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Research Findings on Smoking of Abused Substances

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Research Findings on Smoking of Abused Substances

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Introduction and Overview

C. Nora Chiang and Richard L. Hawks

For centuries, smoking has been a popular route for the self-administration of recreational drugs such as tobacco, marijuana, and opium. Recently, smoking has also gained popularity for the use of phencyclidine (PCP), cocaine, and methamphetamine. It is likely that smoking as a route of administration is being more frequently chosen by drug abusers as a "safer" route because of the dangers of intravenous (IV) transmission of the human immunodeficiency virus. This route of administration is a major contributing factor to the current epidemic of cocaine and methamphetamine abuse. The toxicity resulting from this route of administration is complex. Not only the drug itself but also the resulting degradation products contribute to the pharmacological and toxicological effects. In addition, there are unique pharmacokinetic and behavioral characteristics associated with smoking as a route of administration. To understand the particular toxicity associated with smoking, it is necessary to integrate research findings in several areas. These include chemistry, pharmacology and toxicology, pharmacokinetics, and behavioral and clinical observations. A technical review concerning research finding on smoking of abused substances was held by the National Institute on Drug Abuse on November 10, 1988.

The first presentation by Dr. Donald Wesson on the pattern as well as the techniques for the self-administration of abused drugs revealed that cocaine and methamphetamine smoking is on the rise and that the methods for smoking abused drugs are quite variable. Often, combinations of drugs are smoked. This information is important for the interpretation of clinical observations and for the design of research experiments for the investigation of various aspects of smoking of abused drugs.

Dr. Neal Benowitz discus& the unique pharmacokinetic and pharmacodynamic aspects of the smoked route of drug administration that may contribute to the abuse of tobacco cigarettes. Using nicotine as an example, Dr. Benowitz demonstrated that the inhalation route provides a very rapid delivery of the drug into the brain. The smoker is therefore able to titrate the level of the drug in the brain to achieve a desired mental state. This user control would be expected to enhance the reinforcing nature of the

drug and thus contribute to the preferred use of inhalation for abused substances.

The pharmacokinetic and pharmacodynamic data on cocaine presented by Dr. Reese Jones showed rapid peak effects and peak plasma levels from smoked and IV doses as compared with those from intranasal and oral doses. He explained that the rapidly developed tolerance, which occurs even with the first dose of cocaine, is a contributing factor to the much less intense drug effects perceived from the oral and intranasal doses. As the intense peak effect is important to drug abusers, the differences between addictive potential and lethality of cocaine from different routes of administration undoubtedly could be partly due to the pharmacokinetic differences.

The dynamics of smoking marijuana was presented by Dr. Mario Perez-Reyes. A great portion of tetrahydrocannabinol (THC) is either degraded during pyrolysis or lost in sidestream smoke. Therefore, the manner in which marijuana cigarettes are smoked plays an important role in determining the amount of THC inhaled or the resultant plasma concentrations. Consequently, great variability in both plasma levels and observed effects has been reported for the smoked dose. The assumption that smokers have control over the rate of THC inhaled is also evidenced by the observation that the smoke volume inhaled per puff during smoking progressively decreases.

Lungs are the principal organ exposed to the smoke and pyrolysis products of abused drugs. The long-term effects of such smoking on the lung were discussed by Dr. Donald Tashkin. Chronic smoking of only a few marijuana cigarettes a day has been shown to have long-term adverse effects on the lung. A comparison of habitual smoking of marijuana and tobacco indicates that both cause functional alterations in the respiratory tract but on different sites. The adverse effects on the respiratory tract and the lung caused by marijuana plus tobacco are additive. Preliminary data from studies with free-base cocaine smokers revealed a high proportion of subjects with acute cardiopulmonary symptoms temporally related to cocaine smoking. Further research should be directed at the effect of cocaine smoking on lung function and structure.

Health concerns about passive exposure to tobacco smoke have led to similar concerns about marijuana smoke. Additionally, passively inhaled THC has been raised as an issue in urine testing. As summarized by Dr. Edward Cone, the duration of a positive test for urine for cannabinoids and the intensity of behavioral and pharmacological effects depend upon the amount of THC that actually gets into the body. Passive inhalation of great amounts of smoke could no doubt result in significant behavioral responses as well as positive urine analyses. However, exposures that occur in social situations are unlikely to result in levels that could produce noticeable pharmacological effects or positive results for cannabinoids in urinalysis.

On the other hand, oral ingestion of marijuana in a brownie containing the equivalent of one 2.8 percent cigarette was shown to result in measurable pharmacological effects and positive urines for about a week. It is conceivable that such ingestion could be inadvertent.

Since all the pyrolysis products of abused substances in the mainstream smoke are inhaled and could contribute to the pharmacological effects and toxicities associated with smoking, the identification and quantification of these products are important for understanding the drug's effects. Drs. Edgar Cook and Billy Martin presented comprehensive data obtained from both in vivo and in vitro studies of smoked cocaine, PCP, and heroin. Some of the pyrolytic products from heroin, identified by Dr. Cook, such as 6-acetylmorphine and N-acetylnormorphine, might be expected to contribute to pharmacological and toxicological effects observed with smoking. In addition, the contaminants or adulterants in an abused drug could also pose health risks when smoked. Quantitative determination of the constituents of the smoke actually inhaled by a drug abuser on the street is very difficult. The amounts of the intact drug and its degradation products in mainstream smoke are quite variable and highly dependent upon the physicochemical properties of the drug and the smoking conditions employed. These include temperature, flow rate, and the presence or absence of other materials. The decomposition of a compound generally is much more extensive if it is in its acid-salt form rather than its free-base form. This explains why cocaine free base is the predominant form for smoked cocaine abuse. An exception to this is methamphetamine for which the hydrochloride is typically the smoked form (ice), as this form is extensively vaporized on heating.

When plant materials or abused drugs mixed with plant materials are smoked, the resultant pyrolysis byproducts can be even more complex. Studies by Dr. Charles Sparacino indicate that marijuana smoke condensate contains several thousand compounds. So far, several hundred compounds have been identified, and a significant portion of these are nitrogen containing aromatic compounds. Dr. Sparacino showed that fractions of smoke condensate that contain such amine compounds are mutagenic.

The use of proper animal models has made significant contributions to our understanding of the behavioral pharmacokinetic and pharmacologic effects of abused drugs. Studies by Dr. Martin in rats have shown that behavioral effects and biodisposition for PCP were similar following both smoking and intraperitoneal administration of PCP.

Dr. Ronald Wood explained that animal models for studying self-administration of abused drugs by smoking have been very difficult to devise. In addition to the fact that animals cannot be trained to inhale like humans, the pattern of particulate deposition and absorption is dependent on both species and particle size. Research is currently attempting to develop a special generator to produce reliable exposure concentrations, stable particle size

distribution, and less irritation. This system will be used for studies of behavioral and pharmacological effects from self-administration of smoked drugs

The conclusion can be easily drawn from this technical review that smoking of abused drugs is a very complex issue. So far, a limited body of information has accumulated on some of the unique characteristics of the smoked dose. Much additional research is needed in this area and will involve the collaboration of both basic and clinical researchers to understand sufficiently the fundamental mechanisms, toxicities, and behaviors, a collaboration that can lead to the design of better treatment and prevention strategies.

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Current Patterns of Drug Abuse That Involve Smoking

Donald R. Wesson and Peter Washburn

INTRODUCTION

Smoking is a method of drug use that is acceptable to many people, including many for whom injection of street drugs would not be acceptable. Familiarity with smoking is, of course, an important factor. Because of extensive tobacco and marijuana smoking in this culture, most people are familiar with drug use by smoking, and they do not perceive smoking to be as culturally deviant as self-administration of drugs by intravenous injection. Most people associate intravenous injection of drugs with "hardcore drug abusers."

Smoking is perceived as safer than intravenous injection; however, the safety is somewhat illusionary. Although smoking does not produce many of the medical complications that occur with intravenous drug abuse, e.g., human immunedeficiency virus (HIV) infection, infective endocarditis, or serum hepatitis, the toxicity of some drugs that are smoked is substantial. Cocaine smokers, for example, can ingest lethal quantities of cocaine by smoking. Of particular public health concern is cocaine's toxicity for the fetuses of pregnant women who smoke crack.

The willingness of many people to smoke drugs played a major role in the emergence of the crack cocaine epidemic of the eighties. Table 1 shows the sources of commonly smoked drugs. The introduction of cocaine in small lots ready for smoking opened a large new marketplace for cocaine among people whose former drug choices were alcohol, marijuana, and nicotine.

For drugs that volatilize at low temperature, smoking can be an easy, efficient method of consumption, and large amounts of a drug can be rapidly delivered to the brain. Most psychoactive drugs exert the maximum subjective effects when blood levels of the drug are rapidly increasing. Decause smokable drugs enter the blood stream rapidly through the lungs, an

inhalation can produce a sharp increase in arterial blood concentration of the drug. The drug is carried by the arterial blood directly to the brain. The high concentration of drug in the arterial blood can produce an intense psychoactive drug effect, which can be qualitatively different from the drug's steady state effect. The intense psychoactive drug effect, often called a "rush," is the effect desired by the abuser. This is why, for example, drug abusers prefer to smoke or inject methamphetamine rather than take it orally. Methamphetamine is bioavailable orally; however, when absorbed from the gastrointestinal tract, blood levels rise slowly and do not produce the desired "rush."

TABLE 1. *Drugs that are smoked and their source*

Drug	Source
Freebase cocaine (freebase crystals and crack)	Coca plant
Opium	Poppy plant
Freebase heroin	Opium
Phencyclidine (PCP)	Illicit synthesis
Methamphetamine	Illicit synthesis
Delta-9-tetrahydrocannabinol (THC) marijuana (.1-9.5%) Δ-9-THC* hashish (1040%) Δ-9-THC*	Cannabis indica, Cannabis sativa

*Range of THC values from Mikuriya and Aldrich (1988).

COCAINE

In the United States, cocaine is sold as either the hydrochloride salt or as free base. Cocaine hydrochloride, which is water soluble, is used by snorting or by intravenous injection. Some cocaine abusers smoke cocaine hydrochloride; however, cocaine hydrochloride volatilizes at a high temperature and most of it is destroyed by burning. Most cocaine abusers who smoke cocaine use free-base cocaine. Cocaine free base volatilizes at a lower temperature, and a greater percentage of it is spared during the smoking process. The form of free base called crack has become the most popular form of cocaine that is smoked in the United States.

Figure 1 shows the number of emergency room mentions of cocaine in the Drug Abuse Warning Network (DAWN) between 1982 and 1987. The total number of cocaine mentions per year have increased for all methods of amine use; however, the largest increase has ocurred in the mentions of smoked cocaine-which is now primarily crack.

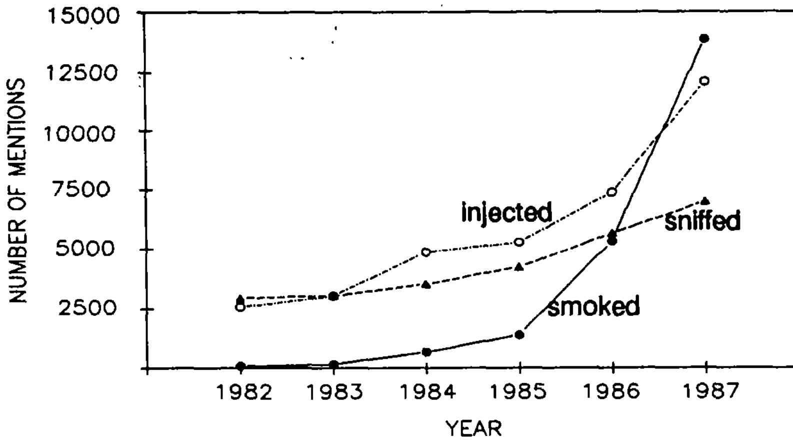

FIGURE 1. *DAWN national emergency room mentions of cocaine*

Note: Data from the annual publication Data from the Drug Abuse Warning Network, 1982-1987. Shown are the national total of emergency room visits in which the administration route of cocaine was specified. The relationship between national prevalence of cocaine use and mentions in DAWN is complex. DAWN cocaine data are consistent with many other indices of cocaine use that support a marked increase in prevalence of cocaine smoking.

Free-Base Cocaine

Freebase cocaine can be prepared from cocaine hydrochloride as either free-base crystals or as crack.

The preparation of free-base crystals involves dissolving the cocaine hydrochloride in water, converting the hydrochloride to the alkaloid form by addition of a strong base (such as sodium or ammonium hydroxide), extraction of the alkaloid with an organic solvent (such as petroleum ether), and crystallization of the free base. Before the current crack epidemic in the United States, most cocaine free-base users made their free base from cocaine hydrochloride. The preparation of free-base crystals was part of the ritual of use. The free-base conversion also removed sugars and other water-soluble diluents used to "cut" cocaine. Until most States passed drug paraphernalia laws that made free-base conversion kits illegal, most users made their free base with the kits, which contained all the ingredients for the conversion-except, of course, the cocaine hydrochloride. The kits were sold in drug paraphernalia shops and by mail order. A history of cocaine free-base use before 1982 is detailed in a theme issue of the *Journal of Psychoactive Drugs* (Siegel 1982).

Crack cocaine is also made from cocaine hydrochloride. The cocaine hydrochloride is dissolved, mixed with baking soda or sodium bicarbonate,

and heated. While the mixture is being heated, the "cooker" gently swirls or rotates the container. The freebase cocaine precipitates and coalesces into a soft mass that becomes hard when it is dried. Sugars and other water-soluble diluents remain in the water, which is then poured off; leaving the solid mass. Now, most crack users buy crack in the form of pellets or "rocks" that are already in the free-base form. Some users prefer to make their own crack from the cocaine hydrochloride because they feel more confident of its purity.

Several years before the current crack epidemic, smoking cocaine paste, an extract of coca leaves containing 40 to 85 percent cocaine sulfate, became a major public health problem in Peru. Subsequently, paste smoking became common in Bolivia, Columbia, and Ecuador (Jeri 1984). Cocaine paste abusers there are mostly people in the lower socioeconomic classes. The paste is generally smoked in marijuana or tobacco cigarettes.

Crack and cocaine free-base crystals are often smoked in glass pipes. The glass pipe has a bowl fitted on the bottom with one or more fine-mesh copper screens, which support the cocaine. The user heats the side of the bowl with a small butane torch or lighter. The free base vaporizes and is drawn through the pipe. A pipe used in this manner gets hot and users often bum fingers or lips. Some free-base smokers have burns on their groins from suddenly hiding hot free-base pipes in their pockets.

Cocaine and Marijuana Combinations

Cocaine free base and occasionally cocaine hydrochloride are mixed with marijuana and smoked. A marijuana cigarette laced with crack is commonly called a "grimmie." Crack is crushed between two coins, mixed with marijuana, and rolled into a "joint." Although smokers of grimmies are smoking crack, they seem to view it as a less intense and leas severe form of use than using crack in a pipe. Many users of grimmies do not realize that they are free basing as they associate free basing with using a pipe. Some free-base abusers report using grimmies as a means of controlling their cocaine use. When cocaine is smoked in marijuana, users report that there is less compulsion for continued use and less difficulty sleeping after use. In the San Francisco Bay area, grimmies are particularly popular among black youth.

Cocaine and Tobacco Combinations

The combination of crack cocaine and a tobacco cigarette is known as caviar (or cavies). Cocaine hydrochloride is also used in this manner. The user makes the combination by reducing the crack to a powder and using the cigarette as a straw, sucking the cocaine inside the cigarette. The advantage of this dosage form is that it can be smoked in public without its being apparent that the person is using cocaine.

Phencyclidine and Cocaine

Phencyclidine (PCP) and cocaine are sometimes mixed together. The combination is known as “whack” or “spacebase.” Some users seek the combination, but PCP is more often included because it is a less expensive substitute for cocaine. Thus, some cocaine users who believe they are using only cocaine are also using PCP.

Cocaine Substitutes

Cocaine substitutes, most often procaine, are still used either as drugs of deception or intentionally as a “poor man’s cocaine substitute.” Sold under names such as Snowcaine or Rock, procaine am be purchased in drug paraphernalia shops or by mail order. Sellers escape drug regulation by selling the substitute as incense. The label disclaimer gives directions for use: “Do not inhale vapors, as it may cause stimulation.”

Acquired Immunodeficiency Syndrome and Crack

Although smoking avoids the intravenous drug abuser’s risk of being infected with HIV by contaminated needles, smoking crack cocaine is still associated with behavior that puts some abusers at risk. Increasingly, women offer sex in exchange for crack.

PCP

Most users of PCP use it by smoking, usually mixing it with parsley, tobacco, or marijuana. The PCP may be dissolved and a cigarette dipped in the solution. A dark-brown colored cigarette is often used for this purpose as the discoloration produced by PCP is not as obvious as it would be on a white cigarette. Sherman cigarettes, because they are dark brown, are often used, thus the street name of this combination is known as “Shermans” or “shermies.”

METHAMPHETAMINE (ICE)

Methamphetamine can be smoked in either the hydrochloride salt or free base form. Smoking of the d-isomer of methamphetamine, called “ice,” has become popular in Hawaii. Drug Enforcement Agency (DEA) seizures of ice in Hawaii began in 1985. Ice sold in Hawaii probably originates in Korea; however, mainland U.S. manufacture is beginning. In 1990, the DEA seized ice crystals manufactured in California.

Methamphetamine can be smoked in one of several ways:

1. Methamphetamine is placed on aluminum foil, the aluminum foil heated from below, and the methamphetamine vapors inhaled. (As with heroin, this mode of use is sometimes called “chasing the dragon.“)

2. Methamphetamine is smoked in small, straight glass pipes. (Unlike free-base cocaine, which is not water soluble, methamphetamine cannot be smoked in water pipes, as the methamphetamine is water soluble and would be trapped in the water.)

3. Methamphetamine is smoked in combination with tobacco or marijuana.

HEROIN AND OPIUM SMOKING

Smoking heroin is a dominant form of opiate use in Asia, where the form of freebase heroin prepared for smoking is designated by the U.S. Drug Enforcement Agency as heroin #3. Smoking free-base heroin also occurs in the United States and England.

In the early seventies, Persian heroin, a freebase form of heroin, was favored by cocaine free-base users on the West Coast, who used it to attenuate cocaine-induced anxiety, agitation, or paranoia. With continued, frequent use, some cocaine users who used Persian heroin as a secondary drug became physically dependent on the heroin, much to their surprise, as they thought that physical dependency could occur only with intravenous injection.

Smoking opium is a primary and stable mode of use among some youthful heroin abusers in England (Gossop et al. 1988). Opium smoking also occurs in the United States, particularly among immigrants from Southeast Asia.

STIMULANT AND OPIATE COMBINATIONS

Drug abusers have long used combinations of stimulants and opiates intravenously. The combination is commonly called a “speedball.” Cocaine freebase abusers also smoke cocaine free base or methamphetamine mixed with freebase heroin. The street term for smoking a mixture of cocaine and heroin is “chasing and basing.”

RECOMMENDATIONS FOR FUTURE RESEARCH

Future research in pyrolytic chemistry should examine both street drugs and drug combinations, emulating as closely as possible the way abusers actually smoke the drugs. Much pyrolytic chemistry research on drugs of abuse involves the use of pure drugs. This is important in delineating the toxicity of specific drugs, but does not completely address clinicians’ need to understand the toxicity of abused drugs combined with street impurities, adulterants, and other drugs. Much of the toxicity information currently available to clinicians is based on case reports that lacked adequate analysis of the drug.

The role of cigarette smoking in drug abuse relapse needs systematic study. Some patterns of drug abuse involve combining drugs with cigarettes. In addition, many patients report that they smoke more cigarettes when using cocaine. The role of continued cigarette smoking in inducing cravings for other drug of abuse and relapse to other drug abuse deserves careful clinical study. If researchers should find that smoking cigarettes contributes to relapse, treatment programs should incorporate smoking cessation as part of drug abuse treatment.

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Clinical Pharmacology of Inhaled Drugs of Abuse: Implications in Understanding Nicotine Dependence

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INTRODUCTION

Why do people abuse drugs by the smoked or inhaled route? How does the pharmacology of inhaled drugs differ from that of drug administered by other routes? This chapter will discuss these and other aspects of the clinical pharmacology of inhaled drugs of abuse using nicotine as an example.

Pharmacokinetic and pharmacodynamic characteristics of a drug are important determinants of dependence liability, the temporal patterns of drug use, and the level of drug use. ultimately, an understanding of pharmacokinetics and pharmacodynamics may be useful in developing effective treatment strategies. Several pharmacokinetic and pharmacodynamic characteristics appear to be necessary or optimal for a drug to produce dependence: (1) the drug must be effectively absorbed into the blood stream; (2) the drug must rapidly enter into the brain; and (3) the drug must be psychoactive and that psychoactivity related to levels of the drug in the brain. These characteristics allow for the drug abuser to manipulate the dose of his or her drug to optimize mood and psychological functioning and are most likely to result in the behavior described as criteria for drug dependence,

Inhalation of a drug provides a unique delivery system that is conducive to producing drug dependence. Inhalation of a drug facilitates rapid absorption into the circulation which, in turn, results in rapid delivery to the brain. Studies on the clinical pharmacology of inhaled drugs of abuse present some particular research problems. For example, the bioavailability of inhaled drugs is highly variable from person to person and difficult to quantitate. Inhaled drugs are delivered and distributed to target organs rapidly. This means that pharmacokinetic and pharmacodynamic behaviors are in

disequilibrium conditions. This makes it difficult to study actions of a drug by the usual techniques of comparing venous concentrations with effects. Finally, tolerance develops rapidly to many or all effects of drug of abuse. Thus, even if the concentration in the brain could be known, the effect will vary over time and as a function of previous drug exposure history.

Approaches to studying these and other research problems concerning the pharmacology of inhaled drugs will be presented.

THE LUNGS AND DRUG ACTION

Inhaled drugs are taken into the lungs, from which they are absorbed into the systemic circulation (Bend et al. 1985) (table 1). Before discussing the pharmacology of inhaled drugs, it is useful to consider how the anatomy and physiology of the lung may influence drug action.

TABLE 1. *Lungs and drug action*

Pharmacologic Function	Action
Absorption of Inhaled Drugs	Rapid transfer of unionized lipophilic drugs from alveolar spaces to circulation High pulmonary blood flow
Accumulation (Uptake) of Drugs	Retention of drugs after inhalation First pass uptake after IV dosing Local toxicity
Metabolism	Clearance of drugs and endogenous chemicals Oxidation, reduction, hydrolysis, biosynthesis

The lung may be conceived as having three major pharmacologic actions. The first is the absorption of inhaled drugs. Absorption of drugs from the lungs is facilitated by a huge alveolar surface area, thin alveolar epithelial and endothelial layers, and an extensive capillary bed. As a result of these anatomical factors, many drugs, particularly un-ionized lipophilic drugs (which includes all of the common drugs of abuse), move rapidly from the alveolar spaces into the systemic circulation. Also of importance is that pulmonary capillary blood flow is high, representing passage of the entire blood volume through the lung every minute. Thus, drugs that are absorbed are carried quickly to various parts of the body and to target organs.

A second function of the lung is the uptake of drugs by the lung parenchyma. Such uptake may involve extracellular binding, intracellular binding, or both to the various proteins, including receptors, and in some cases energy-dependent active transport systems. The results of such uptake may include retention of drugs after inhalation or during the first pass through the pulmonary circulation after intravenous dosing. In these cases, the lung becomes a reservoir for slow and persistent release of drugs into the circulation. Pulmonary uptake may also result in high local concentrations of a drug, which could result in local toxicity, such as is the case with paraquat.

A third pharmacologic function of the lung is the metabolism of some drugs and endogenous chemicals. The lungs have the potential to metabolize drugs by the same pathways as the liver. Although the concentrations of drug-metabolizing enzymes in lung tissues are lower than that in liver, the blood flow to the lungs (and hence the amount of a drug brought into contact with drug-metabolizing enzymes) is much greater. Therefore, metabo lism by the lung can contribute substantially to total drug metabolism. Cytochrome P450 activity is primarily in nonciliated bronchial epithelial (Clara) and Type 2 alveolar cells. These cells are most vulnerable to injury as a result of metabolism of certain drugs and chemicals to reactive metabolites. Examples of how these various lung processes contribute to the pharmacology of inhaled drugs, in particular nicotine, are discussed in subsequent sections.

