	U95	L95	P	U95
130	51	133	204	314	164	232	327
132	52	135	207	319	167	236	333
134	53	137	210	324	170	240	338
136	54	139	214	329	173	243	344
138	54	141	217	334	175	247	349
140	55	143	220	338	178	251	355
142	56	145	223	343	181	255	361
144	57	147	226	348	184	259	366
146	57	149	229	353	187	263	372
148	58	151	232	358	189	267	378
150	59	153	236	363	192	271	383
152	60	155	239	368	195	275	389
154	61	157	242	372	198	280	395
156	61	159	245	377	201	284	400
158	62	161	248	382	204	288	406
160	63	163	251	387	207	292	412
162	64	165	254	392	209	296	417
164	65	167	258	397	212	300	423
166	65	169	261	401	215	304	429
168	66	171	264	406	218	308	435
170	67	173	267	411	221	312	440
172	68	176	270	416	224	316	446
174	69	178	273	421	227	320	452
176	69	180	277	426	230	324	458
178	70	182	280	431	233	328	463
180	71	184	283	435	235	332	469
182	72	186	286	440	238	336	475
184	72	188	289	445	241	341	481

(*Continued*)

Predicted Normal Heart Weight (g) as a Function of Body Height in 392 Women and 373 Men

Body Height		Women[a]			Men[a]		
(cm)	(in)	L95	P	U95	L95	P	U95
186	73	190	292	450	244	345	487
188	74	192	295	455	247	349	492
190	75	194	299	460	250	353	498
192	76	196	302	465	253	357	504
194	76	198	305	469	256	361	510
196	77	200	308	474	259	365	516
198	78	202	311	479	262	369	522
200	79	204	314	484	265	374	527
202	80	206	318	489	268	378	533
204	80	208	321	494	271	382	539
206	81	210	324	499	274	386	545
208	82	212	327	508	276	394	557
210	83	214	330	508	279	394	557

Source: From Kitzman, D. et al., Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity). A quantitative anatomic study of 765 specimens from subjects 20 to 99 years old, *Mayo Clin. Proc.*, 63, 1237–1246, 1988. With permission.

Note: Observed heart weight should be compared to predicted heart weight in all cases, not just those where drug abuse is suspected. Variations of more than 10% are very likely to be clinically significant but not apparent if only wall thickness is determined. Percentage-based formulas (e.g., 0.4% of body weight for men and 0.45% for women) are approximations only and not nearly so accurate or reliable.

[a] L95, lower 95% confidence limit; P, predicted normal heart weight; U95, upper 95% confidence limit.

Predicted Normal Heart Weight (g) as a Function of Body Weight in 392 Women and 373 Men

Body Weight		Women[a]			Men[a]		
(kg)	(lb)	L95	P	U95	L95	P	U95
30	66	133	196	287	162	213	282
32	71	137	201	295	167	220	291
34	75	141	206	302	172	227	300
36	79	144	211	310	177	234	309
38	84	148	216	317	182	240	317
40	88	151	221	324	187	247	325
42	93	154	226	331	191	253	334
44	97	157	230	337	196	259	341
46	101	160	234	344	200	265	349
48	106	163	239	350	205	270	357
50	110	166	243	356	209	276	364
52	115	169	247	362	213	281	371
54	119	171	251	368	217	287	379
56	123	174	255	374	221	292	386

(*Continued*)

Predicted Normal Heart Weight (g) as a Function of Body Weight in 392 Women and 373 Men

Body Weight		Women[a]			Men[a]		
(kg)	(lb)	L95	P	U95	L95	P	U95
58	128	177	259	379	225	297	392
60	132	179	262	385	229	302	399
62	137	182	266	390	233	307	406
64	141	184	270	395	237	312	412
66	146	187	273	401	240	317	419
68	150	189	277	406	244	322	425
70	154	191	280	411	248	327	431
72	159	194	284	416	251	331	437
74	163	196	287	420	255	336	444
76	168	198	290	425	258	341	450
78	172	200	293	430	261	345	455
80	176	202	297	435	265	349	461
82	181	205	300	439	268	354	467
84	185	207	303	444	271	358	473
86	190	209	306	448	275	362	478
88	194	211	309	453	278	367	484
90	198	213	312	457	281	371	489
92	203	215	315	461	284	375	495
94	207	217	318	465	287	379	500
96	212	219	320	470	290	383	506
98	216	221	323	474	293	387	511
100	220	222	326	478	296	391	516
102	225	224	329	482	299	395	521
104	229	226	331	486	302	399	526
106	234	228	334	490	305	403	531
108	238	230	337	494	308	406	536
110	243	232	339	497	311	410	541
112	247	233	342	501	314	414	546
114	251	235	345	505	316	418	551
116	256	237	347	509	319	421	556
118	260	239	350	513	322	425	561
120	265	240	352	516	325	429	566
122	269	242	355	520	327	432	570
124	273	244	357	523	330	436	575
126	278	245	360	527	333	439	580
128	282	247	362	531	335	443	584
130	287	249	364	534	338	446	589
132	291	250	367	537	341	450	593
134	295	252	369	541	343	453	598
136	300	253	371	544	346	456	602
138	304	255	374	548	348	460	607
140	309	257	376	551	351	463	611