ABSORPTION AND BIOAVAILABILITY

Nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring. Nicotine is a weak base with a pKa of 8.0 soluble both in water and in lipids. At physiological pH, about 31 percent of nicotine is non-ionized such that it readily crosses cell membranes. The pH of tobacco smoke is important in determining the absorption of nicotine from different sites within the body. The pH of smoke from flue-cured tobaccos found in most cigarettes is acidic (Brunneman and Huffman 1974). At this pH, the nicotine is primarily ionized. In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes. As a consequence, there is little buccal absorption of nicotine from cigarette smoke, even when it is held in the mouth. The pH of smoke from air-cured tobaccos, such as in pipes, cigars, and a few European cigarettes, is alkaline, and nicotine is primarily unionized. Smoke from these products is well absorbed through the mouth (Armitage et al. 1978).

When tobacco smoke reaches the small airways and alveoli of the lung, the nicotine is rapidly absorbed independent of pH of the smoke. Blood concentrations of nicotine rise quickly during and peak at the completion of cigarette smoking (figure 1). The rapid absorption of nicotine from cigarette smoke through the lungs, presumably because of the huge surface area

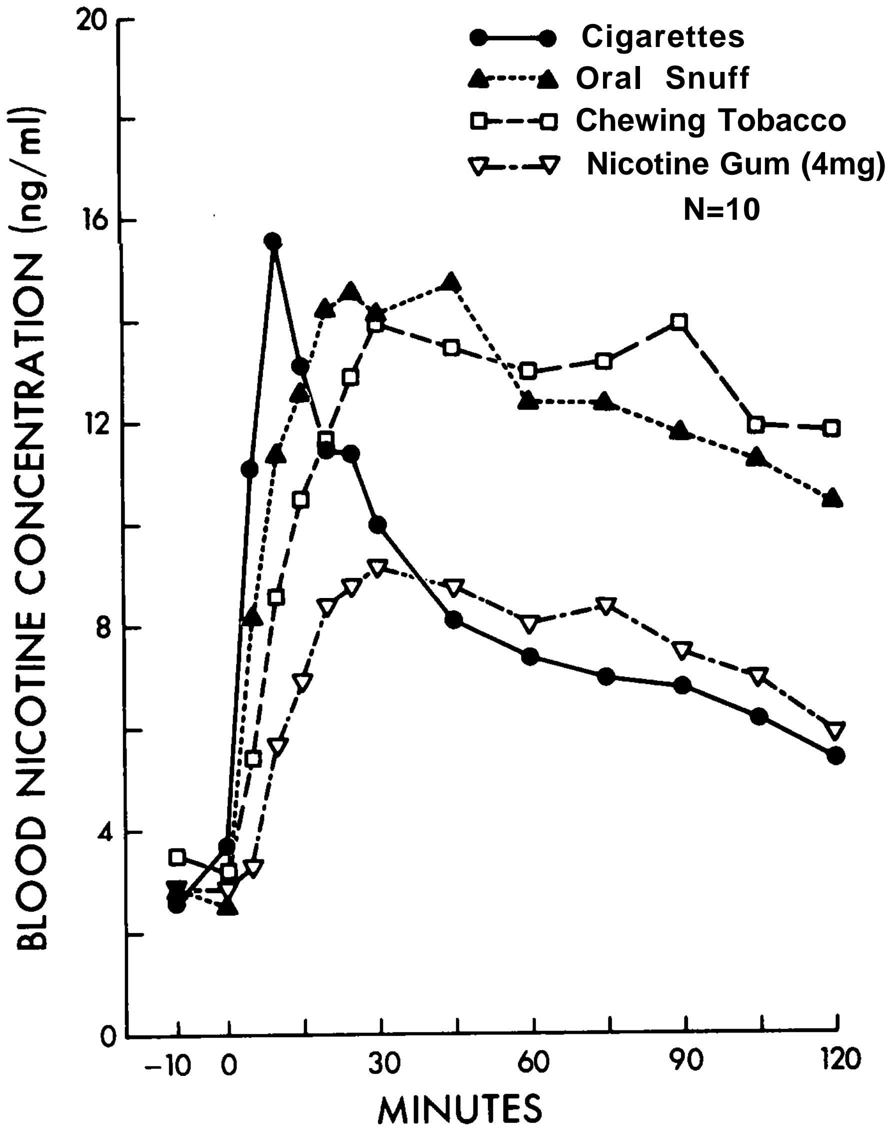

FIGURE 1. *Blood concentrations &ring and after cigarette smoking for 9 minutes (one and one-third cigarettes) and use of oral snuff (2.5 g), chewing tobacco (average 7.9 g), and nicotine gum (two 2-mg pieces) for 30 minutes*

NOTE: Average values for 10 subjects (±SEM).

of the alveoli and small airways and dissolution of nicotine into fluid of pH in the human physiologic range, facilitates transfer across cell membranes.

Blood or plasma concentrations of nicotine sampled in the afternoon in smokers generally range from 10 to 50 ng/ml (0.6 to $3x10^{-7}$ M). The increment in blood nicotine concentration after smoking a single cigarette ranges from 5 to 30 ng/ml, depending on how the cigarette is smoked. Smoking is commonly considered to be a process of intermittent dosing of nicotine, which is in turn rapidly eliminated from the body. There is considerable peak to trough oscillation from cigarette to cigarette. However, consistent with a half-life of 2 hours, nicotine accumulates over 6 to 8 hours of regular smoking and nicotine levels persist overnight, even as the smoker sleeps (Benowitz et al. 1982b). Thus, smoking results not in intermittent exposure but in exposure to nicotine that lasts 24 hours of each day.

Smoking behavior is complex, and the dose of a drug delivered to the circulation is influenced by how the smoker smokes. Thus, the intensity, duration and number of puffs, depth of inhalation, degree of mixing of smoke with air, and other factors influence the dose. Estimation of the dose of nicotine has been attempted by setting up standardized procedures of machine testing, where a smoking machine puffs a cigarette in a pattern similar to that of human smokers. This test is the basis for the cigarette yield data that were formerly published by the United States Federal Trade Commission, and that appear in many cigarette advertisements. However, human smokers smoke differently from machines, and machine deliveries do not correlate very well with biochemical markers of nicotine absorption in people (Benowitz et al. 1983).

Bioavailability can be assesed by methods analogous to those used in other forms of drug testing. For example, we infused nicotine intravenously either on a separate day or, using stableisotope-labeled nicotine, on the same day as ad libitum cigarette smoking. Using the clearance estimated from infusion data and the area under the blood nicotine concentration time curve while smoking, absolute intake of nicotine from cigarette smoking, could be estimated (Jacob et al 1988; Benowitz and Jacob 1984). The intake of nicotine is quite variable, both on the basis of daily intake (range 10 to 80 mg) and on a per cigarette basis (range 0.4 to 1.6 mg per cigarette), among people.

The experimental situation under which smokers are studied may influence self-determined intake of an inhaled drug. For example, we compared the intake of nicotine per cigarette in smokers during restriction of cigarette availability (Benowitz et al. 1986). When smoking was restricted from an average of 37 to 5 cigarettes per day, intake of nicotine per cigarette increased 2.7-fold. It is likely for other drugs that the time since the last use of the drug and other environmental factors will influence the inhaled dose of the drug and that smokers will vary the dose throughout the day.

The bioavailability of a drug from smoking material is in theory influenced by the amount of drug in the material. For tobacco, we examined this relationship by measuring the amount of nicotine in unsmoked tobacco and comparing that to biological markers of absorption of nicotine in smokers (Benowitz et al 1983). On average, modem American cigarettes contain 9 mg nicotine per cigarette. The average dose inhaled into the systemic circulation by a smoker is 1 mg. Therefore, the bioavailability averages 11 percent. However, there was very little correlation between the amount of nicotine in the tobacco or the amount of tobacco in the cigarette burned with the amount of nicotine absorbed by smokers (Benowitz et al. 1983; Benowitz and Jacob 1984). The bioavailability of nicotine can be systematically changed by engineering features such as placement of ventilation holes to allow dilution of tobacco smoke with room air.

In summary, the absorption and bioavailability of inhaled drugs is highly variable from person to person and potentially from smoke to smoke. Smoking behavior is complex and permits the smoker considerable latitude in adjusting the dose to desired levels. Pharmacologic studies of inhaled drugs of abuse need to consider the extent and sources of individual variability in bioavailability in developing dose-response and dose-toxicity for such drugs.

DISTRIBUTION KINETICS

Smoking is a unique form of systemic drug administration in that entry into the circulation is through the pulmonary rather than the portal or systemic venous circulations. Entry via the lung influences the rate and pattern of delivery of drugs to body organs. For example, nicotine can be expected to move quickly from inhaled cigarette smoke to the brain. It is estimated that it takes 11 seconds or less from the start of a puff to the delivery of nicotine to the brain and 19 seconds to complete transit through the brain (table 2). This estimation assumes a 2-second puff, negligible time for diffusion of nicotine across alveolar membranes, and negligible time for

TABLE 2. *Nicotine effects after inhalation: Temporal considerations*

Action	Seconds
Puff time	2.0
Pulmonary circulation time	7.5
Left ventricle to cerebral circulation time	-1.0
Brain transit time	8.5
Total circulation time	19.0

NOTE: Estimates derived from Mapleson (1973).

movement from the arterial blood into the brain. With respect to the latter, nicotine is rapidly and extensively taken up from the blood in its first pass through the brains of rats (figure 2). Likewise, intravenously injected C^{14} nicotine is immediately taken up by the brains of mice, reaching a maximum concentration within 1 minute after injection (Stalhandski and Slanina 1970). Similar findings based on positron emission tomography of the brain were seen after injection of ^{11}C nicotine in monkeys (Maziere et al. 1976). The time from the start of a puff to delivery of nicotine to the brain is similar to the time from intravenous injection to arterial circulation, estimated as from 9 to 16 seconds in healthy people.

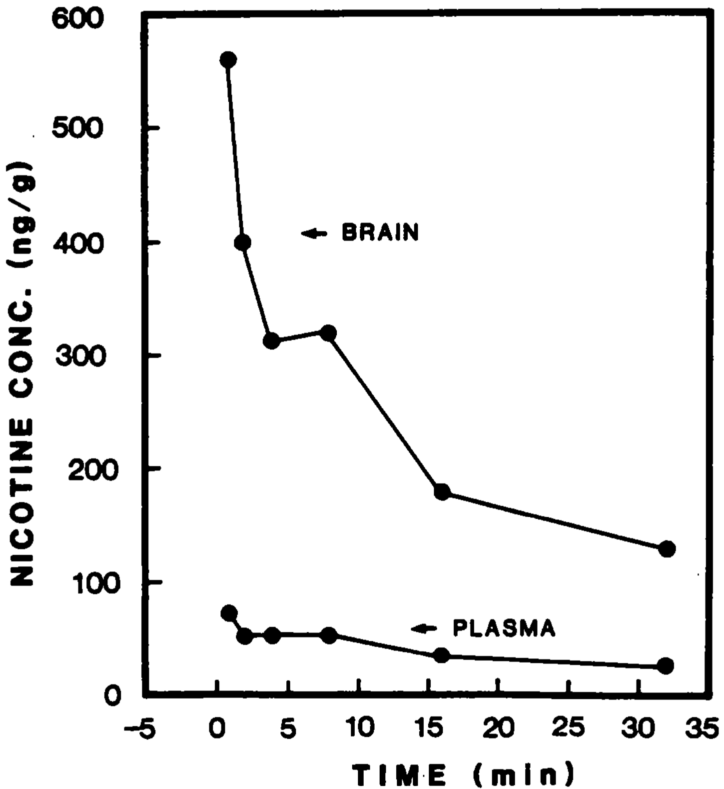

FIGURE 2. *Concentrations of nicotine in the brain and arterial plasma of rats after IV injection of 0.1 mg/kg nicotine over 30 seconds*

NOTE: Concentration of nicotine peak in the brain at or bcfore the earliest sampling time (30 seconds after the time of injetion). Brain concentrations are much highger than arterial concentrations, particularly in the early minutes after injection.

Brain concentrations decline quickly as nicotine is distributed to other body tissues. Using partition coefficients (the ratio of drug concentration in tissue compared to blood at steady state) derived from experiments in rabbits, along with organ weights and blood flows taken from people, perfusion models can be used to stimulate the concentrations of nicotine in various organs after smoking a cigarette (figure 3). Of note is that the lung has a steady state partition coefficient of 2.0, suggesting tissue binding. Studies

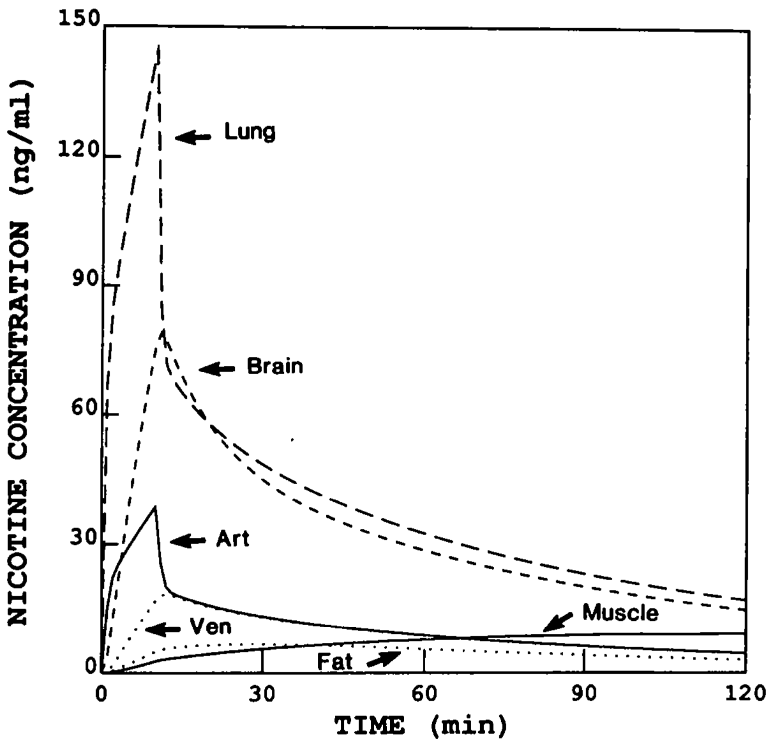

FIGURE 3. *Perfusion model simulation of the distribution of nicotine in various tissues following infusion of 1.5 mg of nicotine into the lung compartment over 10 minutes*

NOTE: The dose, route, and dosing regimen were intended to mimic smoking a cigarette. Typical human organ weighs and blood flows and partition coefficients from rabbits wcre used for the simulation.

with lung slices demonstrate lung binding, which is somewhat dose dependent (Ludden et al. 1976). There was 50 percent more binding of nicotine to lung slices at low concentrations than at high concentrations. As a result of high local concentrations and binding, concentrations of nicotine in the lung are predicted to be extremely high after intrapulmonary arterial injection and would be expected to be similarly high after tobacco, smoking. Such high concentrations could conceivably cause local pharmacologic effects on the lung, such as neutrophil chemcattraction (Totti et al. 1984) at concentrations that would not be expected to be achieved based on the relatively low concentrations of nicotine in the blood.

Concentrations of nicotine in arterial blood and the brain are seen to increase sharply following exposure, then to decline over 20 to 30 minutes as nicotine is redistributed to other body tissues, particularly skeletal muscle. Venous blood concentrations, reflecting outflow of nicotine from body tissues, are predicted to be considerably lower than arterial concentrations for the duration of the infusion and for several minutes afterward. This discrepancy has been observed in rabbits following rapid intravenous injection of nicotine (Porchet et al. 1988b) (figure 4). It is seen from figure 3 that the ratio of nicotine in the brain to that in venous blood is highest during and at the end of the infusion and gradually decreases as the elimination phase is entered. The importance of this disequilibrium between venous and arterial concentrations with respect to pharmacodynamic studies is discussed in a later section.

In summary, inhalation of drugs allows for rapid transfer into the arterial circulation and into the brain. Rapid passage into the brain provides for the possibility of rapid behavioral reinforcement from smoking and allows the smoker to precisely control the concentrations of nicotine in the brain, hence to modulate pharmacologic effects. To better understand this process, the relationship between the level of a drug and its effects on the brain must be examined.

PHARMACODYNAMICS OF NICOTINE IN TEE BRAIN

Nicotine binds to specific receptors in the brain and affects cerebral metabolism in the areas of specific binding (London et al. 1985). Nicotine is known to have a complex dose-response relationship. In experimental preparations, nicotine in low doses causes ganglionic stimulation and in high doses causes ganglionic blockade following brief stimulation. This biphasic response pattern is observed in the intact organism as well, although the mechanisms are far more complex. With regard to central nervous system effects, Ashton has provided evidence that nicotine exerts biphasic actions in humans (Ashton et al. 1980). When contingent negative variation (CNV, a component of the evoked EEG potential) was examined, it was seen that nicotine in low doses increased while high doses decreased the amplitude of the CNV. It was proposed that low doses produced arousal or increased

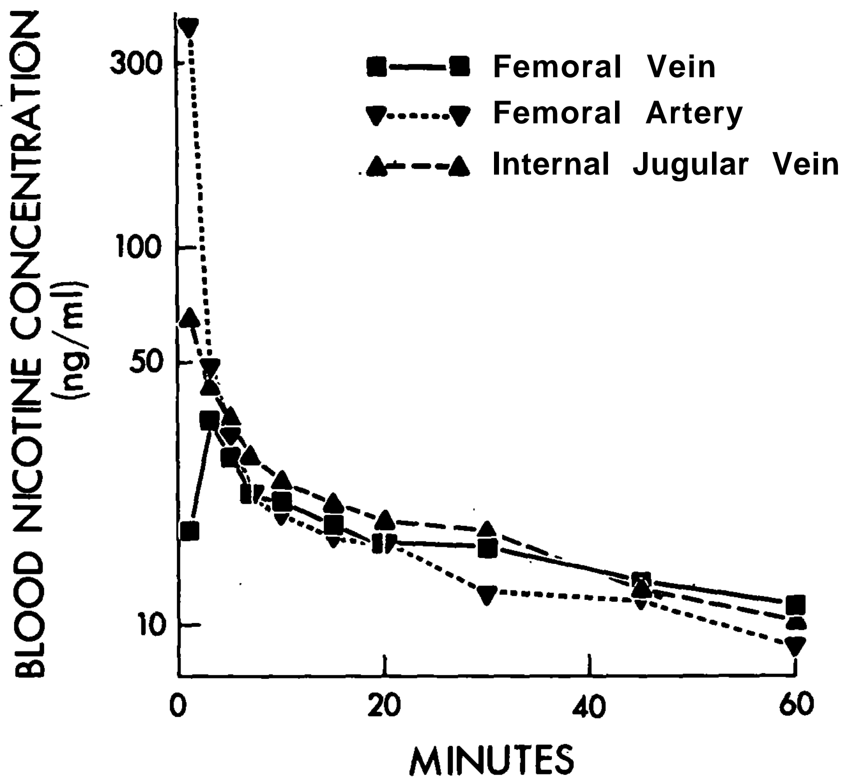

FIGURE 4. *Mean blood concentrations from three vascular sites after an IV bolus of 25 ug/kg of nicotine given over 1 minute in six rabbits*

KEY: Squares=femoral vein; inverted trianguls=femoral artery; triangles=internal jugular vein.

NOTE: Arterial and internal jugular vcnous concentrations (the latter proportional to brain concentrations) were much higher than those in femoral venous blood during the first 5 minutes.

SOURCE: Porchet et al. 1988b, copyright 1988. Rockefeller University Press.

attention and vigilance, while higher doses produced relaxation and reduced stress. Presumably, the smoker, by titrating the level of nicotine in the brain, can achieve the desired mental state.

The time course of nicotine absorption strongly influences the actions of nicotine, although it is not clear whether this is a result of a more rapid

rate of rise or higher absolute levels of nicotine in the brain. We and others have found that heart rate acceleration, a reflection of the level of sympathetic neural discharge and a sensitive physiologic response, tends to correlate with the magnitude of subjective effect (Rosenberg et al 1980; West and Russell 1987). Heart rate acceleration appears to be mediated by the central nervous system either through actions on chemoreceptor afferent pathways or via direct effects on the brain stem. With repeated intravenous injections of nicotine, dosed to simulate cigarette smoking, heart rate acceleration and subjective effects were greatest with the first series of injections and then declined over time with successive injections (Rosenberg et al. 1980).

To examine the importance of rate of dosing of nicotine and resultant actions and the relationship between effects and brain levels of nicotine, we compared the consequences of rapid- and slow-loading infusions of nicotine (Porchet et al. 1988b). The study was performed in seven volunteer smokers who had abstained from smoking overnight. Venous blood concentrations were not markedly different for the two infusion rates except for the first 5 minutes, when the increase in blood concentration was greater with rapid infusion (figure 5). Peak concentrations were seen at 30 minutes and were similar for both infusions. However, the heart rate increased much more quickly and to a greater level and was associated with transient dizziness and euphoria after rapid infusion, compared to slow infusion (figure 6). During the slower infusion, heart rate increased slowly, peaking at 30 minutes, and subjective effects were minimal. Using kinetic data derived from subjects of this study and studies in rabbits and with the use of a perfusion model, we estimated levels of nicotine in the human brain during the rapid and slow infusions. Predicted brain concentrations rose much more rapidly and to a higher level after rapid infusion, with a concentration time course similar to that seen for heart rate acceleration (figure 7). Brain concentrations versus heart rate acceleration tended to change in parallel These data suggest that effects of nicotine track brain concentrations over time.

In summary, for drugs that rapidly enter and act upon the brain, drug dosing such as by inhalation results in higher brain concentrations and greater psychoactive (and possibly cardiovascular) effects than when the same dose is given more slowly. The possibility of titrating the level of a drug in the brain to achieve a desired mental state is also facilitated by inhalation as the route of dosing. These factors may explain why smoking a drug may be preferred over the oral or nasal routes of administration. In addition, as mentioned previously in the discussion of drug distribution, venous blood concentrations substantially underestimate arterial and brain levels after rapid dosing, and such data cannot (without mathematical transformations) be used to study concentration-effect relationships.

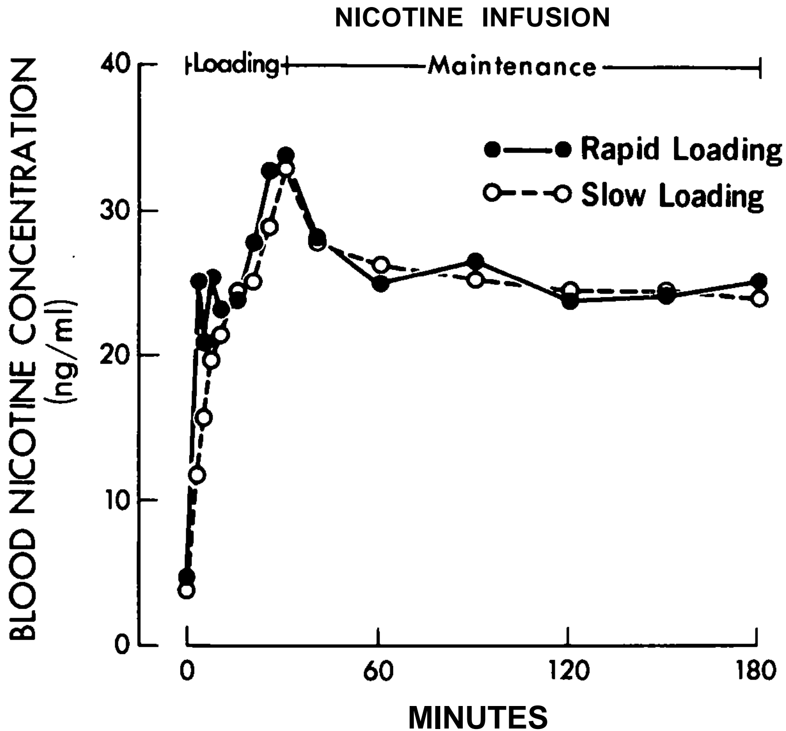

FIGURE 5. *Average blood nicotine concentration in seven subjects*

NOTE: (On the rapid-loading day, subjects received 17.5 ug/kg/min nicotine for 1.5 minutes, then 1.75 ug/kg/min for 30 minutes; on the slow-loading infusion day, 25 ug/kg/min for 30 minutes. Maintenance infusion on both days was 0.35 ug/kg/min from 30 to 180 minutes.

SOURCE: Porchet et al. 1988b. copyright 1988. Rockefeller University Press.

TOLERANCE

An important issue in drug dependence is the development of tolerance. Tolerance is defined as the tendency for a given dose of a drug, after repeated doses, to produce less of an effect. In some cases, increasing the dose of a drug in the presence of tolerance may achieve the same effect that was obtained after the first dose. Pharmacodynamic tolerance can be further defined as the condition that exists when a particular concentration of a drug at a receptor site (in the intact **organism,** approximated by the concentration in the blood) produces less of an effect than it did after a prior exposure.

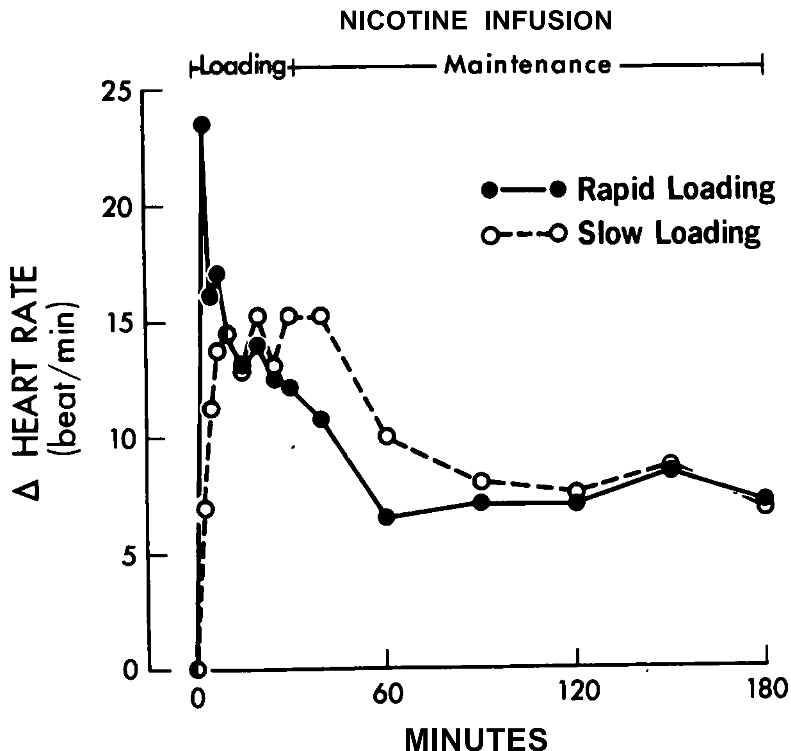

FIGURE 6. *Increase in heart rate in seven subjects*

NOTE: On the rapid-loading day, subjects received 17.5 ug/kg/min nicotine for 1.5 minutes, then 1.75 ug/kg/min for 30 minutes; on the slow-loading infusion day, 25 ug/kg/min for 30 minutes. Maintenance infusion on both days was 0.35 ug/kg/min from 30 to 180 minutes.

SOURCE Porchet et al. 1988b, copyright 1988. Rockefeller University Press.

Studies in animals demonstrate rapid development of tolerance to many effects of nicotine, although tolerance may not be complete (Marks et al. 1985). Smokers know that tolerance develops to some effects of smoking. Smoking the first cigarette as a teenager is commonly associated with dizziness, nausea, and/or vomiting, effects to which the smoker rapidly becomes tolerant. Smoking a cigarette after 24 hours of abstinence increases heart rate, whereas smoking an identical cigarette during the course of the day fails to increase heart rate (West and Russell 1987). Pharmacodynamic tolerance to heart rate acceleration and subjective effects is seen within the course of a 30-minute intravenous infusion of nicotine (Benowitz et al. 1982a).

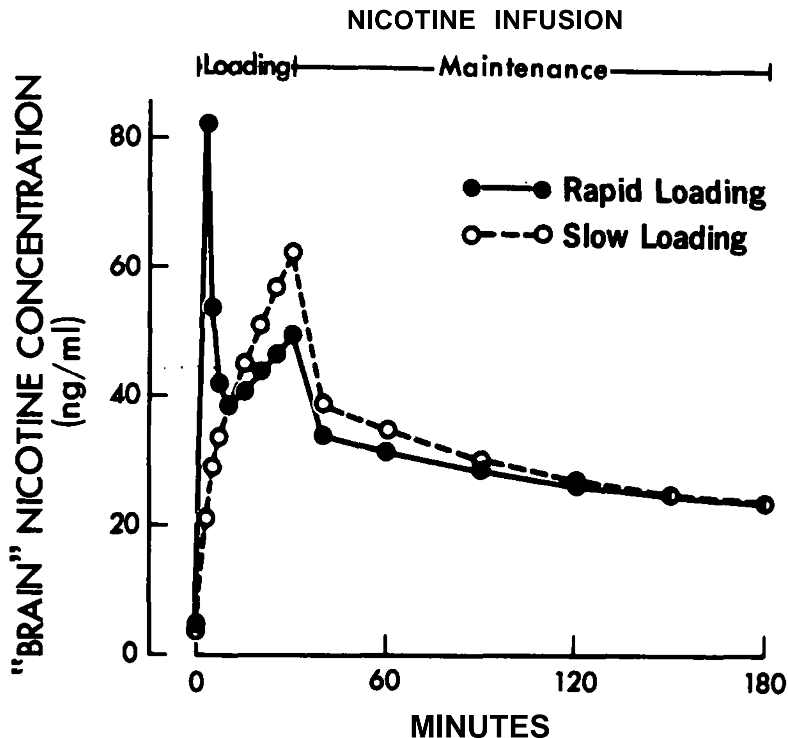

FIGURE 7. *Predicted mean "brain" concentrations of nicotine over time (based on a physiological kinetic model, fit to the rabbit and scaled to humans)*

NOTE: lnfusion rates are as described in figure 3. Note that "brain concentrations" peak at the same time as heart rate (see figure 5).

SOURCE: Porchet et al. 1988b, copyright 1988. Rockefeller University Press.

These and other findings indicate that tolerance is gained rapidly, within minutes (tachyphylaxis). Tolerance is lost, at least to some degree, after overnight abstinence. To characterize the time course of tolerance develop ment and regression, we conducted a study in which subjects received paired intravenous infusions of nicotine, separated by different time intervals (Porchet et al. 1988a). Despite higher blood concentrations, heart rate acceleration and subjective effects were much less when a second infusion was given at 60 or 120 minutes after the first infusion. At 240 minutes, the response was fully restored. The pharmacodynamics were modeled using a pharmacokinetic-pharmacodynamic model. This modeling indicates a half-life of development and regression of tolerance of 35 minutes. Such

modeling suggests that at three half-lives or 1 1/2 hours after a cigarette, nearly full sensitivity should have been regained.

Because tolerance develops so quickly, the rate of drug dosing influences the magnitude of effect. That is, the faster the administration, the less time there is to develop tolerance and the greater the effect for any given dose or maximal level. The interval at which cigarette smokers smoke cigarettes may be determined by two factors: the rate of distribution out of the brain after smoking a cigarette and the kinetics of regression of tolerance to effects of nicotine.

In summary, tolerance develops rapidly to the effects of nicotine as it does to effects of other inhaled drugs of abuse. The kinetics of tolerance are such that tolerance may develop and regress in cycles throughout the day. The interval at which users smoke their drugs may be influenced by two factors: the rate of distribution of drug out of the brain after a particular dose of the drug and the kinetics of regression of tolerance to effects of that drug. Rapid rate of dosing, as by inhalation, also allows the maximal drug effect with the least extent of development of tolerance.

PHARMACOKINETICS AND PHARMACODYNAMICS OF DRUG DEPENDENCE

The reinforcing properties of a drug are expected to be strongest when a psychoactive effect, usually a pleasant one, follows in close temporal proximity to the self-administration of a drug. In pharmacokinetic or pharmacodynamic terms, after dosing, the drug should enter the bloodstream rapidly and move rapidly from the bloodstream into the brain. The appearance of the drug in the brain should be associated with the desired psychoactive effects. If the effect of a drug is delayed after appearance of the drug in the brain or if tolerance to the drug effect is already present, the drug is less likely to be reinforcing.

That the user can easily control the dose of the drug and modulate the resultant psychoactivity would be expected to further strengthen the reinforcing nature of the drug. As discussed previously, nicotine obtained by cigarette smoking demonstrates these characteristics, as do other drugs of abuse that are inhaled, such as crack cocaine or marijuana, and drugs that are intravenously injected, such as heroin and cocaine.

Tolerance is most likely to develop to the effects of a drug when its receptors are continuously exposed to the drug. Frequent and sustained dosing, a long half-life of a drug, or a combination of these factors, would favor development of tolerance. The half-life of nicotine in the brain following a single dose exposure is short, probably about 10 minutes in humans, due to redistribution out of the brain to other body tissues. Some degree of tolerance does develop rapidly (tachyphylaxis), even after brief exposures. Such

tolerance also regresses relatively quickly (half-life, 35 minutes) such that a smoker may learn that smoking a cigarette at particular intervals is more reinforcing than smoking more frequently.

With repetitive dosing, levels of nicotine build up in the body (and in the brain) in accordance with the terminal half-life of 2 hours. Thus, there is substantial accumulation of nicotine in the brain and an increasing general level of tolerance throughout the day. Presumably, however, smoking individual cigarettes still results in peaks of nicotine in the brain, which may overcome the underlying level of tolerance and produce some of the desired effects.

Withdrawal symptoms are mast likely to occur when there has been a sustained effect in the brain and the drug is then rapidly removed. Thus, rapid exit from specific brain regions would favor more severe withdrawal symptoms. Nicotine, as it is dosed by cigarette smokers, exhibits both of these characteristics. Due to repetitive dosing, the brain is exposed to nicotine for prolonged periods of time, so that neuroadaptation occurs during the smoking day. When exposure is terminated, nicotine rapidly exits the brain and withdrawal symptoms are experienced. It is likely, therefore, that relief of withdrawal symptoms plays an increasingly important role in determining smoking behavior as the day progress.

NICOTINE AND THE DAILY SMOKING CYCLE

The pharmacokinetic and pharmacodynamic considerations discussed thus far help us understand the development of nicotine dependence and human cigarette-smoking behavior, as well as adverse effects of cigarette smoking. The daily smoking cycle can be conceived as follows. The first cigarette of the day produces substantial pharmacologic effects, primarily arousal, but at the same time tolerance begins to develop. A second cigarette may be smoked at a later time, at which the smoker has learned there is some regression of tolerance. With subsequent cigarettes, there is accumulation of nicotine in the body, resulting in a greater level of tolerance, and withdrawal symptoms become more pronounced between cigarettes. Transiently high brain levels of nicotine following smoking individual cigarettes may partially overcome tolerance. But the primary (euphoric) effects of individual cigarettes tend to lessen throughout the day. Overnight abstinence allows considerable resensitization to actions of nicotine. Because of the dose-response and dose-tolerance characteristics, habitual smokers need to smoke at least 15 cigarettes and consume 20 to 40 mg nicotine per day to achieve the desired effects of cigarette smoking and minimize withdrawal discomfort throughout the day.

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The Pharmacology of Cocaine Smoking in Humans

Reese T. Jones

INTRODUCTION

Smoking crack is smoking cocaine. Why would people go to the trouble of smoking cocaine when they can so easily snuff it, take it orally, or inject it parenterally? For many of the same reasons that nicotine is pleasurable to a tobacco addict and is so much more addicting when smoked in the form of tobacco cigarettes than when snuffed or chewed, so smoking the alkaloid cocaine is even more addicting than use by other routes (Benowitz, this volume). For similar reasons, most cannabis-dependent people choose to smoke marijuana or hashish even though tetrahydrocannabinol (THC) is psychoactive by oral ingestion. Of course, cocaine, nicotine, and THC pharmacology differ, but the aspects of their pharmacology that lead to preference for the smoked route of ingestion as well as some of the consequences of that choice are similar. If what is known of other inhaled drugs is kept in mind (Benowitz, this volume), it may be easier to understand certain conse quences of cocaine smoking and, perhaps more important, to design proper experiments to study cocaine smoking.

Smoking cocaine has become popular only in recent years, mostly in North America and some Latin American countries (Jeri 1984; Siegel 1985). Like most current patterns of psychoactive drug use, smoking cocaine was not completely unknown to the ancients. For example, in the United States, during the early part of this century, the Parke, Davis & Company pharmaceuticals catalog listed, among other forms of coca and cocaine, coca cheroots and coca cigarettes. The Parke, Davis coca cigars and cigarettes probably contained between 0.5 and 1.0 percent cocaine and were recommended for therapeutic use. A similar product known as the *Coca Leaf Ball* was advertised in England during the same period with the suggestion that "families, mothers and children" would benefit from the therapeutic effects of smoked coca. Famous people from England and Europe testified as to its effectiveness and must have engaged in cocaine smoking, although there is no indication that addiction to coca smoking was common. In

some ways, smoking coca leaf might be analogous to smoking tobacco or cannabis leaf in that a relatively small concentration (0.5 to 2 percent) of active substance (cocaine, nicotine, THC) is contained in a relatively large amount of plant material. Smoking coca leaf never became as popular as smoking tobacco leaf. Chewing coca leaf remained common only in relatively isolated groups, where it had long been part of their culture (Hanna and Hornick 1977).

In more recent times, smoking cocaine in the form of a crude plant extract sometimes called paste and containing about 80 percent cocaine base became popular in Peru and in adjacent countries (Almeida 1978; Jeri 1984; Flores 1986). Up to then, both in medical applications and illicitly, the water-soluble salt, usually cocaine hydrochloride, was the form more commonly used. Currently in North America, a readily available and widely discussed form is cocaine base, usually converted from the cocaine salt by mixing cocaine hydrochloride and a solution containing an excess of sodium bicarbonate usually baking soda) and heating and evaporating off the fluid. This leaves mostly cocaine base in a bicarbonate crystalline mixture. In most large cities in the United States, the availability of illicit ready-to-smoke base facilitates smoking cocaine. Cocaine base melts at a much lower temperature than the salt (about 80 °C instead of 180 °C) and then boils, producing an inhalable aerosol (Snyder et al. 1988; Wood, this volume). Whether forces of the illicit marketplace led to changes in cocaine chemistry and packaging or whether the availability of high concentration, relatively pure cocaine base shaped the market forces to increase consumer demand is not entirely clear. *In any case,* a cocaine user who chooses to smoke can now obtain material containing close to 100 percent cocaine rather than using coca plant material containing 1 percent of cocaine base. Consider the likely consequences if THC was readily available to add to smoking devices or if nicotine addicts began adding additional nicotine to tobacco cigarettes. Although not a perfect analogy, considering such events may help to put cocaine smoking in perspective.

PROBLEMS IN STUDYING COCAINE SMOKING

A variety of devices are used for cocaine smoking: pipes of all sorts, or cigarettes made of admixtures of cocaine base with tobacco, with marijuana, or with other plant material. The use of nonstandardized smoking devices contributes to a situation similar to that seen during the early days of cannabis-smoking research. When programs of controlled laboratory experiments with smoked cocaine are begun, it is not obvious what the typical laboratory smoking conditions should be, what constraints on smoking behavior are proper, and what physical and chemical considerations will maximize the likelihood that laboratory experiments will be relevant to the real world of illicit smoked cocaine, but will maintain adequate research subject safety. In contrast, a researcher of tobacco smoking has the advantages of great standardization of dose, dosage form, and delivery systems developed

by the tobacco industry. Even with that information, smoking behavior procedural issues in tobacco-smoking research, particularly dose regulation, are complex, and not all are resolved (Biding 1987; Kozlowski et al. 1982).

Relatively little has been published describing the human pharmacology of cocaine smoking under controlled or semicontrolled laboratory conditions (Perez-Reyes et al. 1982; Jeffcoat et al. 1989). Experiments in which experienced volunteers smoked from a glass apparatus heated to 260 ºC and containing 50 mg of cocaine base demonstrated the rapid increase in plasma cocaine levels during smoking, as compared to intravenous (IV) cocaine administration, that would be expected from what is known of the smoking process with nicotine and cannabis (Benowitz, this volume; Perez-Reyes, this volume). Other than more rapid onset, the resulting cardiovascular and psychologic changes were not unlike those seen after equivalent IV or nasal cocaine doses. Other experiments with monkeys (Siegel et al. 1976) and with smokers of crude extracts of cocaine often called paste noted a generally similar pattern of effect (Paly. et al. 1982).

Laboratory studies with any smoked drug present great methodologic challenges to the investigator. Proper pharmacologic studies must involve control of dose or at least accurate specification of the dose actually absorbed. The nature of smoking behavior makes dose difficult to regulate and, under many conditions, difficult even to estimate. Although animals can be made to smoke under some conditions (Siegel et al. 1976), animals seem reluctant to smoke the way humans do; thus, human studies are necessary.

Cocaine may present problems that have not been important in tobacco or cannabis smoking research. The rate of cocaine degradation is very temperature dependent, hence, subject to variability because of smoking-apparatus design and variation in conditions. Temperature in most cocaine-smoking devices varies with air flow. Air flow, in the case of humans smoking under natural conditions, depends on both puff and inhalation volumes and duration. The importance of puff and inhalation volume in determining dose is well recognized by tobacco and cannabis researchers (Heming et al. 1983; Benowitz 1986). How to control or regulate dose in psychopharmacologic studies involves compromises, however, and optimal solutions are not evident yet. When cocaine is smoked, the amount of cocaine pyrolyzed to other compounds will be related to the temperature in the smoking device. The temperature, in turn, will be related to the smoker's behavior, making for a complex equation to determine drug delivery.

When researching nicotine smoking, an investigator can depend on a degree of industry- and government-derived standardization of the smoking devices (cigarettes, pipes, etc.). In marijuana smoking research over the years, some consensus has developed as to smoking material and apparatus, at least in the laboratory. Cocaine smoking research, however, is nowhere near that point. The usual and expected "natural" or typical pattern (or

more likely, the range of patterns) of cocaine smoking is not at all established; hence, the relevance of laboratory paradigms is still open to question. For example, typical puff and inhalation parameters obtained during relatively unconstrained cocaine smoking have not been determined as they have with nicotine and with marijuana.

THE PHARMACOLOGY OF COCAINE SMOKING

In experiments trying to describe the pharmacology of cocaine smoking, we attempted to control some aspects of smoking behavior but, in these beginning experiments, left options open for the subjects, all of whom claimed to be experienced cocaine smokers. What is apparent in the laboratory data collected thus far is great variability; both variability in cocaine smoking behavior and, under conditions where smoking behavior parameters ate to some extent constrained, considerable variability in cocaine plasma levels and effects.

For example, in figure 1 the plasma concentrations are shown from 10 different research subjects smoking cocaine base under similar conditions. Peak venous cocaine levels ranged from less than 100 ng/ml to approximately 500 ng/ml, with considerable variability in time of peak as well. These cocaine smokers were allowed to take one or two and occasionally three inhalations spaced 1 minute apart from an electrically heated l-liter glass flask, beginning about 45 seconds after 100 mg of finely powdered cocaine base was dropped into the flask. At that time the cocaine base had melted, and a fine white aerosol was forming in the flask. The clear puddle of melted cocaine at the bottom of the flask was just beginning to yellow in the process that would turn it into a black tarry mass just a few minutes later. The temperature of the glass surface at the bottom of the flask was 260 °C.

The variation in plasma levels is contrasted with those from the same subjects given cocaine IV at a dose of 0.6 mg/kg of body weight injected by infusion pump over 1 minute (figure 2). Perhaps noteworthy is the considerable variability in peak plasma levels of cocaine even with controlled IV administration. Variability in venous plasma levels is not unexpected with a rapidly distributed, relatively rapidly metabolized drug, although it should give pause for thought to those attempting to predict precise relationships between drug effect and blood levels from a particular cocaine dose. It should be kept in mind that, particularly by the smoked route, arterial level of cocaine is more likely to be a much better indicator of brain level than is venous blood level. As is the case after nicotine smoking, venous cocaine levels will underestimate brain levels.

When the mean plasma cocaine levels after IV or smoked doses are compared to those of nasal or oral doses of cocaine hydrochloride, reasons for some of the appeal of the smoked route are evident (figure 3). The time of

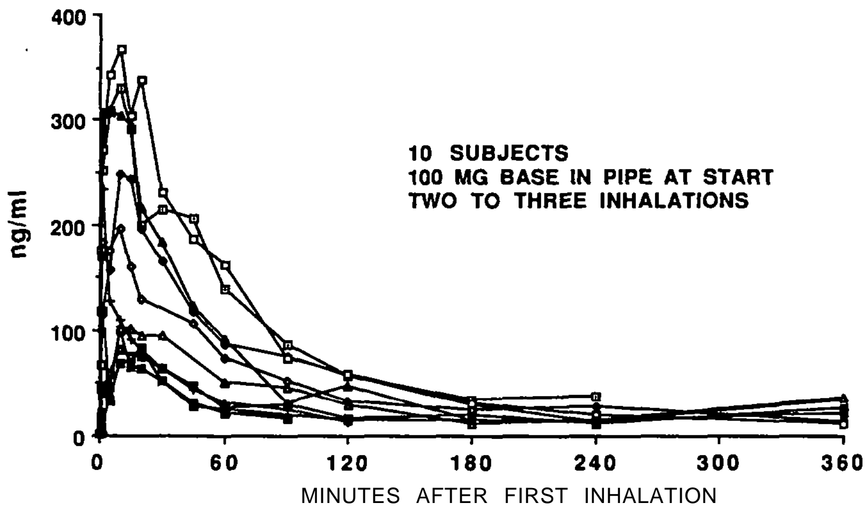

FIGURE 1. *Plasma levels of cocaine after smoking*

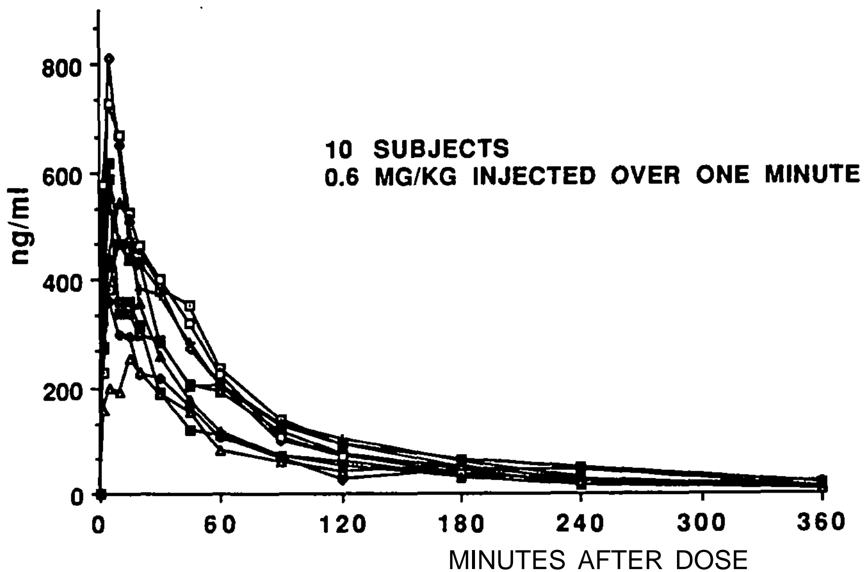

FIGURE 2. *Plasma levels of cocaine: IV administration*

peak level is significantly earlier after dose in the smoked or IV route. Similarly, the time of peak subjective effect is earlier by the smoked route (figure 4). Figure 4 illustrates the peak subjective-high rating in which zero is defined as a normal, sober state, and 100 is as intoxicated as ever experienced after cocaine administration.

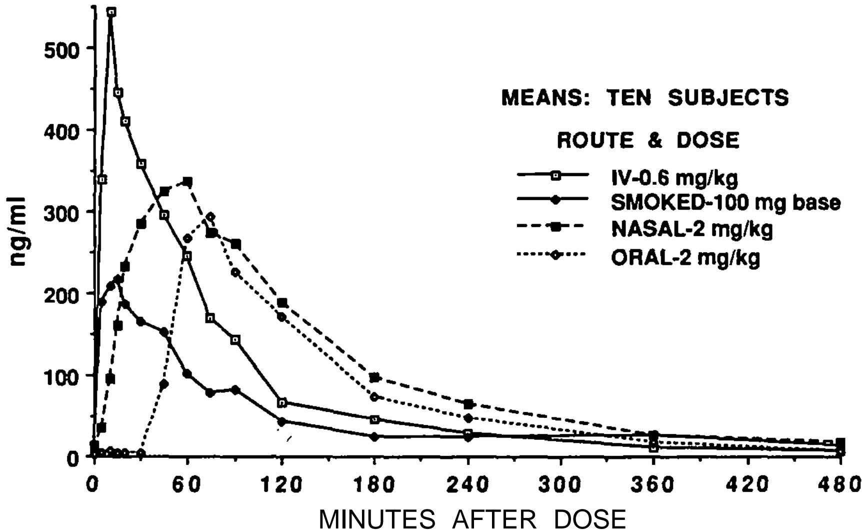

FIGURE 3. *Plasma levels of cocaine*

DEVELOPMENT OF TOLERANCE TO COCAINE

Why should a shorter interval to peak effect be important to a drug user? probably because neuroadaptation (tolerance) to many psychoactive drug effects is a rapidly developing process better timed in seconds and minutes than in hours or days. The slower any drug effect arises, other things being equal the less intense the effect, because tolerance to the effect is in a real sense developing concurrently with the appearance of the drug effect.