(*Continued*)

Predicted Normal Heart Weight (g) as a Function of Body Weight in 392 Women and 373 Men

Body Weight		Women[a]			Men[a]		
(kg)	(lb)	L95	P	U95	L95	P	U95
142	313	258	378	554	353	466	616
144	317	260	381	558	356	470	620
146	322	261	383	561	358	473	624
148	326	263	385	564	361	476	629
150	331	264	387	567	363	479	633

Source: From Kitzman, D. et al., Age-related changes in normal human hearts during the first 10 decades of life. Part II (Maturity). A quantitative anatomic study of 765 specimens from subjects 20 to 99 years old, *Mayo Clin. Proc.*, 63, 1237–1246, 1988. With permission.

[a] L95 = lower 95% confidence limit; P = predicted normal heart weight; U95 = upper 95% confidence limit.

Zeek's Table

The following table was first published by Zeek in 1942 (Heart Weight, I. The weight of the normal human heart. *Arch. Pathol.* 34: 820–832). The numbers were derived from 926 cases and also included wall thickness and valve circumference. This is the largest such series ever to be published, perhaps explaining why the standard variation, though still large, is smaller than the other tables.

Adult Normal Heart Weight in Relation to Body Length

Body Length (cm)	Heart Weight (g)		Body Length (cm)	Heart Weight (g)	
	Males SD ± 40 g	Females SD ± 30 g		Males SD ± 40 g	Females SD ± 30 g
135	254	219	168	317	277
136	256	220	169	319	279
137	258	222	170	321	281
138	260	224	171	323	283
139	262	226	172	325	284
140	264	227	173	327	286
141	266	229	174	329	288
142	268	231	175	330	290
143	270	233	176	332	291
144	272	235	177	334	293
145	273	236	178	336	295
146	275	238	179	338	297
147	277	240	180	340	299
148	279	242	181	342	300
149	281	243	182	344	302
150	283	245	183	346	304
151	285	247	184	348	306

(*Continued*)

Adult Normal Heart Weight in Relation to Body Length

Body Length (cm)	Heart Weight (g)		Body Length (cm)	Heart Weight (g)	
	Males SD ± 40 g	Females SD ± 30 g		Males SD ± 40 g	Females SD ± 30 g
152	287	249	185	349	307
153	289	251	186	351	309
154	291	252	187	353	311
155	292	254	188	355	313
156	294	256	189	357	315
157	296	258	190	359	316
158	298	259	191	361	318
159	300	261	192	362	320
160	302	263	193	365	322
161	304	265	194	367	323
162	306	267	195	368	325
163	308	268	196	370	327
164	310	270	197	372	329
165	311	272	198	374	331
166	313	274	199	376	332
167	315	275	200	378	334

Source: Zeek, P., Heart weight: The weight of the normal human heart, *Arch. Pathol.,* 34, 820–832, 1942.

This table was compiled from data collected in 1942 and is thought by some to be more accurate than Kitzman's table, as Zeek's series was based on nearly 1000 hearts. In most instances, the standard deviation in Zeek's series is smaller than in Kitzman's study. Over the years, many other norms have been proposed, and some of the more important are listed here:

1. Dadgar, S. K., Tyagi, S. P. et al. (1979). Factors influencing the normal heart weight—A study of 140 hearts. *Jpn Circ J* 43(2): 77–82.
2. Eckner, F. A., Brown, B. W. et al. (1969). Dimensions of normal human hearts after standard fixation by controlled pressure coronary perfusion. *Arch Pathol* 88(5): 497–507.
3. Howell, T. H. (1978). Organ weights in nonagenarians. *J Am Geriatr Soc* 26(9): 385–390.
4. Hutchins, G. M. and Anaya, O. A. (1973). Measurements of cardiac size, chamber volumes and valve orifices at autopsy. *Johns Hopkins Med J* 133(2): 96–106.
5. Reiner, P., Mazzoleni, A. et al. (1959). The weight of the human heart. I. Normal cases. *Arch Pathol* 68: 58–73.
6. Schenk, K. E. and Heinze, G. (1975). Age-dependent changes of heart valves and heart size. *Recent Adv Stud Cardiac Struct Metab* 10: 617–624.
7. Smith, H. (1928). The relation of the weight of the heart to the weight of the body and of the weight of the heart to age. *Am Heart J* 4: 79–93.
8. Westaby, S., Karp, R. B. et al. (1984). Adult human valve dimensions and their surgical significance. *Am J Cardiol* 53(4): 552–556.