Rapidly developing tolerance to cocaine is demonstrated in a hysteresis plot of plasma level vs. subjective intoxication rating in figure 5. If little or no tolerance (neuroadaptation) developed over the 4 hours summarized in figure 5, the plot following the ascending and descending phases of the time/ plasma level function would be more or less a straight line. One index of the magnitude of tolerance is the divergence of those ascending and descending functions (Holford and Sheiner 1981). Note that the subjects had the option of rating their level of dysphoria with a minus value, and

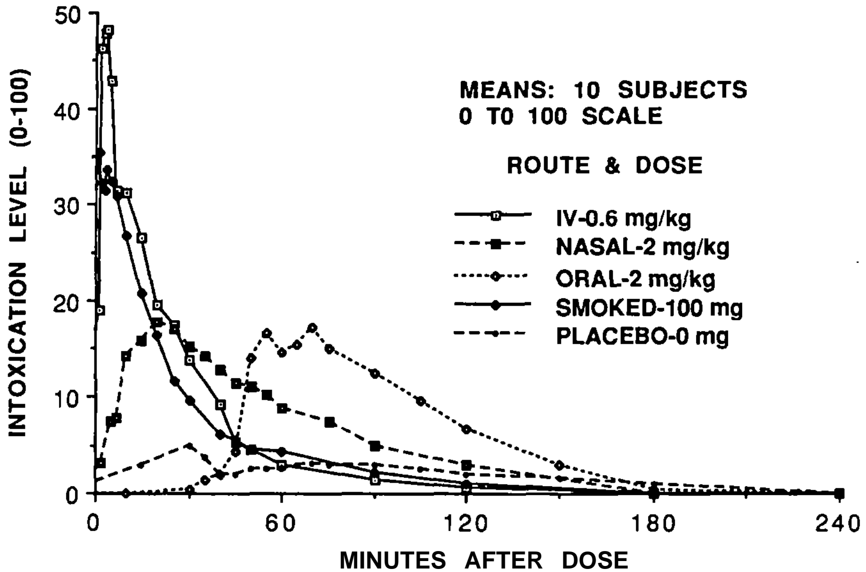

FIGURE 4. *Subjective high ratings*

some did so as the cocaine effects wore off. Figure 6 shows the clockwise hysteresis plots of subjective intoxication levels vs. cocaine concentrations in plasma after smoking cocaine and after an IV dose (0.6 mg/kg) in the same 10 research patients whose time vs. concentration data are shown in figures 1 and 2. Quantitative comparisons between the smoking and the IV hysteresis loops involve many assumptions, but a variety of semiparametric methods seem useful for a quantitative pharmacokinetic-pharmacodynamic descriptive model (Verotta et al. 1989). The similarity to the rapid tolerance that develops to many effects of nicotine is striking (Benowitz, this volume).

PHYSIOLOGIC AND SUBJECTIVE EFFECTS

Assuming that most of cocaine's subjective effects are related to its brain effects, the more rapid transit time from lung to brain (5 to 10 seconds) after inhalation of cocaine smoke would more likely provide a steeper and higher gradient of cocaine brain levels than could oral, nasal, or even IV administration. Table 1 summarizes the magnitude and time of some peak effects after cocaine was given to the same 10 experienced cocaine users by various routes, in balanced order, with about 3 days between doses.

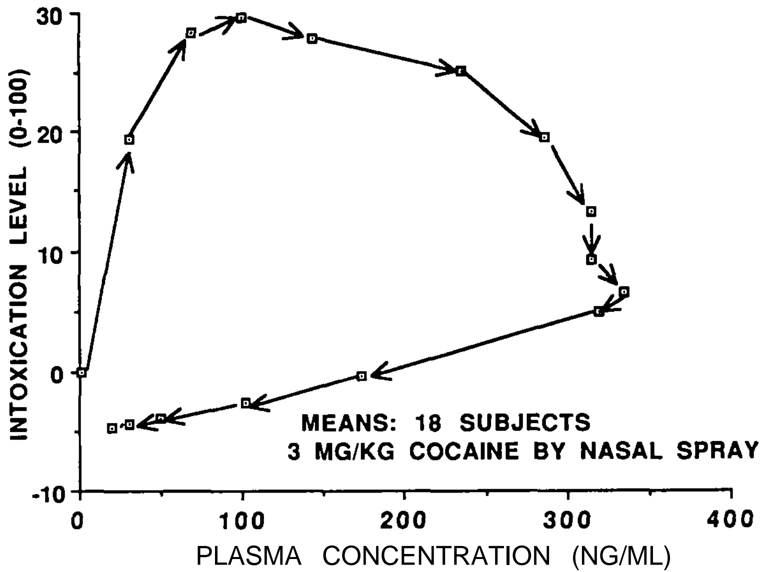

FIGURE 5. *"High" vs. cocaine levels in plasma after nasal cocaine (20% solution)*

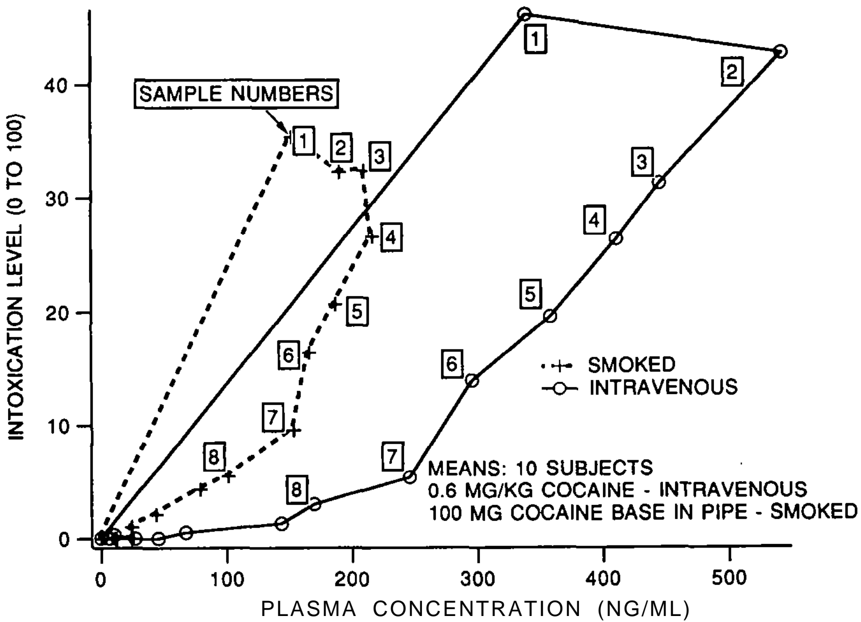

FIGURE 6. *"High" vs. cocaine levels in plasma after IV and smoked cocaine*

That both the peak venous plasma cocaine levels and the peak intoxication levels are greater when cocaine is taken IV, compared with the smoked route, might suggest more advantage to the IV route. To make proper comparisons between smoked dose and effect, the dose actually delivered to the individual subject must be considered. Therein lies one of the problematic considerations in human research with any smoked material. Traditional procedures to characterize bioavailability do not precisely fit drug-smoking situations. How much of that 100 mg of cocaine base placed in the pipe actually was absorbed by the subject?

TABLE 1. Mean physiologic and subjective changes after cocaine

	Route (Dose)			
Changes	IV (0.6 mg/kg)	Smoked* (0.4 mg/kg)	Nasal (2mg/kg)	Oral (2 mg/kg)
Heart rate increase, BPM (time to max, minutes)	46 (10)	32 (2)	26 (40)	20 (55)
Systolic BP increase mmHg (time to max)	28 (10)	32 (2)	24 (25)	19 (70)
Diastolic BP increase mmHg (time to max)	16 (10)	22 (1)	11 (25)	14 (75)
Pupil diameter increase mm (time to max)	0.8 (4)	1.1 (5)	0.6 (45)	0.5 (90)
Skin temperature decrease °C (time to max)	-2.8 (30)	-1.8 (20)	-4.7 (30)	-5.1 (75)
Subjective high 1-100 scale (time to max)	48 (4)	35 (1)	18 (20)	18 (70)

*Smoked dose is estimate, see text for discussion.

On average, 70 (range 10 to 85) mg of the cocaine base remained in the flask and was recrystallized on the surface of the tubing after smoking. Thus, on average, a maximum of about 30 mg of cocaine base was delivered to the mouth of the inhaling research subject. An indeterminant amount of cocaine was broken down to poorly characterized pyrolytic products in the smoking process. The biological activity of those products is not well established. Thus, in fact, considerably less than 30 mg of cocaine base was probably delivered to the mouth of some smokers. Our electrically heated l-liter flask was, of course, a rather special and not a typical free-base pipe, but experience with cocaine pipes brought into the laboratory by research subject volunteers is similar. Much, perhaps even most, of the

cocaine base placed in the pipe does not reach the mouth of the smoker. How much cocaine aerosol actually reaches lung alveoli depends on heating conditions, air flow, number of inhalations, and other things determined largely by the smoker, who only reluctantly yields control to the researcher, if cocaine smoking is like other smoking behavior.

If all the cocaine not remaining in the pipe was delivered to the lung alveoli and thus probably completely absorbed, cocaine dose could be better estimated. However, pyrolysis occurs at variable rates during the smoking process. Although breakdown is not completely quantified yet, there is strong evidence that anhydroecgonine methyl ester is one of the major breakdown products under our smoking conditions. Thus, under the smoking conditions leading to the plasma levels in figures 1 and 2, only a small fraction of the 100 mg of cocaine base in the smoking apparatus actually was delivered to the alveolar surface, where rapid absorption is most likely.

The relative percentages of cocaine base delivered, what remains in the pipe, and what is destroyed vary greatly with the smoking conditions encountered with various smoking apparatus. Although all the 10 subjects in the experiment discussed claimed to be experienced and reasonably confirmed cocaine base smokers, they exhibited a varied repertoire of smoking styles ranging from very big puffs with very little and brief inhalation to, at the other extreme, maximum and rapid forced inhalation and valsalva maneuver with little or no intermediate puff behavior. The variability in smoking behavior we have encountered among cocaine smokers is much greater than the variability commonly seen in otherwise similar groups of marijuana or tobacco smokers.

CONCLUSION

Inferring what is likely to be happening in the real world of crack smoking from laboratory studies is risky until the range of possible cocaine-smoking behavior in real-life situations is better understood and better described. Much can be gleaned from what we know of nicotine and THC smoking. The principles that guide the smoking of those drugs and the unresolved problems of researching nicotine and THC smoking apply to cocaine as well.

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Marijuana Smoking: Factors That Influence the Bioavailability of Tetrahydrocannabinol

Mario Perez-Reyes

INTRODUCTION

Marijuana smoking is the most commonly used method for the self-administration of delta-9-tetrahydrocannabinol (THC) the active principle of marijuana. The inhalation of marijuana smoke induces subjective effects ("high") that are rapidly perceived by the smokers. The perception of these effects allows smokers the capacity to achieve, within certain limits, their desired level of high by changing the puff volume, the interval between puffs, and the number of puffs taken. Therefore, the manner in which marijuana cigarettes are smoked (smoking dynamics) is probably the mast important factor in determining the bioavailability of THC. However, the following factors are also important: (1) the potency of the marijuana smoked; (2) the amount of unchanged THC present in the smoke inhaled (i.e., the amount of THC not destroyed by pyrolysis); (3) the amount of THC lost in side-stream smoke; (4) the method of smoking (cigarette vs. pipe smoking); and (5) the amount of THC trapped in the mucosa of the upper respiratory tract. A review of the experiments we have conducted that permit evaluation of the relative importance of these factors is presented in this chapter.

METHODS COMMON TO ALL CLINICAL STUDIES

Subjects

Male and female, healthy, paid volunteers who used marijuana no more than two times per week participated in the studies. They were thoroughly informed about the experimental procedures, the purpose of investigation, and the potential risks. All signed an informed consent approved by the Committee on the Protection of the Rights of Human Subjects of the University of North Carolina at Chapel Hill.

Experimental Variables

Subjective Ratings of Intoxition. The major psychologic effect of marijuana is a temporary euphoric effect with diversely perceived sensory, somatic, affective, and cognitive changes that are commonly described as a high. To measure this effect, we asked the subjects to rate their degree of high at frequent intervals during the experiment on a graph form provided for them. For this rating, the subjects were asked to estimate their level of high on a scale of 0 to 100; zero represented no effect and 100 represented the highest they had ever been after smoking marijuana. Every time a rating was to be made, subjects were given their previous rating to use for comparison. This technique allowed the subjects to rate themselves as experiencing relatively more, less, or the same effects as those rated in the previous time interval.

Physiologic Effects. Since the most consistent physiologic effect of marijuana in humans is cardiac acceleration, the ECG was continuously recorded on a polygraph before the beginning of smoking (baseline) and throughout the duration of the experiment. The results are reported as. the percentage of heart rate acceleration over baseline values.

Determination of the THC Plasma Concentration. Blood samples were drawn through an indwelling needle before the initiation of smoking and at frequent intervals thereafter. The plasma obtained was analyzed by standard radioimmunoassay procedures (Owens et al. 1981; Cook et al. 1982).

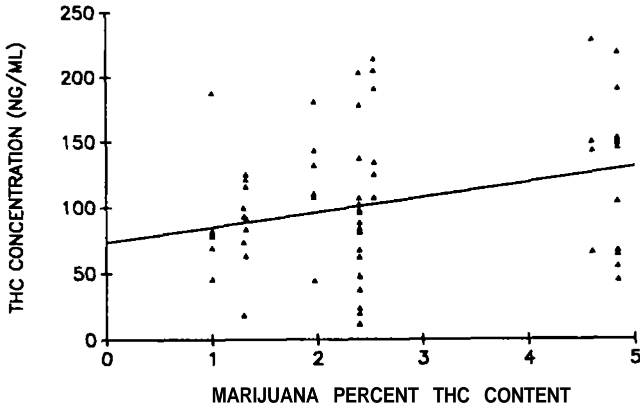
Statistical Analyses

For comparison, the means of the data plus or minus the standard errors of the mean (SEM) were calculated and are reported as such throughout the text. Areas under the curve (AUCs) were calculated by the trapezoidal rule method. The results were analyzed statistically by means of regression analysis and t test for either dependent paired data or independent nonpaired data as appropriate.

MARIJUANA POTENCY

To investigate the influence that marijuana potency per se has on the peak THC plasma concentrations produced, a regression analysis was done on the results of experiments in which National Institute on Drug Abuse (NIDA) marijuana cigarettes of eight different potencies (range 1 percent to 6.24 percent THC) were smoked. The marijuana potencies investigated, the number of subjects tested, the mean peak THC plasma concentrations, and the range of individual peak THC plasma concentrations are illustrated in figure 1. The results indicate the presence of a statistically significant correlation between the potency of the marijuana smoked and the peak THC plasma concentrations ($r=.269$, $r=2.27$, one-tailed $p<.025$). However, as

shown in this figure, wide individual variations in peak THC plasma concentrations occurred consistently across all of the marijuana potencies investigated. This variability in THC plasma concentrations indicates that factors other than the potency of the marijuana smoked, particularly the smoking dynamics, influence the magnitude of the resulting THC plasma concentrations.



THC CONTENT	SUBJECTS	THC (ng/ml)	RANGE
1.00%	6	90.4±20.2	45.6-187.8
1.30%	4	71.3±18.4	18.7-99.6
1.32%	6	100.0±10.1	62.8-125.3
1.97%	6	119.8±10.6	44.5-180.9
2.40%	18	63.0±8.6	11.7-137.0
2.40%	6	119.0±23.0	81.0+203.0
2.54%	6	162.6±18.7	107.4-204.7
4.60%	4	146.3±33.1	65.7-227.6
4.84%	12	124.2±16.2	44.8-218.0

FIGURE 1. *Relationship between marijuana potency and THC plasma concentration (top) and marijuana potency and peak THC plasma concentration (ng/ml) (bottom)**

*r=269; p=.025

NOTE: In the upper part of the figure, the Individual peak THC plasma concentrations of 68 volunteers are plotted against the THC content of the NIDA marijuana cigarettes that they smoked. In the lower part of the figure, the numerical values measured are summarized.

PYROLYTIC DESTRUCTION

When marijuana is smoked, a portion of its THC content is destroyed by pyrolysis before reaching the mouth of the smoker. Information regarding the amount of THC destroyed by pyrolysis is derived from both cigarettes and water-pipe smoking.

Cigarette Smoking Study

Davis and colleagues (1984) conducted a study in which standard NIDA marijuana cigarettes containing 1.6 percent and 3.1 percent THC were smoked by a cigarette-smoking machine in a constant draft mode, i.e., the cigarettes were artificially inhaled in a single puff until nothing was left. The percent of the original THC not recovered in the smoke condensate collected and probably lost to pyrolysis was 30.6±1.4 and 30.9±1.2 for the 1.6 percent and the 3.1 percent THC content cigarettes, respectively. Since no measurement of the THC trapped in the smoking apparatus was done, the total amount actually lost to pyrolysis, under their experimental conditions, was probably less than 30 percent.

Water-Pipe Smoking Study

Marijuana water-pipe smoking replicates artificial single-draft smoking because the amount of plant material placed in the bowl of the pipe is entirely combusted, and the smoke produced is inhaled in a single puff. One milligram of Vitiated THC was added to 70 mg of placebo marijuana (0.004 percent THC) and placed in the bowl of a commercially available water pipe. One bowl was smoked every 2.5 minutes, for a total of 10 bowls. The six volunteers who participated in this study were instructed to draw air until the total amount of marijuana placed in the pipe's bowl was combusted and to inhale the total amount of smoke produced in a single puff. A Cambridge filter, to trap the THC content of the smoke produced, was placed between the mouth of the pipe and the mouth of the subjects. The amount of THC recovered from the pipe, the plant material residues, and the Cambridge filters was measured by gas liquid chromatography. It was found, in this unpublished study, that 40.4±1.5 percent of the original amount of THC was trapped in the Cambridge filters (i.e., approximately the amount of THC that would have reached the mouth of the subjects if the marijuana smoke had not been filtered), and that 40.2±4.5 percent of the original amount of THC remained in the pipe and plant material residues (figure 2). Therefore, since approximately 80 percent of the original amount of THC was recovered, it can be concluded that 20 percent was destroyed by pyrolysis.

The results of these smoking studies, given the large margin of error introduced by differences in methodology, compare favorably and indicate that between 20 percent and 30 percent of the THC contained in the plant

material is destroyed by pyrolysis during single-draft smoking conditions, irrespective of the marijuana potency smoked.

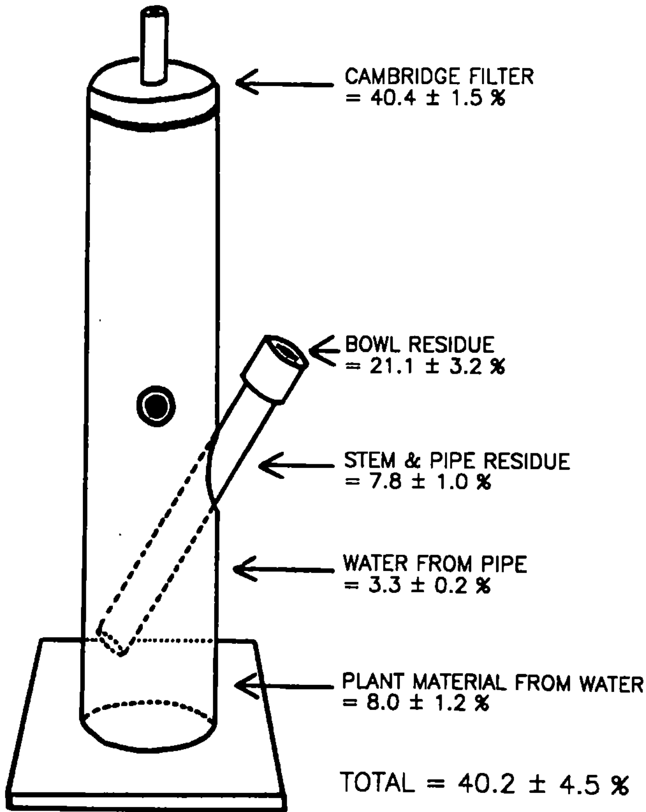


FIGURE 2. *Diagram of the water pipe used in THC studies*

NOTE: The amount of THC recovered from the pipe components and the amount of THC that was potentially inhaled by the subjects (i.e. the THC trapped in the Cambridge filter) are indicated.

SIDESTREAM SMOKE

When marijuana cigarettes are combusted, the smoke produced is either inhaled when puffs are taken (mainstream smoke) or escapes to the

atmosphere during the interval between puffs (sidestream smoke). To have an approximate measure of the amount of THC present in mainstream smoke and lost in sidestream smoke when marijuana cigarettes are smoked by machine, experiments were conducted by Davis and associates (1983). In these experiments, standard MDA marijuana cigarettes containing 1.6 percent and 3.1 percent THC were smoked by a cigarette-smoking machine. Two modes of smoking were investigated, continuous inhalation (i.e., the cigarettes were inhaled in a single puff until nothing was left) and intermittent inhalation (the cigarettes were inhaled at a rate of two 40-ml puffs per minute). The results of these experiments indicate that marijuana potency did not significantly influence the percent of THC present in mainstream smoke within either mode of artificial smoking, i.e., 69.4±1.4 and 69.1±1.2 percent for continuous smoking and 19.0±1.2 and 16.1±0.6 percent for intermittent smoking, respectively, for the 1.6 percent and 3.1 percent THC-content cigarettes. Assuming that the amount of pyrolytic destruction of THC is the same between constant and intermittent smoking, the difference in the amount of THC present in mainstream smoke between the two modes of smoking indicates that between 40 percent and 50 percent of the original THC content of the cigarettes was lost in side stream smoke (figure 3).

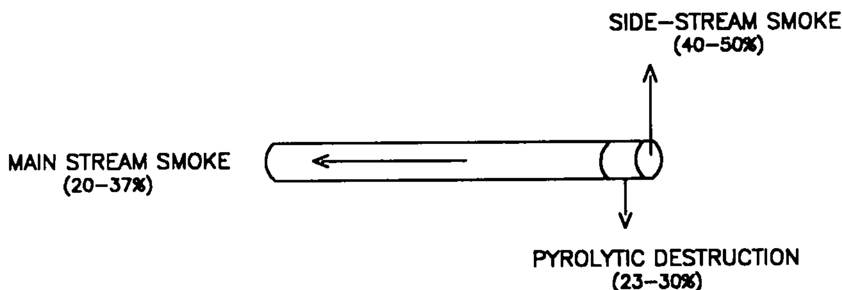


FIGURE 3. *Fate of THC during marijuana cigarette smoking*

NOTE: The percent of THC that potentially reach the mouth of the subject (mainstream smoke) and that lost to pyrolysis and sidestream smokc when marijuana cigarettes are smoked. Percent values are approximations.

The results of clinical studies of marijuana cigarette smoking (Perez-Reyes et al. 1982) indicate that, on the average, smokers puff more often (2.6 puffs per minute) than the puff frequency used in the Davis cigarette-smoking study (2 puffs per minute) (Davis et al. 1983). Therefore, it is reasonable to assume that when subjects smoke marijuana cigarettes, the amount of THC lost in sidestream smoke is not as large as that found in the cigarette-smoking-machine experiments. Nevertheless, this loss should

still be of considerable magnitude because the cumulative interpuff interval (the time when the cigarette is idly smoking) accounts for 90 percent of the total smoking time (table 1).

TABLE 1. *Smoking parameters for two modes of marijuana cigarette smoking*

	Accelerated Smoking (n=4)		Slow Smoking (n=6)	
	1.51% THC	2.87% THC	1.32% THC	254% THC
Smoking time (min)	5.2±0.9	6.2±1.0	17.9±2.0	17.2±2.1
Number of puffs	18.3±2.3	17.0±2.3	30.7±5.1	31.5±4.0
Puff duration (sec)	2.0 ± 0.2	1.8±0.1	2.0±0.4	2.3±0.3
Interpuff interval (sec)	18.3±2.5	22.5±4.2	20.7±2.8	21.0 ±2.4
Total puff duration (sec)	31.9±3.8	30.4±5.4	56.2±6.3	74.3±13.8
Total interpuff interval (rain)	4.7±0.9	5.7±0.9	16.9±2.0	15.9±2.0
Plasma THC, time to peak (min)	6.5±1.0	5.0±1.2	6.7±0.6	7.7±0.8
Plasma THC, peak value (ng/ml)	210.8±44.0	229.6±46.4	100.0±10.1	162.6±18.7
Mean puff volume (ml)	55.8±10.6	49.2±6.8		
Total puff volume (ml)	889.0±92.0	818.0±106.0		

NOTE: Data obtained in studies in which marijuana cigarettes of comparable potency were smoked by the volunteers in one instance through a puff-volume measuring instrumcnt (acceleratcd smoking), and in the other instance without it (slow smoking).

SMOKING DYNAMICS

Marijuana Cigarette Smoking

We have conducted studies in which the number of puffs, the puff duration, the interval between puffs, and the duration of marijuana cigarette smoking of similar (Perez-Reyes et al. 1981) or different (Perez-Reyes et al. 1982) potencies were measured. However, in these studies, the critically important puff volume parameter was not measured. To fill this gap in the knowledge of the dynamics of marijuana cigarette smoking, we conducted experiments in which the puff volume was measured throughout the period of smoking.

To measure the puff volume, a cigarette-holder flow-rate transducer based on the Venturi's flow meter was used (Hutcheson et al. 1984). The signal from this pressure transducer was amplified, filtered, and then routed to a PDP 11/34 Laboratory computer for processing by an online program that

computed puff volume, puff duration, inter-puff interval, cumulative puff volume, and cumulative smoking time.

Using this technology, NIDA marijuana cigarettes containing 1.51 percent and 2.87 percent THC were smoked at weekly intervals in a double-blind crossover design by four male volunteers. To imitate natural conditions as much as possible, the volunteers were instructed to smoke the cigarettes in their customary fashion and to smoke until they had reached their desired level of high or for as long as they wished. Blood samples for the determination of the THC plasma concentrations were collected.

Results

Smoking Time. For comparison, the results obtained in this study in which the cigarettes were smoked through the puff-volume transducer and those obtained in a study in which cigarettes of comparable potency were smoked without this device are illustrated in table 1. Examination of the data shows a profound difference in mean smoking time between the two studies. We hypothesized that the presence of the cigarette-holder puff-volume transducer in front of the subjects' faces provoked them into an unintended accelerated rate of smoking. Accelerated smoking did not affect the mean puff duration or the mean interval between puffs. The mean number of puffs was decreased, however, and as a consequence the mean cumulative interval between puffs was shorter.

Puff Volume. As shown in table 1, the mean puff volume and the mean cumulative puff volume were not significantly influenced by the potency of the marijuana smoked. The mean puff volumes observed during the period of smoking are shown in figure 4. The similarity of the smoking pattern between the groups is apparent, as is the progressive decrease in puff volumes over time. To investigate the significance of this pattern, the cumulative puff volume inhaled by each subject during the first half was compared to that which occured during the second half of their smoking period (figure 5). The differences in mean cumulative puff volumes between cigarette halves are statistically significant: $t=3.22$, $p<.05$, and $t=4.53$, $p<.02$ for the 151 percent and the 2.87 percent THC content marijuana, respectively.

THC Plasma Concentration. For each comparable marijuana potency smoked, the magnitude of the THC plasma concentrations during the time of observation (figure 6) and the mean peak THC plasma concentrations measured (table 1) were larger in response to accelerated smoking. This finding may be due to the large individual variation in THC plasma concentrations (figure l), as different volunteers participated in the studies. This could also be due to a faster rate of THC delivery during accelerated smoking studies.

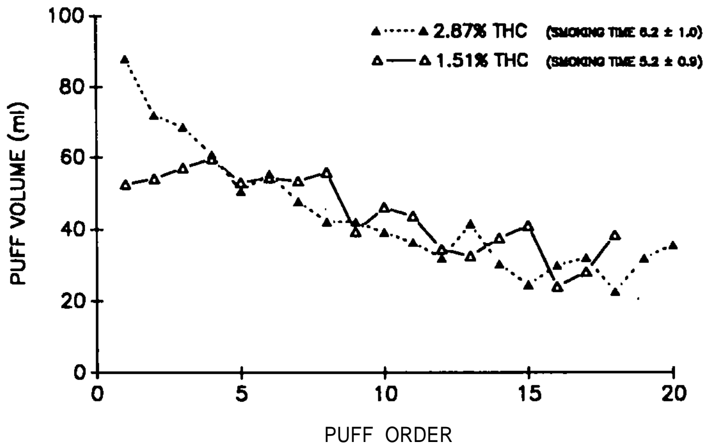


FIGURE 4. *Comparison of mean puff volume*

NOTE: Mean smoke volumes inhaled per puff during the period of smoking. It can be seen that the twofold difference in marijuana potency did not change the smoking profile.

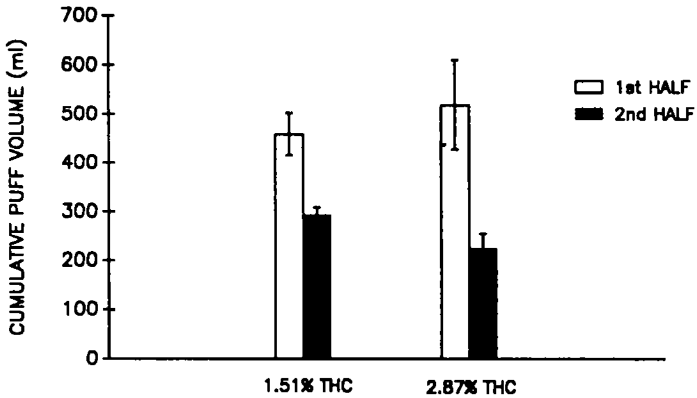
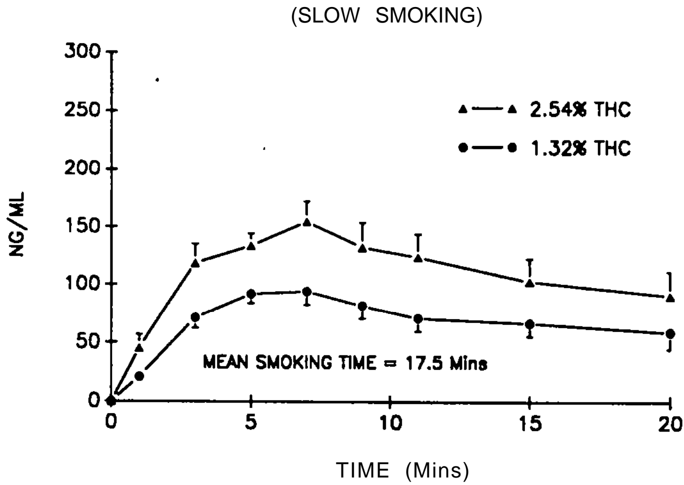


FIGURE 5. *Comparison of the cumulative puff volume (total smoking time divided into halves)*

NOTE: A larger mean cumulatlve smoke volume was inhaled during the first half of the cigarettes. The difference between marijuana potencies is not statistically significant.



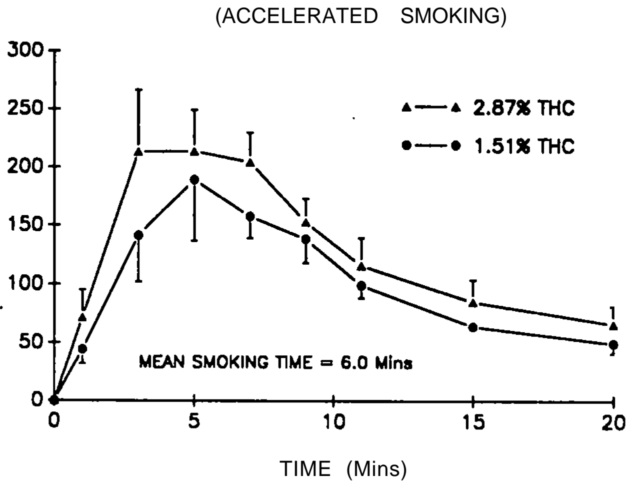


FIGURE 6. *Comparison of the mean THC plasma concentration between slow and accelerated smoking*

The relationship between THC time to peak and the smoking time observed in response to slow smoking reported in table 1 indicates that THC plasma concentrations sometimes peaked several minutes before the termination of smoking. As shown in figure 7, this relationship has been consistently

observed in the many marijuana-smoking experiments that we have previously conducted. ThiS observation was always intriguing because it was contrary to the reasonable expectation that for as long as the smoke produced by the combustion of marijuana cigarettes was inhaled, the resulting THC plasma concentrations should have continued to increase until smoking was terminated. Therefore, it was unexpected to find that, during accelerated smoking, the THC plasma concentrations peaked at approximately the same time that smoking was terminated. We hypothesized that rapid inhalation of marijuana smoke resulted in a rate of delivery of THC to the bloodstream that was faster than the drug's rate of disappearance into the extravascular compartment. In contrast, when the rate of inhalation of marijuana smoke was slow, the drug's rate of disappearance into the extravascular compartment was faster than the rate of delivery of THC to the bloodstream.

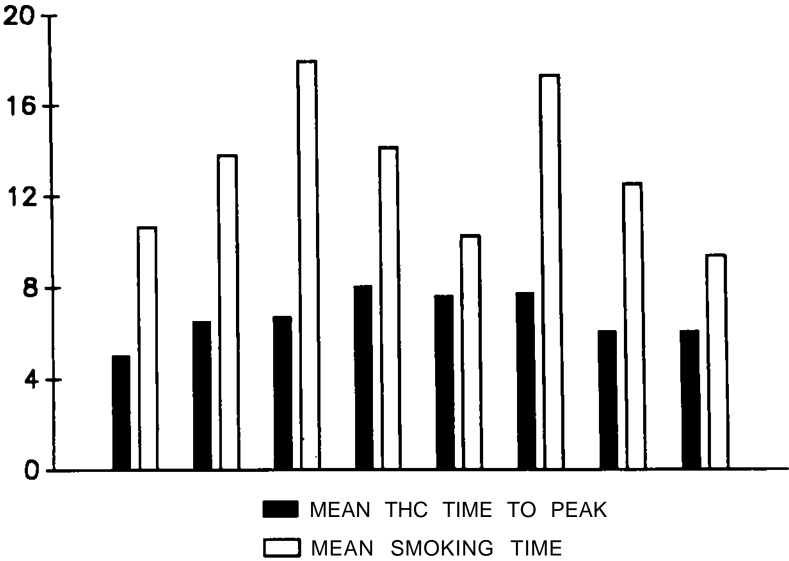


FIGURE 7. *Relationship between smoking time and THC time to peak*

NOTE: The results of eight separate studies in which marijuana cigarettes were smoked in the volunteers customary manner are shown. It can be seen that peak THC plasma concentrations cosistently occurred before the termination of smoking.

Marijuana Water-Pipe Smoking

Marijuana is frequently smoked in pipes of different sixes and configurations, particularly in water pipes, because they cool the smoke and facilitate its inhalation. This method of smoking has become more popular in recent years because it permits the combustion of smalI portions of high-potency marijuana (sometimes as high as 13 percent THC in Sinsemilla plant material).

As discussed, marijuana cigarette smoking of identical THC content results in large interindividual variations in THC plasma concentrations. This is because the number of puffs taken, the interval between puffs, and the volume of smoke inhaled per puff vary greatly from person to person. In contrast, marijuana pipe smoking permits control of the number of puffs taken, the interval between puffs, and the volume of smoke inhaled per puff. Moreover, at least theoretically, the amount of plant material placed in the bowl of the pipe should be entirely combusted, all of the smoke produced should be inhaled, and practically no THC should be lost in sidestream smoke. We hypothesized that under these controlled smoking circumstances, less interindividual variation in the magnitude of THC plasma concentrations should occur.

To verify this hypothesis, we conducted three dose-response studies, using a single-blind crossover design in which marijuana of different potencies was smoked in a 250-ml capacity water pipe (figure 2). A wire screen was placed at the bottom of the pipe's bowl to prevent uncombusted plant material from dropping into the pipe. A constant amount of water was used to fill the pipe, which provided a consistent 200-ml volume of air space between the mouth of the subject and the water level (the puff volume).

The volunteers were instructed to inhale the total amount of smoke produced by the combustion of the marijuana placed in the pipe's bowl ("toke") and to hold the smoke in their lungs for 10 seconds. Smoke inhalation was facilitated by the drawing of atmospheric air through a small hole located above the water level. Tokes were taken at 0, 2.5, 5, 7.5, and 10 minutes. This rate of administration was selected because in our previous studies in which radiolabeled ’H-THC was smoked in a water pipe, the resulting THC plasma concentrations were sustained during the period of smoking.

Experimental Variables. To determine the peak THC plasma concentrations produced, blood samples were drawn through an indwelling needle at appropriate intervals. The subjective rating of marijuana high was measured at frequent intervals throughout the experiments, and the heart rate was recorded before and after marijuana smoking in only the second and third studies.

Drug Dosage. In our initial study, 0.625 mg/kg of marijuana containing 1.68 percent or 2.51 percent THC was combusted per toke. The amount of smoke produced by the combustion of this amount of marijuana was large, and some of the six volunteers who participated in the study had difficulties combusting the tokes completely or inhaling the total amount of smoke generated. For this reason, a second dose-response study was conducted in which the amount of plant material combusted per toke was reduced to 0.5 mg/kg for marijuana containing 2.51 percent THC and to 0.4 mg/kg for marijuana containing 3.12 percent THC. The latter marijuana potency was obtained by mixing equal parts of 6.24 percent and 0.004 percernt THC. The amount of smoke produced by the combustion of tokes containing 0.5 mg/kg was still large enough that some of the six volunteers who participated in this study had difficulty inhaling the smoke completely. However, the amount of smoke generated by the combustion of the 0.4 mg/kg tokes was completely inhaled without difficulty. The pharmacologic effects obtained in this study were of greater magnitude than those obtained in the first study, but they were leas pronounced than those produced by the smoking of marijuana cigarettes. In an effort to obtain larger pharmacologic effects, a third study was conducted in which 0.4 mg/kg of marijuana containing 3.12 percent and 6.24 percent THC was combusted per toke. Originally, as in the previous studies, we planned to teat six volunteers. However, the smoking of the high-potency marijuana induced postural hypotension and bradycardia (vasovagal syndrome) in the third and fourth subjects tested. Because of this undesirable side effect, the study was terminated after testing only three subjects. We are perplexed by the occurrence of this syndrome because we have never observed it in response to marijuana cigarette smoking. We have, however, observed the occurrence this syndrome in some subjects after the oral administration of high doses of THC (35 mg) or after the intravenous infusion of THC.

Results

THC Plasma Concentrations. As illustrated in figure 8, the THC plasma concentrations increased progressively for as long as tokes were taken. As a result, peak THC concentrations occurred in close proximity to the end of the smoking period (table 2). This finding suggests that the inhalation of a large volume of smoke (approximately 200 ml) every 2.5 minutes delivers enough THC to the blood stream to balance the drug's rate of disappearance into the extravascular compartment. In this respect, the results of marijuana water-pipe smoking are comparable to the results of accelerated marijuana cigarette smoking.

Contrary to our expectations, peak THC plasma concentrations varied widely among the subjects who participated in the studies (table 2). This result is disappointing because it demonstrates our failure to reduce the variability in peak THC plasma concentrations, despite controlling the amount of marijuana combusted per toke, the number of tokes taken, and the interval

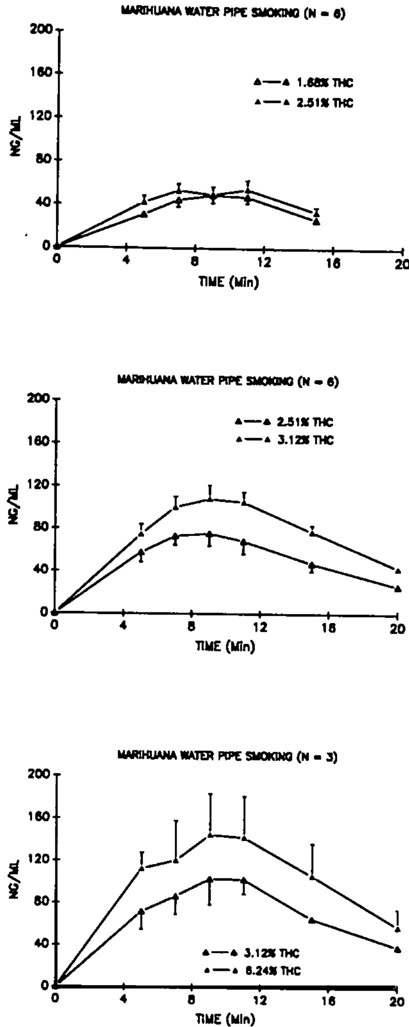


FIGURE 8. *Comparison of the magnitude of THC plasma concentrations produced by marijuana water-pipe smoking over time*

between tokes. It should be noted, however, that smoking factors other than those controlled in this study may have influenced the magnitude of the THC plasma concentrations observed. For example, although the subjects were instructed to hold the smoke inhaled for a period of 10 seconds, this requirement was not enforced. Therefore, according to their custom,

many subjects held the smoke for longer intervals of time. Likewise, it was not possible to maintain a constant puff volume because uncontrolled amounts of atmospheric air were drawn to facilitate inhalation of the smoke produced by the combustion of each toke.

TABLE 2. *Comparison of the THC plasma concentrations produced by marijuana water-pipe smoking*

Potency	Time to Peak (min)	Peak Value (ng/ml)	Range (ng/ml)
	First Study (n=6)		
1.68% THC	9.7±0.8	53.7±6.0	40.9-72.7
2.51% THC	8.3±1.0	58.8±7.6	34.9-81.8
	Second Study (n=6)		
2.51% THC	7.7±0.7	79.7±10.8	39.0-116.0
3.12% THC	9.0±0.7	114.5±10.4	80.8-150.0
	Third Study (n=3)		
3.12% THC	10.3±0.7	109.6±20.8	81.0-150.0
6.24% THC	8.3±1.8	151.7±37.0	90.2-218.0

NOTE: Despite controlled conditions, THC plasma concentrations varied widely among the subjects tested.

Subjective Effects. The mean subjective rating of high over the time of observation is illustrated in figure 9. It can be seen that the magnitude of subjective high increased progressively during smoking and for several minutes after its termination. Peak values occurred approximately 20 minutes after the beginning of smoking, i.e., approximately 10 minutes after the occurrence of peak THC plasma concentrations (table 3). Although peak subjective effects and AUC (0-35') values increased proportionally to the magnitude of the THC plasma concentrations obtained, statistical analysis of this limited amount of data indicates that no significant correlation is present between peak THC plasma concentrations and peak subjective effects (r=.153, t=0.82, p NS). This lack of correlation is probably due to the large intersubject variations in both plasma THC concentrations and subjective effects.

Heart Rate Effects. The mean heart rate acceleration over the time of observation is illustrated in figure 10. The changes in heart rate produced by marijuana pipe smoking mimic in all respects the subjective rating of high. Thus, the heart rate accelerated progressively during smoking and for

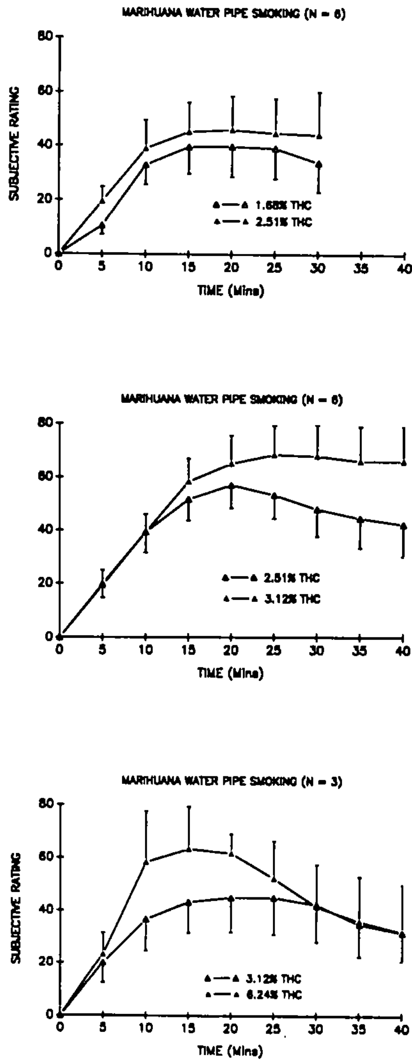


FIGURE 9. *Comparison of the magnitude of the subjective rating of high produced by marijuana water-pipe smoking over time*

several minutes after its termination. Peak values occurred approximately 20 minutes after the beginning of smoking i.e., approximately 10 minutes after the occurrence of peak THC plasma concentrations (table 3). Likewise, mean peak heart rates and AUC (0-35') values increased proportionally to the magnitude of the THC plasma concentrations obtained.

TABLE 3. *Comparison of the pharmacologic effects produced by marijuana after pipe smoking*

Time to Peak	Peak Value	AUC (0-35')
	Subjective Rating of High	
	First Study (n=6)	
18.3±2.5	41.7±10.5	980±221
18.3±1.7	48.3±11.9	1,071±265
	Second Study (n=6)	
20.0±2.2	57.5±8.9	1,457±249
28.3±4.0	73.0±10.4	1,754±282
	Third Study (n=3)	
2.0±5.0	50.7±12.9	1,270±368
13.3±3.3	68.3±13.6	1,595±409
	Percent Heart Rate Acceleration	
	Second Study (n=6)	
20.8±2.7	34.4±6.2	492±151
21.7±2.1	53.6±5.5	862±96
	Third Study (n=3)	
14.0±0.0	70.4±3.0	1,377±293
18.0±2.0	74.9±18.0	1,589322

NOTE: The heart rate was not measured in the first study.

Nonetheless, statistical analysis indicates that no significant correlation is present between peak THC plasma concentrations and peak heart rate acceleration (r=.385, t=1.67, p NS). This is probably due to the large intersubject variations in plasma THC concentrations and heart rate accelerations.

AMOUNT OF THC TRAPPED IN TEE UPPER RESPIRATORY TRACT MUCOSA

The results of several independent studies indicate that THC is present in saliva and that its concentration declines with time after marijuana smoking (Hawks 1984; Cross et al. 1987; Maseda et al. 1986; Thompson and Cone 1987). The presence of THC in saliva after marijuana smoking is not due to its secretion by the salivary glands. This is demonstrated by the results of experiments in which we administered radioactively labeled THC orally

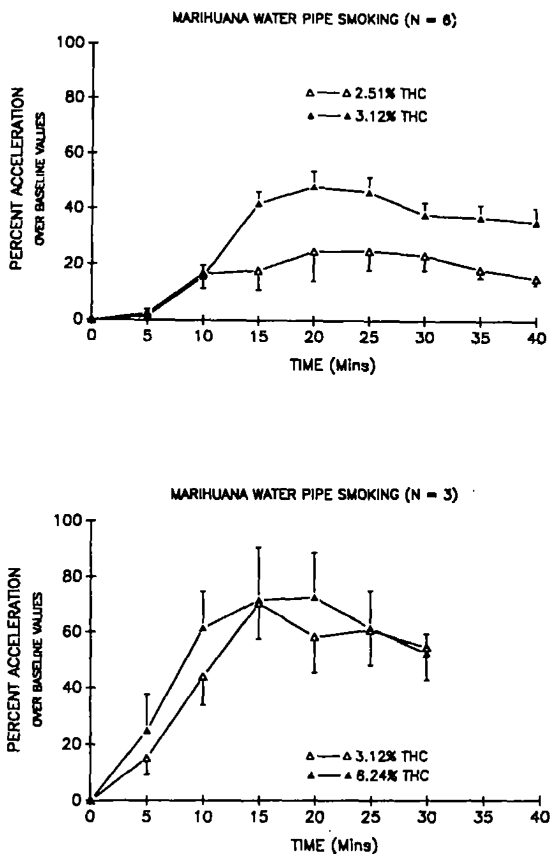


FIGURE 10. *Comparison of magnitude of mean hem rate acceleration produced by marijuana water-pipe smoking over time*

and intravenously to normal volunteers (Perez-Reyes et al. 1972; Perez-Reyes et al. 1973). In these experiments we collected timed saliva samples after drug administration. No radioactivity was detected in any of the saliva samples assayed. This observation indicates that neither THC nor its me tabolities are excreted by the salivary glands. Therefore, the THC present in saliva derives from that sequestered in the buccal mucosa during smoking.