In a study of 232 cases, almost all trauma deaths, Molina and DiMaio found the average age was 29 years, the average height 173 cm, and the average weight 76.4 kg (168 lb). Regression analysis disclosed no relationship between body weight, body mass, and body length to allow for predictions. They suggested a reference range (95% inclusion) of 233–383 g for the adult male human. This does seem reasonable, but the total number of patients studied was smaller than Kitzman's study and may be underpowered.

Population-Based Estimates of Heart Size

As this edition was going to press, two Swedish researchers published a new paper. The findings of that paper may, finally, settle the ongoing debate of what constitutes a normal heart weight. The researchers, Carl Wingren and Anders Ottoson (2015) were given access to the database maintained by the Swedish National Board of Forensic Medicine. They reviewed 76,778 forensic autopsies, but included only those cases where an external underlying cause of death, appropriately coded, was included. Cases missing the data required to calculate a BMI, cases of decomposition, and cases where the weights recorded were simply implausible, were all excluded, leaving 27,645.

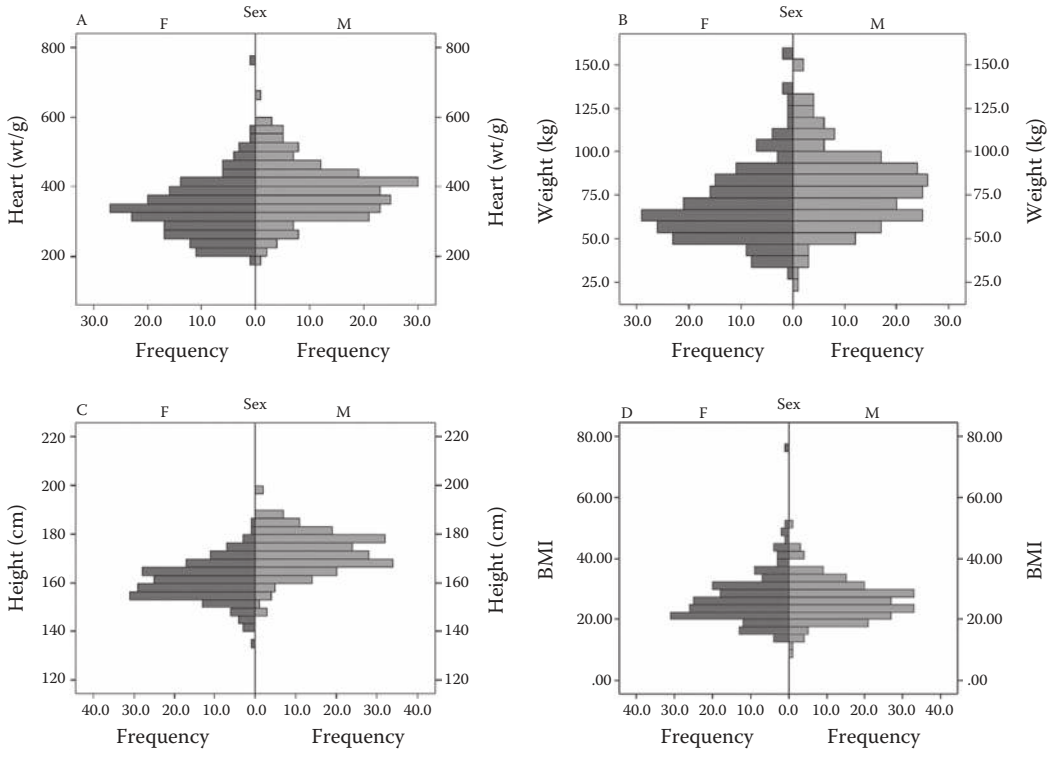
This subgroup was divided into three additional subgroups—(1) Underweight, (2) normal overweight, and (3) obese—and then characterized the groups using linear regression models. They found that approximately 50% of the variation in heart weight was explained by age, sex, and body size, although these three variables were less important in explaining the variation in heart weight in individuals who were underweight and obese compared to in those who were of normal weight or overweight, based on the linear regression models that calculated the predicted heart weight, with reference intervals, using age, sex, body weight, and height. Most importantly, they created an algorithm and put it online.