The amount of THC trapped in the buccal mucosa or present in mixed saliva samples is time and dose dependent. This is demonstrated by a study in which 30 volunteers smoked NIDA marijuana cigarettes containing either

2.49 percent (18 subjects) or 4.84 percent (12 subjects) THC blindly. The mean amount of THC present in swabs of the buccal mucosa and the mean amount of THC present in 25 microliters of mixed saliva samples are shown in table 4. Because the THC present in the swabs cannot be accurately related to a volume of saliva, we have reported them as amounts instead of concentrations. The largest amounts of THC in swab and saliva, irrespective of the marijuana potency smoked, were observed at the first time of measurement (i.e., 1 hour after the initiation of smoking). After this time, mean THC amounts declined progressively to minimal but still detectable values at the 4-hour end of the study.

TABLE 4. *Comparison of the mean amounts of THC (ng) measured in 25 microliters of saliva and swabs of the roof of the mouth and tongue*

	2.40% THC		4.84% THC	
	Swab	Saliva	Swab	Saliva
1 Hour	16.9±7.2	20.9±8.2	59.8±27.5	60.5±24.9
2 Hours	7.8±3.7	5.8±1.8	26.5±12.3	26.7±14.8
3 Hours	7.1±3.8	4.1±1.3	16.6±8.9	6.9±2.7
4 Hours	3.8±1.7	1.9 ±0.8	13.5±7.4	4.2±1.7

Since THC is trapped in the buccal mucosa, it is reasonable to assume that THC is also trapped in the mucosa of the other parts of the upper respiratory tract, i.e., pharynx, larynx, trachea, and bronchi. Obviously, it is not possible to know the amount of THC that is trapped in the combined surface. area of these anatomical structures during marijuana smoking. However, judging by the relatively small amounts of THC trapped in the buccal mucosa (i.e., less than 100 ng in either swabs or 25 microliters of saliva 1 hour after smoking) the total amount of THC trapped in the mucosa of the upper respiratory tract can be considered to be a factor of minor importance with regard to the bioavailability of THC

CONCLUSION

Although smoking is a practical method for the delivery of marijuana's active principle (THC), many factors inherent in the smoking process decrease the drug's bioavailability. For example, whether marijuana is smoked in pipe or cigarette form, 23 to 30 percent of its THC content is destroyed by pyrolysis; if marijuana is smoked in cigarette form, as much as 40 to 50 percent of THC can be lost in sidestream smoke.

There is a significant correlation between the potency of the marijuana smoked and the magnitude of the THC plasma concentrations obtained. However, large interindividual variations in peak THC plasma concentrations occurred across all of the marijuana potencies investigated. These results were obtained in experiments in which the volunteers smoked marijuana cigarettes in their customary fashion (uncontrolled smoking). To reduce this variability, experiments were conducted in which marijuana was smoked in a water pipe. This method of smoking permits control of the amount of marijuana combusted per toke, the number of tokes taken, and the interval between tokes. Despite controlling these important smoking factors, large interindividual variations in the magnitude of the THC plasma concentrations obtained continued to be observed.

During marijuana smoke inhalation, THC is trapped in the mucosa of the upper respiratory tract, thereby reducing the drug's bioavailability. Judging by the amounts of THC measured in saliva or swabbings of the buccal mucosa, however, the total loss of THC by this factor can be considered of minor importance.

In summary, marijuana smoking is a complex process that does not permit controlled dosing. Therefore, for research or therapeutic purposes, it is indispensable to measure the THC plasma concentrations to know how efficiently a given individual smoked.

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Effects of Habitual Use of Marijuana and/or Cocaine on the Lung

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INTRODUCTION

Marijuana remains one of the most widely abused substances in our society (Johnstone et al. 1987). As with tobacco, smoking is the preferred route of administration of marijuana, so the lungs are the principal organ exposed to its combustion products. The latter include a number of known respiratory 'irritants and carcinogens (Hoffman et al. 1975; Novotny et al. 1982), as well as numerous other compounds the pulmonary effects of which are unknown. Experimental animal (Roy et al. 1976; Fleischman et al. 1975; Fleischman et al. 1979) and *in vitro* (Huber et al. 1975; Leuchtenberger and Leuchtenberger 1976) studies have demonstrated that exposure to marijuana smoke produces significant toxic, inflammatory, and even carcinogenic effects on lung tissue and cells. Few studies in man, however, have systematically addressed the long-term effect of real-life habitual marijuana smoking on the lung. Moreover, the few published clinical studies of the pulmonary effects of regular marijuana smoking (Rubin and Comitas 1975; Cruickshank 1976; Coggins et al. 1976; Boulougouris et al. 1976) have yielded conflicting results, possibly because of small sample sixes, failure to control adequately for confounding factors, such as concomitant tobacco smoking, and the cross-sectional nature of these studies, which may not be sensitive to intraindividual changes in respiratory status. In 1983, a longitudinal study was initiated at the University of California, Los Angeles (UCLA) in an attempt to determine (1) whether habitual smoking of marijuana alone and with tobacco leads to pulmonary symptoms, lung dysfunction, or respiratory illness and (2) whether such harmful effects persist or progress with continued (or increased) use and decline with cessation of use. Another objective of this study was to evaluate the effect of regular smoking of marijuana with and without tobacco on tracheobronchial

histopathology, alveolar cell populations, and the structure and function of alveolar macrophages, key cells in the lung's defense against infection and other noxious insults. Results of this continuing study are presented below.

PULMONARY EFFECTS OF HABITUAL MARIJUANA USE: RESPIRATORY SYMPTOMS AND LUNG FUNCTION

Cross-Sectional Findings

In 1983 and 1984 a convenience sample of Caucasian men and women (ages 25 to 49 years) who reported smoking 10 or mote marijuana joints (or joint-equivalents) per week, on the average, over the previous 5 years or more was recruited from the greater Los Angeles area by means of newspaper and radio advertisements. Exclusionary criteria included intravenous drug abuse more than 2 times per lifetime, smoking of illicit substances other than cannabis more than 12 times per lifetime or within the previous 6 months, significant occupational exposure to dusts or fumes potentially hazardous to the lungs, a history of chronic respiratory disease, such as asthma, cystic fibrosis, kyphoscoliosis, or tuberculosis, or previous chest surgery. A comparison sample of 167 nonmarijuana-smoking control subjects of similar age was recruited from the same general population.

All subjects underwent a detailed respiratory and drug use questionnaire, modified from the American Thoracic Society/National Heart, Lung, and Blood Institute questionnaire (Ferris 1978) and the National Institute on Drug Abuse National Survey on Drug Abuse questionnaire (Fishburne et al. 1980), which included questions concerning respiratory symptoms, general health, residence, occupational history, socioeconomic status, and current and past history of use of alcohol, tobacco, marijuana, and other illicit drugs. In addition, an extensive battery of pulmonary function tests was performed, including maximal expiratory flow-volume curves breathing air and 80 percent helium:20 percent oxygen, subdivisions of lung volume, single-breath diffusing capacity for carbon monoxide (D_LCO), single-breath nitrogen washout, plethysmographically determined airway resistance, and specific airway conductance and methacholine inhalation challenge. Peripheral venous blood was obtained for measurement of hemoglobin and carboxyhemoglobin (for correction of D_LC0 measurements) and for determination of serum cotinine and Δ^9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-THC for verification of self-reported daily smoking of tobacco and marijuana.

Characteristics of the study population are shown in table 1. The smokers of marijuana (MS) and the control subjects who did not smoke tobacco (NS) were of similar age, while the tobacco-smoking control subjects (TS) were 3.4 years older than the smokers of both marijuana and tobacco (MTS). The MS smoked only slightly more marijuana than the MTS ($p>.6$), while the TS smoked significantly more tobacco than MTS ($p<.01$).

TABLE 1. *Subject characteristics*

Subjects	N	Mean Age Yrs	Marijuana		Tobacco	
			Joints/Wk	Joint-Yrs’	Cigrrettes/D	Pk-Yrs'
Marijuana Plus Tobacco Smokers (MTS)	135	33.6	25.9	54.0	18.8	16.1
Marijuana- Only Smokers (MS)	144	31.6	28.0	56.7	0.0	2.7
Current Tobacco Smokcrs (TS)	70	37.0	0.0	0.1	28.9	22.0
Nonsmokers (NS)	97	31.0	0.0	0.0	0.0	0.0

[a]Number of joints per day times number of years smoked.
[b]Number of cigarcttes per day times number of years smoked.

Respiratory symptom data and results of lung function obtained in this study population at the time of initial evaluation have been presented previously (Tashkin et al. 1987a). Prevalence of chronic respiratory symptoms by smoking category is shown in table 2. Regular smokers of marijuana alone had significantly more symptoms of chronic bronchitis (cough, sputum, wheeze) and a significantly greater frequency of episodes of acute bronchitis than nonsmoking control subjects. No difference in prevalence of chronic cough, sputum production or wheeze was noted between the marijuana and tobacco smokers, nor were additive effects of combined smoking of tobacco and marijuana on the prevalence of acute or chronic respiratory symptoms apparent.

TABLE *2. Prevalence of chronic respiratory symptoms (percent)*

		Acute			
		Bronchitic			Shortness
Subjects	Cough[a]	Sputum[a]	Episodes[b]	Wheeeze[c]	of Breath
MTS	23.1*	25.6*	14.1*	34.8*	4.4
M S	18.4*	19.7*	13.4*	24.8*	0.7
T S	24.3*	25.7*	10.0*	37.1*	8.6**
N S	1.1	5.6	2.2	7.8	2.2

*$p<.01$ (comparison with NS; x^2).
**$p<.05$ (comparison with MS; x^2).
[a]Present on most days for more than 3 months for 2 or more consecutive years.
[b]More than 1 episode of increased cough and sputum lasting more than 3 weeks within past 3 years.
[c]Present on more than 21 days during past year.

Results of analysis of lung function are presented in tables 3 and 4. Tobacco smokers, irrespective of concomitant marijuana smoking, showed significantly poorer values and/or a significantly greater frequency of abnormality for several tests of lung function, including D_LCO (a measure of alveolar gas transfer, which is deranged in early emphysema) and $Vmax_{50}$, $\Delta Vmax_{50}$, Visoflow, $\Delta N_2/L$, CV, and CC (teats reflecting mainly small airways function), in comparison with nonsmokers or with smokers of marijuana alone. On the other hand, marijuana smokers, regardless of

TABLE 3. *Significantly different between-group comparisons of lung function tests from covariance analysis**

Test	Men (n=259)		Women (n=128)	
VisoV	MTS>NS MTS>MS			
ΔN_2L**	MTS>NS MTS>MS		TS>NS TS>MS	MTS>NS
CV**	TS>MS TS>MTS TS>NS	MTS>MS MTS>NS	TS>NS	
CC**	TS>MS TS>MTS	NS>MS	TS>NS TS>MS TS>MTS	
D_LCO**	MTS<NS MTS<MS	TS<NS	TS<NS TS<MS	MTS<NS
R_{aw}+	MS>NS MS>TS	MTS>NS MTS>TS		
SG_{aw}+	MTS<NS	MS<NS		

*p<.05 (least significant difference testing using adjusted group means from ANCOVA).
**Two-way ANCOVA, with age and/or height as covariates: significant tabacco effect (p<.03); no adverse marijuana effect.
Two-way ANCOVA: significant marijuana effect, males only (p<.03); no tobacco effect.

KEY: VisoV=volume of isoflow; $\Delta N_2/L$=change in nitrogen concentration per liter in Phase III of single-breath nitrogen washout curve; CV=closing volume.; CC=closing capacity; D_LCO=single-breath diffusing capacity of the lung for carbon monoxide; R_{aw}=airway resistance SG_{aw}=specific airway conductance.

SOURCE: Tashkin et al. 1987a.

concomitant tobacco smoking, demonstrated worse values and a greater frequency of abnormality for airway resistance (R_{aw}) and specific airway conductance (SC_{aw}) (tests of mainly large airways function) in comparison with nonsmokers or smokers of tobacco alone. No adverse interactive effects of habitual heavy marijuana use and regular tobacco smoking on lung function could be demonstrated. These findings indicate that habitual smoking of marijuana or tobacco causes functional alterations at different sites in the respiratory tract, with marijuana affecting mainly the large airways and tobacco predominantly the peripheral airways and alveolated regions of the lung.

TABLE 4. *Significantly different between-group comparisons of frequency of abnormality on lung function tests*

Test	Comparison	
$Vmax_{50}$	TS>NS	
Δ' $Vmax_{50}$	TS>NS TS>MS	
VisoV	MTS>NS	
CC	TS>NS TS>MS TM>MTS	
D_LCO	TS>NS TS>MS	MTS>NS MTS>MS
R_{aw}	MS>NS MS>TS	MTS>TS
SG_{aw}	MTS>NS	

p<.05, x^2 or Fisher's exact test (men and women combined).

KEY: $Vmax_{50}$=flow rate at 50 percent of forced vital capacity;Δ'$Vmax_{50}$=change in maximal expiratory flow between curves obtained breathing 80 percent helium:20 percent oxygen and breathing air at 50 percent of the vital capacity.

SOURCE Tashkin et al. 1987a.

Longitudinal Findings

Approximately 50 percent of the cohorts of young habitual smokers of marijuana alone (MS) or with tobacco (MTS) and of control nonsmokers (NS)

and smokers of tobacco alone (TS) who were initially tested in 1983 and 1984 (Time 1) returned for retesting in 1985 and 1986 (Time 2) after a 2 to 3 year interval. On repeat testing, the following proportion of retested subjects remained in the same smoking category at both Time 1 and Time 2: MTS 40 of 54; MS 60 of 71; TS 30 of 32; and NS 56 of 58. Table 5 shows the prevalence, in percent, of abnormal respiratory symptoms at Time 1 and Time 2 in the retested participants, by smoking group, for individuals who remained in the same smoking category. prevalence of cough, sputum, and wheeze was significantly higher in all smoking groups (MS, TS, and MTS) compared to nonsmokers at both Time 1 and Time 2 ($p<.05$, Chi square). Among smokers of tobacco or marijuana alone (TS and MS), the prevalence of all respiratory symptoms tended to decrease in the interval between the two examinations; in contrast, among the dual smokers (MTS), frequency of reported cough, sputum, wheeze and shortness of breath tended to increase. Symptom prevalence was not statistically different, however, between Time 1 and Time 2 among participants in any smoking category who remained in the same category ($p<.05$; McNemar's test of symmetry).

TABLE 5. *Prevalence (percent) of respiratory symptoms at Time 1 (1983 to 1984) and Time 2 (1985 to 1986)*

Smoking Group	Cough		Sputum		Acute Bronchitic Episodes		Wheeze		Shortness of Breath	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
MTS	28*	33*⁺	23*	33*	13*	13*	32*	40*⁺	10	13*
MS	26*	16*	21*	15*	12*	6	28*	19*	4	0
TS	37*	33*	33*	23*	23*	17	37*	20*	17*	10
NS	0	4	4	4	0	0	5	4	2	2

*Significantly greater than values in NS; $p<.05$; x^2 or Fisher's exact test.
⁺Significantly greater than values in MS; $p<.05$; x^2 or Fisher's exact test.

Table 6 shows the prevalence, in percent, of abnormality in several lung function measures in the retested cohort at both Time 1 and Time 2 for subjects in each smoking group who did not change smoking categories. These findings show persistent or increasing prevalence of abnormality in measures mainly of small airways function (e.g., FEF_{25-75}, CC) and in D_LCO (an indicator of early emphysema) among smokers of tobacco, especially those who also smoked marijuana, compared to nonsmokers. Over the same time interval, the higher prevalence of abnormality in measures reflecting large airways function (e.g., Vmax, R_{aw} among marijuana smokers compared to tobacco-only smokers and nonsmokers increased even further.

TABLE 6. *Prevalence (percent) of abnormality in tests of lung function at Time 1 and Time 2*

Group	FEF_{25-75}		$\dot{V}max$		CC		R_{aw}		D_LCO	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
MTS	11	20[a]	8	28[a]	6	31[ac]	18[ab]	22[a]	24[a]	26
MS	12	15	12	14	8	13	19[ab]	25[ab]	7	5
TS	10	14	0	7	43[a]	43[a]	0	7	30[ab]	37[ac]
NS	8	8	2	2	7	7	4	5	8	6

[a]Significantly greater than values in NS; $p<.05$, x^2 or Fisher's exact test.
[b]Significantly greater than values in TS; $p<.05$, x^2 or Fisher's exact test.
[c]Significantly greater than values in MS; $p<.05$, x^2 a Fisher's exact test.

KEY: FEF_{25-75}=forced expiratory flow rate between 25 and 75 percent of the forced vital capacity; Vmax=peak maximal expiratory flow rate; CC=closing capacity; R_{aw}=airway resistance; D_LCO=single-breath diffusing capacity.

The results of this prospective study support previous evidence from cross-sectional studies (Tashkin et al. 1980; Tashkin et al. 1987a) of (1) a significant relationship between habitual smoking of marijuana and/or tobacco and symptoms of chronic bronchitis, (2) a deleterious- effect of tobacco on both small airways and alveolar function, and (3) an adverse effect of marijuana primarily upon the large airways. Continuing tobacco and/or marijuana smoking was associated, in general, with persistent or worse abnormalities in airway and alveolar function.

One hundred and thirty-eight of the study participants returned for a third round of testing in 1986 and 1987 after an interval of 3 to 4 years from the initial examination. Results in this subgroup not only support the above findings but also suggest an additive effect of habitual smoking of marijuana and tobacco on both respiratory symptoms and lung function, in agreement with findings from a recent community-based epidemiological study (Bloom et al. 1987). Thus far, too few subjects discontinued use of marijuana with or without tobacco to permit meaningful analysis of the possible effect of cessation of marijuana use on respiratory status.

EFFECTS OF HABITUAL SMOKING OF MARIJUANA ALONE OR WITH TOBACCO ON NONSPECIFIC AIRWAYS HYPERREACTIVITY

Nonspecific airways hyperreactivity (AHR) has been found to be associated with regular tobacco cigarette smoking and may be a risk factor for the

development of chronic obstructive pulmonary disease in tobacco smokers (Barter and Campbell 1976; Kanner 1984; Vollmer et al. 1985). Bronchodilator drugs, by inhibiting airway reactivity to nonspecific inhaled spasmogens, could provide protection against chronic obstructive pulmonary disease. Marijuana smoke contains many of the Same irritants found in tobacco smoke (Hoffman et al. 1975; Novotny et al. 1982), in addition to THC and other cannabinoids. THC causes dose-dependent bronchodilation (Vachon et al. 1973; Tashkin et al. 1973; Tashkin et al. 1974) and reverses experimentally induced bronchospasm (Tashkin et al. 1975). THC is also a bronchial irritant, however, at least when inhaled as an aerosol (Tashkin et al. 1977). Therefore, because of its bronchodilator or irritant properties, THC could inhibit or augment the tendency of other noxious components in marijuana smoke (or in tobacco smoked in addition to marijuana) to cause AHR. The net influence was therefore examined of heavy, habitual smoking of marijuana, alone or with tobacco, on nonspecific AHR in comparison with nonsmoking or smoking of tobacco alone (Tashkin et al. 1988).

Airway reactivity to methacholine was measured in 281 subjects (67 MTS, 116 MS, 34 'IS, and 64 NS) using a breath-activated dosimeter and a protocol modified from that of Chai et al. (1975). Nonspecific AHR was considered to be present if the forced expired volume in 1 second (FEV_1) declined by 20 percent or more from the postdiluent control value after inhalation of five deep breaths of methacholine in a concentration equal to or less than 25 mg/ml. The following proportion of subjects in each smoking category exhibited AHR: MTS 37.3 percent; MS 23.3 percent; 'IS 29.4 percent; and NS 17.2 percent. Although a greater proportion of smokers of marijuana or tobacco only exhibited AHR compared to nonsmokers, these differences were not statistically significant. On the other hand, the proportion of dual smokers of both marijuana and tobacco who had AHR was more than twice that of nonsmokers, and this difference was significant ($p<.01$, x^2). While the present small sample of tobacco-only smokers did not have a significantly higher prevalence of AHR compared to the nonsmoking control subjects, two-way ANOVA did show a statistically significant tobacco effect on the degree of airways activity, most pronounced in smokers of both marijuana and tobacco ($F=10.14$; $p=.002$). Therefore synergism between the effects of tobacco and marijuana was suggested, but the interaction did not achieve statistical significance.

It was concluded that the bronchodilator properties of THC do not protect against the development of AHR in smokers of both tobacco and marijuana. In contrast, these data suggest that respiratory irritants within marijuana smoke (including THC and possibly other cannabinoids) augment the effect of tobacco in increasing airways reactivity. These findings are consistent with histopathologic evidence of an additive effect of marijuana plus tobacco in causing tracheobronchial epithelial injury.

PULMONARY EFFECTS OF HABITUAL MARIJUANA USE: TRACHEOBRONCHIAL HISTOPATHOLOGY AND ALVEOLAR MACROPHAGE ULTRASTRUCTURE

Bronchial mucosal biopsies and alveolar macrophages were obtained by fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) from young, heavy habitual smokers of marijuana alone (MS, n=30), marijuana plus tobacco (MTS, n=17), tobacco alone (TS, n=15), and control nonsmokers (NS, n=11) selected at random from participants in the above study of the pulmonary effects of habitual marijuana use (Fligiel et al. 1988). For subjects in each smoking category undergoing bronchoscopy, smoking histories and prevalence of respiratory symptoms and lung function abnormality were comparable to those for all participants in the main study who belonged to the same smoking group. During bronchoscopy, mucosa from the primary carina and randomly selected secondary or tertiary carinae of the right middle or lower lobe was biopsied (Gong et al. 1987). Biopsy specimens from each subject were processed for light microscopy and examined by a single pathologist unaware of the subject's smoking category. Histopathologic features were assessed according to the criteria suggested by Auerbach and colleagues (1957; 1961), as indicated in table 7. Results are shown in table 8. Histopathological alterations in the bronchial mucosa were significantly more common and severe in all groups of smokers than in nonsmokers. Furthermore, most of the mucosal abnormalities (with the exception of squamous metaplasia and the presence of mitotic figures) were more frequently observed in marijuana-only than in tobacco-only smokers. Most notable, however, was the finding that virtually all of the epithelial and basement membrane alterations were most prevalent in subjects who habitually smoked both marijuana and tobacco.

Bronchial biopsies were also examined using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) for further, indepth characterization of mucosal morphology. TEM also permitted a thorough examination of the basement membrane region. The light microscopic findings were confirmed and expanded by both SEM and TEM. TEM revealed frequent and early squamous metaplastic changes in all types of smokers as compared to nonsmokers. Moreover, approximately one-third of all the marijuana smokers (MS and MTS) showed disruption of basement membrane continuity, which most likely occurred as a result of basement membrane permeation by inflammatory cells from submucosal blood vessels, a finding not observed in tobacco-only smokers.

The findings are important for several reasons. First, all subjects studied were young adults, most of whom were asymptomatic with no or minimal lung function abnormality, indicating that habitual marijuana smoking frequently causes significant, albeit clinically silent, airway injury manifested by extensive morphologic changes at a relatively early age when symptoms

TABLE 7. *Criteria for light microscopic evaluation of bronchial biopsies*

Criterion	Definition
Epithelial Changes	
Basal (reserve) cell hyperplasia	≥ 3 rows of basal cells
Stratification	flattening of surface epithelium and/or absence of cilia
Squamous metaplasia	stratification involving any portion or all of the mucosa as well as the total loss of cilia
Goblet cell hyperplasia	goblet cells constituting >25% of all epithelial cells
Variation of nuclear size, shape, and chromatin content	
Presence of mitotic figures	
Increased nuclear-to-cytoplasmic ratio (>1:4)	
Intraepithelial inflammation	4 or more inflammatory cells per high power field
Basement Membrane Thickening	>7 μm
Submucosal Inflammation	≥ 8 inflammatory cells per high power field

and pulmonary dysfunction are absent or uncommon. Furthermore, the marijuana smokers we studied (MS and MTS) were several years younger than the TS, and the MTS smoked nearly half as much tobacco as did the TS subjects, suggesting that habitual marijuana smoking may be at least as damaging, if not more damaging, to the epithelium of the central airways than the smoking of tobacco. The latter possibility is further suggested by the similar, if not greater, frequency of histopathologic abnormalities among MS compared to TS despite a far smaller daily number of marijuana joints smoked by the MS (3 to 4 per day) than the daily number of tobacco cigarettes smoked by the TS (more than 20 per day). Finally, the observation that bronchial mucosal changes were most prevalent in MTS suggests an additive effect of marijuana and tobacco smoking on airway injury, consistent with findings from the only other study of histopathologic abnormalities in the airways of chronic cannabis smokers (Tennant 1980). It is of interest, however, that while Tennant (1980) found extensive histopathologic changes in airways of heavy hashish smokers, all of his subjects had respiratory symptoms and many had abnormal lung function, in contrast to our subjects, most of whom were asymptomatic with normal lung function.

TABLE 8. *Histopathologic changes in bronchial biopsies examined by light microscopy*

	MTS (n=17)		MS (n=30)		TS (n=15)		NS (n=11)		Significant Differences*	
	n	%	n	%	n	%	n	%	Groups	p Value**
Epithelial Changes										
Basal Cell Hyperplasia	15	88	22	73	6	40	0	0	MS>NS MTS>NS MTS>TS	.00002 .000003 .005
Stratification	14	82	16	53	6	40	0	0	MS>NS MTS>TS	.0014 .00002
Squamous Metaplasia	11	65	8	27	6	40	0	0	MTS>NS MTS>MS	.0006 .010
Goblet Cell Hyperplasia	12	71	25	83	8	53	3	27	MS>NS	.001
Cellular Disorganization	13	76	14	47	4	27	1	9	MTS>NS MTS>TS	.0007 .006
Nuclear Variation	14	82	12	40	7	47	0	0	TS>NS MTS>NS MTS>MS	.010 .00002 .005
Mitotic Figures	4	24	4	13	4	27	0	0		
Increased Nuclear-to-Cytoplasmic Ratio	8	47	13	43	6	40	0	0	MS>NS MTS>NS	.007 .008
Inflammation	8	47	10	33	6	40	0	0	MTS>NS	.008
Basement Membrane Thickening	14	82	22	73	5	33	0	0	MS>NS MTS>NS MS>TS MTS>TS	.00002 .00002 .010 .006
Submucosal Inflammation	3	18	7	23	4	27	4	36		

*By Fisher's exact test (one-tailed).
**Only values within the 99% confidence levels are reported.

SOURCE :Fligiel et al. 1988, copyright 1988, Australian Government Press.

The true potential for the type and extent of the bronchial epithelial histopathology noted in the marijuana smokers examined to produce clinically significant chronic airway disease or to progress to malignant change is unknown, but certainly possible (Auerbach 1957; Knudtson 1960). Prolonged followup of these subjects may help answer this question.

Alveolar macrophages obtained by BAL from the same three groups of smokers (MS, n=15; MTS, n=8; TS, n=7) and nonsmokers (NS, n=7) were compared with respect to morphologic and morphometric ultrastructural cellular features (including an evaluation of cell membrane, nucleus, and cell plasma contents, especially cytoplasmic inclusions). Compared to NS, both TS and MS had frequently larger cytoplasmic inclusions, most of which contained needlelike structures. While TS showed an increased nuclear contour index and decreased cell membrane ruffling, MS demonstrated increased blebbing and an increased ruffling index. Macrophages of NS showed the greatest ruffling index and frequently contained surfactant material. These findings indicate definite ultrastructural differences between alveolar macrophages of smokers in general and nonsmokers, as well as be tween the macrophages of MS and TS. These morphological differences may indicate differences in functional activity of these important cells.

The numbers of alveolar macrophages and other inflammatory cells (lymphocytes, neutrophils, and eosinophils) recovered in BAL fluid from 16 MTS, 14 MS, 13 TS, and 19 NS were determined from Giemsa-stained cytopreps (Barbers et al. 1987). Results are indicated in table 9. These findings indicate that smoking of marijuana or tobacco alone induces an inflammatory cellular response in the alveoli consisting mainly of increased numbers of alveolar macrophages and that habitual smoking of both marijuana and tobacco causes a much greater inflammatory response, implying an adverse effect of marijuana smoking on the lung that is independent of and additive to that of tobacco. 'The mechanism of the inflammatory response in the lungs of marijuana and/or tobacco smokers is not known but may include cellular stimulation by physicochemical irritants or antigenic substances within the smoke leading to release of mediators with chemoattractant properties, release of enzymes, or generation of toxic oxygen radicals.

TABLE 9. *Mean number ± SEM of each type of white blood cell ($WBC/ml x 10^{4}$) recovered in bronchoalveolar lavage from smokers of marijuana and/or tobacco and from nonsmokers*

Subject Group	Alveolar Macrophages	Lymphocytes	Neutrophils	Eosinophils
MTS	57.9±6.4[abc]	3.0±0.7[a]	1.16±0.42[a]	0.11±0.11
MS	27.1±5.1[a]	1.9±0.7	0.50±0.18	0.04±0.03
TS	33.3±4.2[a]	2.0±0.5	0.28±0.05	0.02±0.02
NS	10.6±1.5	0.9±0.1	0.07±0.03	0.04±0.02

[a]Significantly different from NS; p<.01, unpaired Student's t test.
[b]Significantly different from MS; p<.01, unpaired Student's t test
[c]Significantly different from TS; p<.01, unpaired Student's t teat.

RELATIONSHIP BETWEEN SMOKING DYNAMICS AND RESPIRATORY DELIVERY OF INSOLUBLE PARTICULATES (TAR) DURING THE SMOKING OF MARIJUANA VS. TOBACCO

As already noted, the frequency of chronic respiratory symptoms and tracheobronchial epithelial histopathology observed in young smokers of only 3 to 4 marijuana joints a day was similar to that of smokers of more than 20 tobacco cigarettes a day (Tashkin et al. 1987a). One possible explanation for these findings is that more particulates and gaseous irritants may be delivered to the respiratory tract in the smoke from marijuana than in the smoke from a comparable amount of tobacco, perhaps due, in part, to differences in the dynamics of smoking these two substances. To evaluate this possibility, the dynamics of smoking a single tobacco cigarette were compared in 11 habitual smokers of both marijuana and tobacco, 9 habitual smokers of marijuana only, and 10 regular smokers of tobacco only. At the same time, the amount of particulates delivered to and retained by the smoker following the smoking of each type of cigarette was quantified. Wu and colleagues previously reported results obtained in dual smokers of *both* marijuana and tobacco in relation to smoking marijuana of low potency (0.004 and 1.24 percent THC) (Wu et al. 1988). Since "street" marijuana is currently estimated to be of higher potency, further experiments were performed involving the smoking of marijuana containing 2.74 percent THC. In addition, these studies were extended to habitual smokers of marijuana only or tobacco only to ascertain whether or not concomitant tobacco or marijuana smoking affects the smoking dynamics and delivery of smoke particulates for each of the other types of smoked substance.

Subjects were studied on one or two separate days at the same time of day. They were asked to refrain from smoking tobacco for at least 2 hours and cannabis for at least 6 hours before visiting the laboratory. During the first visit, the dual smokers of marijuana and tobacco initially smoked their own brand of tobacco, followed, in a single-blind fashion, by a placebo marijuana joint (0.004 percent THC) and then by a marijuana cigarette containing 1.24 percent THC. On the second visit., subjects smoked placebo marijuana followed by a joint containing 2.74 percent THC. An interval of about 30 minutes separated the smoking of each cigarette. The habitual smokers of marijuana only followed the same protocol except that they did not smoke tobacco. The regular smokers of tobacco only were studied in a single session during which they smoked a tobacco cigarette of their own brand. All subjects were asked to smoke each cigarette in a manner as similar as possible to their customary method of smoking each type of substance.

Characteristics of each type of cigarette smoked during these sessions are shown in table 10. Tobacco cigarettes were invariably filter tipped and consisted of different brands with varying FTC-estimated yields ranging from low to high nicotine and tar contents. The tobacco cigarettes on the

average were slightly longer and weighed slightly more than the marijuana joints. The tar yields, measured in mainstream smoke generated by a syringe with a 50-ml puff volume, 2-second puff duration and 30-second inter-puff interval to butt lengths of 35 mm for tobacco and 25 mm for marijuana, indicated an average 2.2-fold greater tar delivery from marijuana than from tobacco. The draw resistance for marijuana was about one-third that for tobacco because of a much looser packing of the marijuana cigarette.

TABLE 10. *Cigarette characteristics*

	Length (mm)	Weight (43)	FTC Yield Nicotine (mg)	FTC Yield Tar (mg)	Tar[a] (mg)	Draw[b] Resistance (cm H_2O/ml/sec)
Tobacco (various brands, filter tipped)	80-120 (89.6±10.0)	878-1,126 (986±81)	0.4-1.4 (0.84±0.32)	4.6-23 (12±5.7)	14.1-20.9 (17.8±2.9)	0.51
Marijuana						
0% THC	85	741-940	-	-	38.5±0.8	
1.24% THC	85	849-985	-	-	46.3±2.2	0.17
2.74% THC	85	710-795	-	-	33.6±3.4	

Measured in mainstream smoke generated by syringe with a 50-ml puff-volume, 2-second puff duration, and 30-second inter-puff interval to butt lengths of 33 mm for tobacco and 25 mm for marijuana.
The pressure difference between two ends of the cigarette (between mouth and the distal end).

Puff volume, duration, number, and interval were measured using a sensitive Fleisch pneumotachograph (linear between 5 and 100 ml per second) connected through Tygon tubing to the distal end of a glass cylinder that contained two ventilation ports and was sealed at its proximal end by a rubber stopper (figure 1). A tobacco or marijuana cigarette was held in a small plastic holder inserted through the rubber stopper. Between puffs, the ventilation ports prevented excessive accumulation of carbon monoxide and extinction of the lighted cigarette. During a puff, the ventilation holes were occluded to allow the air drawn through the cigarette to be measured by the pneumotachograph. Inhaled volume and smoke retention time were measured using inductive plethysmography. Volume changes were calculated from the weighted sums of the chest wall and abdominal signals by the "least-squares" calibration method and showed agreement with spirometric measurements to within plus or minus 10 percent.

A proportional smoke-trapping device interfaced to the puff-volume measuring apparatus measured the amount of smoke particulates delivered to the smoker's mouth (figure 1). In this device, the mainstream smoke split into two parallel pathways, a low resistance pathway containing several parallel capillary tubes (pathway B) and a high resistance pathway with only one

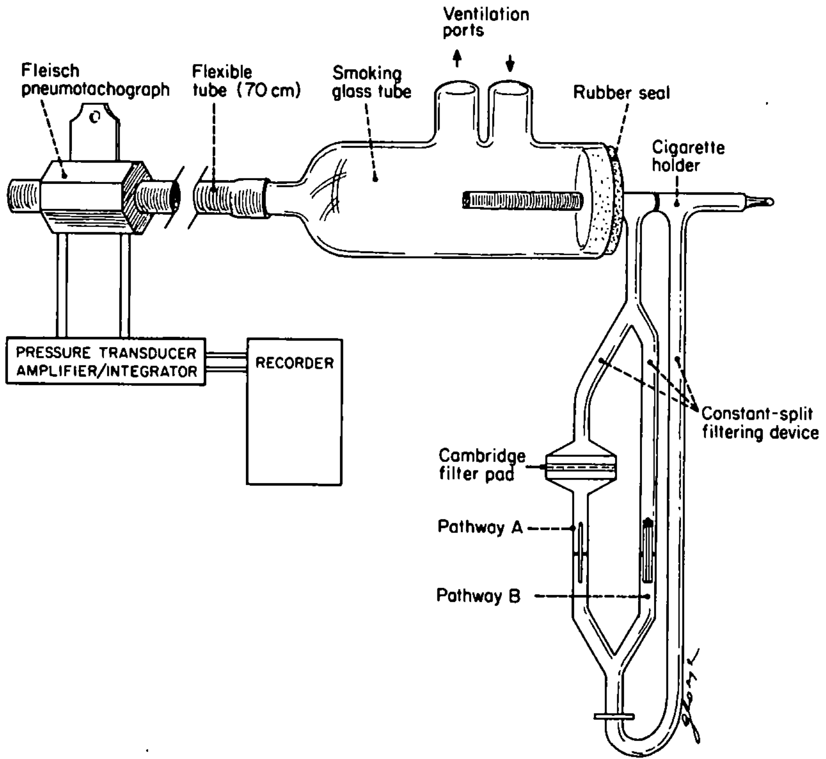

FIGURE 1. *Puff volume measuring apparatus interfaced to proportional smoke trapping device for measuring delivery of smoke particulates*

capillary tube (pathway A). A Cambridge filter pad trapped the smoke passing through pathway A. The amount of tar trapped by the filter was extracted with methanol and analyzed spectrophotometrically at a wave length of 400 nm. Gravimetric and spectrophotometric studies have shown that a fairly constant fraction of the tar (approximately 12.5 percent or one-eighth) is retained in the Cambridge filter over a range of puff volumes, durations, and flow rates (Rose et al. 1987). Therefore, the actual quantity of smoke particulates delivered to the mouth can be calculated simply by multiplying the amount of particulates trapped in the filter pad in pathway A by 7. Smoke exhaled after each puff was collected using another filter attached to the distal end of a megaphone device connected to a vacuum system, as described by Hinds et al. (1983). The amount of smoke deposited in the respiratory tract can then be calculated by simple subtraction and expressed as a percentage of the amount inhaled.

Mean data for smoking dynamics are shown in table 11 for MIS. Compared to tobacco, marijuana smoking was associated with a 30 to 40 percent smaller number of puffs, a 30 to 78 percent greater puff volume, a 33 to 50 percent longer puff duration, a 40 to 54 percent greater inhaled volume and a 3.9- to 5.2-fold longer retention time. Similar differences were noted comparing MS with TS, suggesting that among habitual MTS, the dynamics of smoking one type of substance does not influence smoking topography for the other substance. It is also noteworthy that, despite marked differences in the degree of intoxication, the different strengths of marijuana were associated with a similar smoking profile, except for puff volume, which was smaller during the smoking of 2.74 percent marijuana compared to the lower strengths of marijuana, but still larger than the puff volume associated with tobacco smoking.

TABLE 11. *Mean smoking topographic data for smokers of marijuana plus tobacco (n=11)*

Parameter	Tobacco	Marijuana 0% THC	1.24% THC	274% THC
Puff No.	12.6±4.7	7.4±2.2[b]	8.3±3.1[b]	8.4±3.2[b]
Puff Volume, ml	47.8±14.4	84.9±34.0[b,c]	82.6±20.4[b,c]	62.4±23.1
Puff Duration, sec	2.4±1.1	3.6±2.1[b]	4.4±2.2[b,c]	3.1±2.1
Puff Interval, sec	32.1±16.9	38.8±14.2	43.1±18.4	36.6±12.6
Inhaled Volume, 1	1.24±0.31	1.91±0.74[b]	1.74±0.60[b]	1.73±0.72[b]
Retention lime, sec	3.0±0.8	14.0±11.1[b]	15.7±11.6[b]	11.6±8.0[b]
No Smoking Interval, sec	28.9±17.4	22.2±9.5	24512.3	22.7±5.7
Butt Length, mm	38±14	28±17	29±17	27±10
"High," % (maximum 100%)	-	17±14	69±17[d]	74±18[d]

[a]Significant differences across tobacco and marijuana cigarettes (0-2.74 percent THC); p<.006, two-way ANOVA.
[b]Significantly different from tobacco; p<.03.
[c]Significantly different from 2.74 percent THC; p<.02.
[d]Significantly different from 0 percent THC; p<.001.

For the dual smokers, the mean quantity of inhaled particulates measured in optical density (OD) units and the percentage of inhaled particulates deposited in the respiratory tract for each type of cigarette are shown in table 12. Compared to tobacco, marijuana was associated with the delivery of an average of 2.9 times more particulates and a 40 percent greater retention of the inhaled particulates in the respiratory tract. Similar differences were noted comparing MS with TS.

In summary, in smokers of both marijuana and tobacco, a nearly threefold greater delivery of particulate matter to the smoker's mouth was found

during the smoking of a single marijuana joint of varying potency up to 27.74 percent THC compared to a single tobacco cigarette of the smoker's own brand. We also found the respiratory retention of particulates inhaled from marijuana smoke to be 40 percent greater than that of particulates inhaled from the smoke of tobacco. Therefore, the net respiratory burden of particulates was approximately fourfold greater during marijuana than tobacco smoking. Similar differences were noted comparing smokers of marijuana alone with smokers of tobacco alone.

TABLE 12. *Mean quantity of inhaled particulates and percent of inhaled particulates retained in the respiratory tract &ring the smoking* of marijuana *or tobacco by habitual smokers of both substances (n=11)*

Parameter	Tobacco	Marijuana		
		O%THC	1.24% THC	274% THC
Inhaled Particulates, Optical Density	4.8±2.1	12.9±5.8*	14.9±5.8*	13.7±4.6*
Particulate Deposition	59.3±19.6	84.3±15.6+	84.4±17.4+	86.7±13.2+

* Significantly different from tobacco, p<.001, ANOVA multiple comparison test.

Factors that might account for the greater particulate burden to the lungs from smoked marijuana than from a comparable quantity of smoked tobacco include differences in the combustion characteristics of the two plant substances and a greater filtration of the tobacco cigarettes, which were filter tipped and more densely packed than the marijuana joints. These differences could largely account for the more than twofold greater tar yield from marijuana than tobacco that was measured using syringe-simulated puffs of similar volume and duration (table 10). Another possible factor involves consumption of a larger proportion of the marijuana than tobacco cigarettes, leaving a smaller butt length. Nonetheless, although marijuana was smoked to a smaller average butt length (28 mm) than tobacco (38 mm), the tobacco cigarettes were longer and heavier to begin with, so that a similar quantity of the two types of cigarette was consumed. A fourth factor, differences in smoking dynamics, probably contributed significantly to the observed differences in tar delivery to and retention by the smoker. Differences in smoking dynamics included a 30 to 78 percent larger puff volume, a 40 to 54 percent greater inhaled volume, and a 3.9 to 5.2 times longer retention time for marijuana than for tobacco, which appeared to more than offset any effect of the 30 to 40 percent smaller number of puffs of marijuana than of tobacco. The deeper inhaled volumes and, in particular, the much longer breathholding times during marijuana vs. tobacco smoking could have accounted for the greater fractional deposition

of inhaled marijuana smoke particulates in the respiratory tract due to gravitational sedimentation and interception of surfaces in the lower airways and air spaces during molecular diffusion.

In conclusion, the fourfold greater respiratory burden of particulates observed during the smoking of a single marijuana cigarette compared to a single tobacco cigarette may partly explain earlier findings that daily smoking of only a few joints of marijuana without tobacco was associated with chronic respiratory symptoms and tracheobronchial epithelial histopathology of frequency and extent similar to that noted in daily smokers of more than 20 cigarettes of tobacco without marijuana. These observations provide further support for the concept that the habitual smoking of only a few marijuana cigarettes a day can have adverse long-term effects on the lung.

On the other hand, the mechanism of the relatively greater impact of marijuana than tobacco smoking on *central* airway function and the apparently greater frequency of some histopathologic changes in the epithelium of the central airways among marijuana-only than tobacco-only smokers, in contrast to the predominant effect of tobacco smoking on the *peripheral* airways, remains unclear. Possible explanations for these differences could be a relatively greater fractional deposition of marijuana smoke particulates in the central airways due to inertial impaction, greater absorption of irritating gases within marijuana smoke by the mucosa of the central airways leading to proportionally less particulate deposition or gaseous absorption more peripherally in the respiratory tract, or a combination of these factors.
Since particle size is an important determinant of the mechanism and rate of deposition of particulates in the respiratory tract (Brain and Valberg 1974), the aerodynamic size of particulates was measured in the smoke of filtered and unfiltered tobacco cigarettes (Marlboro) and marijuana joints containing 0.004, 1.24, and 2.75 percent A9-THC using a multistage cascade impactor (Andersen, Series 290). Puff volumes and durations of air similar to those observed in smokers of marijuana and tobacco (100 ml, 4 sec and 50 ml, 2 sec, respectively, at a flow rate of 25 ml/sec) were delivered through the marijuana or tobacco cigarette in a combustion chamber. The cigarette smoke was immediately diluted 10 to 30 times by a high volume of ambient air at 21 °C and 15 percent relative humidity in a mixing chamber to prevent agglomeration of smoke particulates. The smoke was drawn into the cascade impactor at a flow rate of 4.0 liters per minute. The excess smoke mixed with air was exhausted to the atmosphere through a vent. Methanol extracts of the impaction plates were assayed spectrophotometrically at 400 nm. Measurements were performed in quintuplicate for each type of cigarette. Mass mean aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were calculated based on the assumption of a log normal distribution of particle sixes by the least squares method. Results are shown in table 13.

#TABLE 13. *Mean mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of smoke particulates from tobacco and marijuana cigarettes*

Type of Cigarettes	Number of Measurements	MMAD Mean	GSD	Standard Deviation
Tobacco, Filtered	5	0.490	1.71	0.039
Tobacco, Nonfiltered	5	0.539	1.79	0.061
Marijuana, 0% THC*	5	0.579	1.85	0.032
Marijuana, 1.24% THC**	5	0.927	1.71	0.039
Marijuana, 2.74% THC**	5	0.957	1.66	0.072

* Significantly larger than filtered tobacco (p<.05).
**Significantly larger than filtered or unfiltered tobacco and 0 percent ‘MC marijuana (p<.001).

The findings indicated that marijuana (1.24 and 2.74 percent THC) smoke particulates, although approximately twice as large as particles in tobacco smoke, are still in the submicronic range that favors peripheral deposition in the respiratory tract by gravitational sedimentation and molecular diffusion (Brain and Valberg 1974). Another factor that could account for excessive central deposition of smoke particulates due to inertial impaction is a rapid rate of inhalation of the smoke mixed with air (Valberg et al. 1978). However, although the smoking topography studies did not permit precise measurements of inhaled flow rates, estimates of the latter from inductive plethysmographic tracings did not suggest a substantially greater inhaled flow rate during marijuana compared to tobacco smoking. Thus it appears unlikely that excessive central airway deposition of marijuana smoke particulates occurs during marijuana smoking (compared to tobacco smoking) as a result of either large particulate sixes or unusually rapid rates of inhalation of the smoke mixed with air. Further studies involving lung imaging of the respiratory distribution of deposited smoke particulates labeled with a suitable gamma-emitting radionuclide are required to further evaluate this question.

PULMONARY EFFECTS OF FREEBASE COCAINE

Because of the increasing prevalence of cocaine smoking (freebasing) and reports in small numbers of cocaine freebase users of respiratory symptoms and abnormalities in diffusing capacity of the lung (Weiss et al. 1981; Itkonen et al. 1984), the relationship between self-reported cocaine use by freebasing and respiratory symptoms and lung dysfunction was analyzed. Subjects included habitual smokers of marijuana with and without tobacco who denied intravenous drug abuse and were participants in a study of the chronic pulmonary effects of habitual marijuana use (Tashkin et al. 1987a).

Of 356 marijuana smokers in the study sample, 84 (67 males, 17 females, mean age 30 years) reported freebasing cocaine from 1 to 992 times in their lifetimes. Comparisons were made between tobacco-smoking cocaine freebase users (CTS, n=36) and tobacco-smoking, cocaine nonusers (TS, n=136) and between tobacco-nonsmoking, cocaine-freebase users (CS, n=48) and total nonusers (NS, n=36). "Moderate" (more than 10 times, mean 131 times) and "mild" (fewer than 10 times, mean approximately 3 times) cocaine users were also analyzed separately. Marijuana use was similar (52 to 53 joint-years) but tobacco use less (12 vs. 17 pack-years) in CTS than T'S, marijuana use was less in CS than NS (43 vs. 62 joint-years), and cocaine smoking was comparable in CTS and CS (lifetime use frequency 56 vs. 57 times). No significant differences in frequency of chronic cough, sputum, wheeze, or shortness of breath were noted across any of the above groups. However, moderate freebase cocaine users reported hemoptysis (14 percent), black sputum (43 percent) and chest pain (78 percent) after smoking cocaine. Also, moderate freebase cocaine use had an adverse effect on several tests of airway function (FEV_1, FEV_1/FVC, Vmax, Viso-flow, ΔN2/1, closing volume, and RV), which was significantly greater than that which could be attributed to tobacco ($p<.05$). On the other hand, moderate freebase cocaine use, unlike tobacco, had no demonstrable effect on D_LCO. These findings suggest that, among habitual marijuana smokers, moderate cocaine smoking (1) does not appear to have an effect *on chronic* respiratory symptoms that is additive to that of marijuana alone; (2) not uncommonly causes *acute* respiratory symptoms shortly after smoking cocaine; (3) irritates large and small airways, as reflected by functional changes that are not obscured by concomitant marijuana use and appear to he at least additive to those produced by tobacco; and (4) has no demonstrable effect on the pulmonary microcirculation.