The website for the formula is http://lundforensicmedicine.com. There is no charge for its use. Using this formula a 40-year-old man whose height is 5′10″ and weight is 165 lbs (178 cm, 73 kg) would have a BMI of 23 and a mean heart weight of 364.4 g, roughly in line with most practitioners' expectations.

Zeek's formula from the 1940s would yield a projected heart weight of 336 g. The Kitzman formula would yield a weight of 320 g for men and 223 g for women. The values are less than a standard deviation apart, but have the virtue of being based on a massive sample. Clearly, this algorithm will take some years to come into general use, but would appear to be the approved standard. Of course, no gross weight will ever replace microscopic examination for the diagnosis of true hypertrophy. It is not clear if this approach will prove equally effective in Asians and other noncaucasoid populations.

Gaitskell's Histograms

This figure is taken from Gaitskell et al.'s 2011 study (Derivation of new reference tables for human heart weights in light of increasing body mass index. *J Clin Pathol* 64: 358–362).



Reference

Wingren, C. and Ottosson, A. (2015). Postmortem heart weight modeled using piecwise linear regression in 27,645 medicolegal autopsy cases. *Forensic Sci Int* 252: 157–163.

Appendix E: Normal Organ Weights

The data are from a table included in a paper by Geoffroy Lorin de la Grandmaison et al. It was first published in October 2001 and is reproduced with permission (*Forensic Sci. Int.* 119(2): 149–154). Since the study was so recently published and was based on 684 autopsies, it no doubt reflects weights as they are observed today.

Comparative Data of Organ Weight (g) of Males and Females

	Males (n = 355)		Females (n = 329)	
	Mean ± SD	Range	Mean ± SD	Range
Heart	365 ± 71	90–630	312 ± 78	174–590
Right lung	663 ± 239	200–1593	546 ± 207	173–1700
Left lung	583 ± 216	206–1718	467 ± 174	178–1350
Liver	1677 ± 396	670–2900	1475 ± 362	508–3081
Spleen	156 ± 87	30–580	140 ± 78	33–481
Pancreas	144 ± 39	65–243	122 ± 35	60–250
Right kidney	162 ± 39	53–320	135 ± 39	45–360
Left kidney	160 ± 41	50–410	136 ± 37	40–300
Thyroid	25 ± 11	12–87	20 ± 9	5–88

Note: SD, standard deviation.

Mean and Standard Deviation of Organ Weight (g) according to Height (cm)

	Males			Females		
	144 ≤ H ≤ 165	165 ≤ H ≤ 175	176 ≤ H ≤ 190	126 ≤ H ≤ 155	156 ≤ H ≤ 165	166 ≤ H ≤ 180
Heart	344 ± 75	360 ± 75	381 ± 56	320 ± 88	308 ± 79	311 ± 67
Right lung	616 ± 210	625 ± 207	741 ± 274	494 ± 202	545 ± 183	597 ± 243
Left lung	523 ± 190	551 ± 178	658 ± 257	450 ± 146	472 ± 181	491 ± 204
Liver	1455 ± 370	1637 ± 369	1831 ± 384	1275 ± 321	1496 ± 331	1624 ± 380
Spleen	120 ± 51	150 ± 88	180 ± 90	122 ± 67	139 ± 79	160 ± 82
Pancreas	138 ± 35	143 ± 39	147 ± 39	111 ± 25	122 ± 35	138 ± 41
Right kidney	150 ± 49	157 ± 36	170 ± 37	117 ± 32	137 ± 40	148 ± 36
Left kidney	155 ± 53	164 ± 38	175 ± 38	120 ± 41	136 ± 35	148 ± 33
Thyroid	25 ± 7	25 ± 13	25 ± 9	20 ± 11	18 ± 6	20 ± 11

Note: H, height.

Appendix F: Collecting Leftover Pills from the Scene of a Death*

Case #		To maintain a chain of possession, sign and date each transfer of evidence. *I certify that I recovered and identified this evidence as being linked to the listed case on this date.* Date	
Name			
Date			
Date of death		Name:	
Investigator		Name:	
Signature		Name:	
Investigator		Name:	
Signature		Destroyed by:	

List Medications 1st by Rx Name and 2nd by Rx Date (oldest to newest)

Rx Date	Rx Name	Dosage (i.e., mg)	Rx Quantity (# of Pills/ Tabs)	Refills (No or #)	Dosage Instructions	# Remaining	Doctor/ Pharmacy Contact Phone #s

Note: po, per ora (by mouth); qd, once a day; bid, twice a day; tid, three times a day; qid, four times a day; prn, as needed; qod, every other day; qhs, at bedtime. Refer to a medical abbreviation reference for others.

* As designed by Judith Melinek. With permission.

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