Preliminary data in 39 additional cocaine smokers who reported heavy use of cocaine (mean of 11.7 grams per week) with, a history of regular freebase smoking for an average of 34 months reveal a high proportion of subjects with acute cardiopulmonary symptoms temporally related to cocaine smoking. Of this group of heavy cocaine smokers, 44 percent reported coughing up black sputum from their chests and 48 percent noted hemoptysis within 12 hours after smoking cocaine, while 60 percent reported chest pain (mostly pleuritic in quality) and 40 percent experienced palpitations within 1 hour after freebasing.

Of the 272 non-freebase users in the sample of heavy, habitual marijuana smokers, 242 admitted to "snorting" cocaine. However, analysis of respiratory symptoms and lung function in nonsmokers of cocaine classified into groups of "snorters" and "nonsnorters" of cocaine who did and did not smoke tobacco failed to show any significant relationship between snorting cocaine and either respiratory symptoms or lung dysfunction among either smokers or nonsmokers of tobacco. More detailed studies are needed to examine the impact of frequent cocaine smoking on lung structure and

function and the relationship between frequency, intensity, recency, and method of cocaine freebase use on respiratory alterations, adjusting for effects of concomitant use of other drugs that can have a damaging effect on the lungs.

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Marijuana Effects and Urinalysis After Passive Inhalation and Oral Ingestion

Edward J. Cone

INTRODUCTION

The psychoactive properties of marijuana in humans have been the basis of considerable scientific scrutiny. Over the last two decades, numerous studies have been performed on the pharmacokinetic and pharmacodynamic properties of marijuana and tetrahydrocannabinol (THC), the major active constituent of marijuana. The majority of these studies involve drug administration by subjects smoking marijuana plant material or orally ingesting pure THC. Recently, the effects of passive exposure to marijuana smoke and the oral ingestion of marijuana plant material have been the subject of considerable speculation as a result of extensive urine testing of individuals who claim exposure by these alternate routes of administration.

Absorption of sufficient THC by oral ingestion of marijuana plant material to produce behavioral effects and detectable concentrations of urine metabolites was considered likely if free THC was present or conversion of THC precursor acids in the plant material to THC occurred. Wall and colleagues (1983) found that the bioavailability of orally administered THC was 10 to 20 percent for male and female college students. Also, Law and associates (1984) reported detection of cannabinoid metabolites in blood and urine after administration of cannabis resin to human subjects in a meat sandwich. Studies of subjects who were passively exposed to marijuana smoke also indicated the possibility for absorption of sufficient THC to test positive by urinalysis for cannabinoid metabolites (Perez-Reyes et al. 1983; Mason et al. 1983; Morland et al. 1935; Law et al. 1984; Ferslew et at 1983).

However, two basic pharmacologic questions remained essentially unanswered regarding the effects of marijuana administered by these two routes of administration. These questions were:

1. Ate behavioral effects produced and are they dose related?

2. What is the urine excretion profile of cannabinoid metabolites?

Clinical studies at the Addiction Research Center, in collaboration with the U.S. Navy, were designed to answer these and related pharmacologic and toxicologic questions. In separate studies, male subjects either were passively exposed to marijuana smoke under highly controlled conditions or ingested marijuana-laced brownies. Behavioral measures and all urines were collected and analyzed. This chapter summarizes the results from these studies (Cone and Johnson 1986; Cone et al. 1987; Cone et al. 1988).

METHODS

Subjects

Subjects were healthy, drug-free males with a history of marijuana use. Criteria to enter the study included a documented period of cannabinoid-negative urines (EMIT dau, 20 ng/ml cutoff). Four negative days were required for the brownie study and 14 days for the passive-inhalation study. During the study, subjects were housed on a closed research ward under close surveillance. Two additional male subjects, from the staff, with no history of marijuana use participated in one phase of the passive-exposure study. The study was conducted under the guidelines for the protection of human subjects (45CRF46), and each subject gave informed consent.

Passive Marijuana-Exposure Conditions

Five subjects were passively exposed under double-blind conditions to side stream smoke from 16 marijuana cigarettes (2.8 percent THC) for 1 hour each day (0830 to 0930) for 6 consecutive days. Preceding and following the days of marijuana smoke exposure, the subjects were exposed for 2 days to the smoke of 16 placebo cigarettes (0 percent THC). During exposure, the subjects wore goggles and sat quietly in a small, unventilated room (2.1 m x 2.5 m x 2.4 m). The approximate room volume after adjustment for contents was 12,225 L. Right of the cigarettes were burned during the first 12 minutes, and the remaining eight were burned 30 to 42 minutes into the exposure session. The cigarettes were burned in a constant-draft smoking apparatus designed to release only sidestream smoke. Flow rates for the smoking apparatus were adjusted for an average cigarette bum time of 12 minutes. Room air samples were withdrawn at timed intervals and analyzed for THC content. A second exposure study was performed with the subjects for 6 consecutive days under similar conditions but with exposure to the smoke of four marijuana cigarettes (2.8 percent THC). This was preceded and followed by only 1 day of placebo smoke exposure. A third study was performed with two marijuana-naive subjects who were exposed for 6 consecutive days to the smoke of 16 marijuana cigarettes

(2.8 percent THC) without blind conditions to placebo smoke. Individual urine specimens were collected from all subjects throughout the entire study and were frozen until analyzed.

Marijuana-Laced Brownie Study

Each of the five subjects who participated in the study received single treatments of the following three doses of marijuana brownies, assigned in random order under double-blind conditions: two brownies containing an amount of cannabis plant material equivalent to two marijuana cigarettes (2.8 percent THC); two brownies containing the equivalent of one marijuana cigarette (2.8 percent THC) and one placebo marijuana cigarette (0 percent THC); and two brownies containing the equivalent of two placebo marijuana cigarettes (0 percent THC). The brownies were consumed within 5 minutes starting at 0830. All individual urine specimens were collected and frozen until analyzed.

Behavioral, Physiological, and Chemical Measures

Subjects completed subscales of the Addiction Research Center Inventory (Haertzen 1974) as follows: marijuana scale (Mar 15); morphine-benzedrine group (MBG) scale, which reflects feelings of euphoria and well-being; pentobarbital-chlorpromazine-alcohol group (PCAG) scale, which reflects sedation and intoxication; and the lysergic acid diethylamide (LSD)-specific scale. Additional measures collected included scales from the Single-Dose Questionnaire (Feel Drug and Liking) and a visual analog scale (VAS) for measurement of good feelings and physiological effects (pulse, blood pressure). All measures were taken at 0730 and 0800 before each test session (0830 to 0930) and thereafter at 0930, 1030, 1130, and 1230. For the marijuana brownie study, additional measures also were collected at 1430,1630, and 2030.

Urinalysis

All urines were analyzed for cannabinoids by EMIT dau with a 20 ng/mL cutoff and by Abuscreen radioimmunoassay (RIA) with a 10 ng/mL level of quantitation. Urines also were analyzed quantitatively for 11-nor-Δ^9-THC-9-carboxylic acid (9-carboxy-THC) by gas chromatography/mass spectrometry (GC/MS) with a 5 ng/mL level of quantitation.

RESULTS

Behavioral Effects and Urinalysis After Passive Inhalation of Marijuana Smoke

Mean responses after passive exposure to the smoke of 16 marijuana cigarettes were significantly ($p<.05$) elevated when compared to placebo smoke exposure (day 2) on the MAR 15, LSD, VAS, Feel Drug and Liking scales

(figure 1). Responses appeared to be elevated on the PCAG scale, but were not significantly different from placebo and were unchanged on the MBG scale (not shown). Modest elevations also were obtained on these scales with the same subjects after exposure to the smoke of four marijuana cigarettes. The mean response on these scales by the same subjects after actively smoking one marijuana cigarette (2.8 percent THC) is included in figure 1 for comparison.

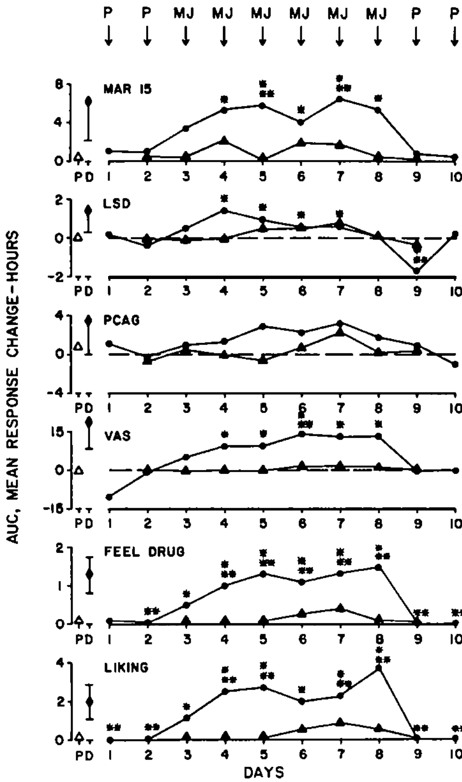

FIGURE 1. Subjective effects induced by passive inhalation of *marijuana smoke*

***Significant difference (p<.05) compared with day 2 response.**
****Significant difference (p<.05) compared with day 3 response.**

NOTE: Mean AUC data (n=5) for the response (minus control) over time are presented after exposure to the smoke of **16 (●) and 4 (▲)** marijuana (MJ) or placebo (P). Mean (±SE) drug (D) AUC data in the same subjects also are shown after active smoking of one marijuana and one placebo cigarette (♦) and after active smoking of two placebo cigarettes (A).

Source: Cone and Johnson 1986, copyright 1986, C.V. Mosby Company.

The behavioral effects after passive exposure to marijuana smoke were time related, with peak behavioral responses being obtained immediately at the end of the smoke-exposure session. Responses were diminished thereafter and generally were completely dissipated within 3 hours. Generally, neutral or negative responses were obtained to the placebo-smoke-exposure sessions.

Assay of urine specimens by immunoassay for cannabinoids and by GC/MS for 9-carboxy-THC provided information on the amount of THC absorbed during the passiveexposure sessions with marijuana smoke and the time course of excretion of marijuana metabolites. Following exposure to the smoke of 16 marijuana cigarettes, 6 of the 7 subjects in the 2 separate experiments produced positive EMIT urine specimens after the first exposure session. The remaining subject produced his first positive after the second exposure session. A high percentage (50 to 100 percent) of all specimens produced thereafter during the 6 consecutive passive-exposure days were positive for cannabinoids. Following exposure to the smoke of four marijuana cigarettes, the results were more variable, with only two subjects producing positive EMIT specimens after the first exposure. Several more positive specimens were produced during the remaining sessions by all subjects with the exception of one subject who produced no positive specimens throughout exposure at the lower passive-smoke-exposure dose. The ranges in concentration of 9-carboxy-THC in urines collected during the consecutive smoke-exposure days, determined by GC/MS assay, are shown in table 1 in comparison to those determined for the same subjects after actively smoking one or two marijuana cigarettes. Following the 6 consecutive days of passive marijuana-smoke exposure, subjects continued to excrete cannabinoids in urine for varying periods, as shown in table 2.

Behavioral Effects and Urinalysis After Oral Ingestion of Marijuana-Laced Brownies

After the ingestion of brownies containing cannabis plant material equivalent to one or two marijuana cigarettes (2.8 percent THC content), subject responses on behavioral measures for Feel Drug, Liking, and the VAS scales were elevated over that of placebo (figure 2). Heart rate was elevated after administration of the two marijuana-cigarette-equivalent brownies. The AUC measures for Feel Drug and Liking scales were significantly ($p<.05$) different from placebo drug administration. The onset of behavioral responses was somewhat slow and variable, with some subjects reporting elevated responses after 30 minutes, but peak responses generally occurred 2.5 to 3.5 hours after dosing. The responses were prolonged and continued to be elevated above placebo through 11.5 hours.

Cannabinoid metabolites were detected in the first urine voids after oral ingestion of marijuana plant material and continued to be detectable for 3 to 14 days. Peak concentrations of 9-carboxy-THC were equivalent to or

higher than those measured after smoking (table 1). Mean detection times to the last positive specimen after dosing are shown in table 2.

TABLE 1. *Individual ranges in peak urinary concentration of 9-carboxy-THC by GC/MS assay after marijuana exposure*

Marijuana Exposure	Dose[1]	Number of Subjects	9-carboxy-THC (ng/ml)
Marijuana brownie[2]	1	5	108-325
(Oral)	2	5	177-436
Marijuana smoke[3]	4	5	0-12
(Passive inhalation)	16	5	15-35
	16	1;1	10;87
Marijuana cigarettes[3]	1	5	9-140
(Active smoking)	2	5	19-152

[1]Dose is expressed in marijuana cigarette equivalents; each cigarette contained 2.8 percent THC. The average cigarette weight was 875 mg.
[2]Data are from Cone et al. 1988.
[3]Data are from Cone et al. 1987.

TABLE 2. *Hours to detection of last positive urine specimen for cannabinoid metabolites after marijuana exposure*

Marijuana Exposure	Dose'	Number of Subjects	Mean Hours + SEW EMIT (20)	RIA (10)	GUMS (5)
Marijuana brownie'	1	5	121.6±27.9	140.3±31.9	149.0±36.2
(Oral)	2	5	142.6±34.3	190.3±42.7	156.3±49.0
Marijuana smoke'	4	5	1.7±1.3	30.9±10.4	9.4±5.8
(Passive inhalation)	16	5	44.2±6.0	129.4±27.5	65.2±15.4
	16	1;1	25.4;141.0	69.2;144.6	25.4;141.0
Marijuana cigarettes'	1	10	53.0±12.2		
(Active smoking)	2	10	69.1±13.5		

[1]Dose is expressed in marijuana cigarette equivalents; each cigarette contained 2.8 percent THC. The average cigarette weight was 875 mg.
[2]The cutoffs (ng/ml) for each assay are shown in parentheses
[3]Data are from Cone et al. 1988.
[4]Data are from Cone et al. 1987.
Unpublished data.

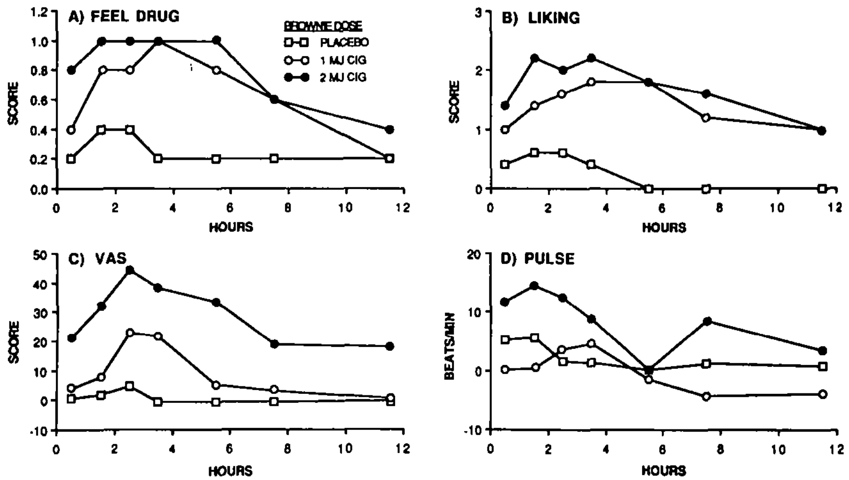

FIGURE 2. *Mean behavioral and physiological* effects *produced in jive subjects after consumption of marijuana-laced brownies*

NOTE: Marijuana brownie content is shown in inset. 1MJ CIG=brownie dose that contained plant material equivalent to one marijuana cigarette (2.8 percent THC) and one placebo cigarette (0 percent THC); 2MJ CIG=brownie dose that contained plant material equivalent to two marijuana cigarettes (2.8 percent THC).

SOURCE Cone et al. 1988 copyright 1988, Preston Publications.

CONCLUSION

Dose-Related Behavioral Effects Produced After Passive Marijuana-Smoke Exposure and After Ingestion of Marijuana Plant Material

Behavioral responses on the Feel Drug and Liking scales were significantly elevated over placebo responses after passive exposure to the smoke of 16 marijuana cigarettes and after the oral ingestion of the equivalent marijuana plant material of 1 or 2 cigarettes. Responses on these measures after lower doses were elevated, but did not differ significantly from placebo. It is clear from these studies that as the level of exposure to marijuana increased, so did the behavioral response. In addition, the behavioral response observed after passive exposure to the smoke of 16 marijuana cigarettes in a small, unventilated room was both qualitatively and quantitatively equivalent to that observed after active smoking of 1 marijuana cigarette. Behavioral response after oral ingestion was both qualitatively and quantitatively similar to that observed after active smoking, but with a delay of 1 to 3 hours.

Urine Excretion Profile of Cannabinoid Metabolites After Passive Marijuana-Smoke Exposure and After Ingestion of Marijuana Plant Material

Passive exposure to the smoke of 16 marijuana cigarettes led to excretion of substantial amounts of cannabinoid metabolites in urine after each exposure with some evidence of accumulation after multiple exposures. Occasionally, levels in urine were sufficiently concentrated to exceed the 100 ng/mL cut-off calibrator commonly used in drug testing. After passive exposure to the smoke of 4 marijuana cigarettes, an exposure level that seems more likely to be encountered in social environments than is the extreme exposure to the smoke of 16 marijuana cigarettes for 1 hour, urines tested positive for cannabinoid metabolites at levels only slightly above the 20 ng/mL cutoff. None of the urines collected after exposure to the smoke of four marijuana cigarettes tested positive by the 75 ng/mL EMIT calibrator.

Oral ingestion of marijuana plant material in cooked brownies led to excretion of highly concentrated amounts of cannabinoid in urine, as seen in table 1. Although marijuana smokers potentially can titrate their drug effect and thus their amount of marijuana exposure, the effects of oral ingestion of marijuana are slow in onset and do not allow the subject sufficiently rapid feedback for dosage adjustment. Hence, the amounts of 9-carboxy-THC in urine after oral ingestion were, at times, substantially higher than those observed after smoking equivalent doses.

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Pyrolytic Degradation of Heroin, Phencyclidine, and Cocaine: Identification of Products and Some Observations on Their Metabolism

C. Edgar Cook and A. Robert Jeffcoat

INTRODUCTION

Smoking of drugs offers certain advantages to the user, Purity of the drug is less important for smoking than it is for intravenous use, the extensive capiIlary network in the lungs facilitates rapid absorption, and the blood circulation pathway ensures that absorbed substances will be rapidly transported to the brain, where they can exert their effects. Thus, rapid onset of action (immediate, positive feedback) combined with some ability to exert control over the dosage and rate of onset of activity combine to produce powerful incentives for smoking of abused drugs. These incentives have long been obvious to smokes of marijuana, opium, and tobacco, but have in recent years also led to smoking of other drugs, such as phencyclidine (PCP) and cocaine. As drug users become more aware of the dangers of acquired immunodeficiency syndrome (AIDS) associated with intravenous drug use, it is possible that smoking will increase in popularity as a means of drug taking.

The advantages enumerated above are also accompanied by disadvantages that may not be so readily apparent to the smoker. Many organic compounds undergo pyrolytic or oxidative reactions when exposed to high temperatures in the presence of air. The resulting compounds may have acute toxicities differing from those of the parent drug, as well as having more long-term effects, such as the induction of lung cancer associated with tobacco smoking. It thus becomes necessary to learn as much as possible about' the nature of these pyrolytic products, their structures, their absorption into the body, and their routes of metabolism as well as toxicity associated with them. Over the past few years, our laboratory has been involved in studies of this type. In this chapter we summarize our work on the

pyrolysis of three drugs of abuse-heroin, cocaine, and PCP. This work demonstrates the diversity of structures that can be obtained and illustrates that smoking of drugs can lead to demonstrable absorption of pyrolysis products.

PYROLYSIS PRODUCTS OF HEROIN

Opium has been smoked for centuries in the Middle and Far East. Therefore, when in the early 1900s heroin was introduced into China and started replacing opium as a narcotic, it also was usually smoked (Huizer 1987). Although in western countries intravenous injection is a frequent mode of heroin abuse, there are still many reports of its continued use by smoke inhalation. These have been reviewed by Cook and Brine (1985) and by Huizer (1987). Smoking of heroin-suffused marijuana or tobacco cigarettes has been reported, but apparently the inhalation of smoke from heroin heated on aluminum foil (chasing the dragon) is currently the most popular method of administration (Huizer 1987). A number of cases of leukoencephalopathy have been attributed to a toxic compound or compounds obtained from heroin smoking (Walters et al. 1981; Walters et al. 1982), but no specific compound has yet been implicated. The problem could have arisen from either pyrolysis products of heroin or of diluents used in the particular heroin smoked. Since relatively little was known about the pyrolysis of heroin or its salts, we investigated the structure of some of the major products of this process (Cook and Brine 1985).

Materials and Methods

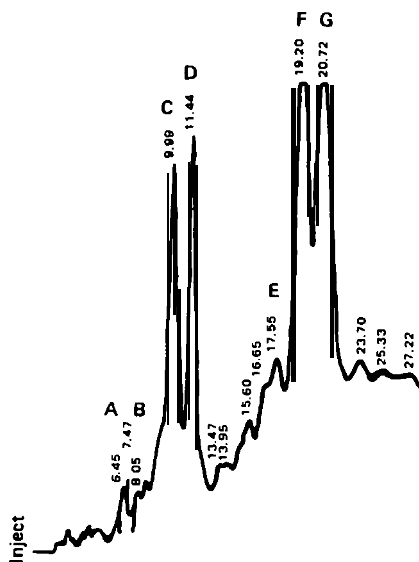
Chemicals. Heroin hydrochloride and not-morphine hydrochloride were obtained from the National Institute on Drug Abuse. Free heroin was obtained by base conversion of the hydrochloride and extraction into butyl chloride solution. The resulting product was shown to be pure by high performance liquid chromatography (HPLC). Normorphine was similarly obtained. It was acetylated with acetic anhydride in pyridine to N-acetylnor heroin. Codeine, pseudocodeine, and isocodeine were acetylated in a similar manner. Methylation was carried out by use of diazomethane in ether containing 10 percent methanol. Allopseudocodeine acetate was prepared from ß-bromocodide by refluxing in acetic acid (Cook and Brine 1985).

Pyrolysis. Pyrolysis was carried out in a quartz boat placed in a quartz furnace tube and connected to a dry trap and two bubbler traps containing 100 mL of absolute ethanol, each at -70 “C. Air was passed through the system at 30 mL per minute. A tube furnace was heated to the desired temperature and placed quickly around the quartz tube. Pyrolysis products were isolated from the various parts of the apparatus by dissolving in solvent and were chromatographed on HPLC. Products were isolated on a Partisil-10/PAC column, eluted with a linear gradient of hexane/ethanol. Like fractions were combined and analyzed by HPLC on Partisil-10/ODS-2

and, if necessary, purified further in this system. The solvent was a mixture of ammonium acetate buffer and acetonitrile. For final purification, ammonium acetate and acetic acid were removed by absorbing material on a C-18 SepPak cartridge (Cook and Brine 1985).

Results and Discussion

Four major ultraviolet (UV)-absorbing peaks and a large number of minor UV-absorbing peaks were observed when heroin hydrochloride pyrolysate was subjected to HPLC (figure 1). One peak (F of figure 1) was attributable to heroin itself. Six other components were isolated in purities exceeding 90 percent. The three major pyrolysis products (peaks C, D, and G of figure 1) were then identified conclusively by means of physicochemical properties and comparison with standard compounds.



Solvent: Linear gradient of 10-70% EtOH in hexane
Column:' Partisil 10 / PAC

FIGURE 1. ***HPLC of a pyrolysate of heroin hydrochloride***

NOTE: Partisil-10 PAC column, eluted with a linear gradient of hexane/ethanol.

SOURCE: Cook and Brine 1985, copyright 1985. American Society for Testing and Materials.

As figure 2 shows, product G was 6-0-acetylmorphine. The UV spectrum showed a slight bathochromic shift from heroin consistent with conversion of the 3-acetate to a phenol. Proton and ^{13}C nuclear magnetic resonance (NMR) revealed that the N-methyl of heroin was still present and the 6a-acetoxy group was unchanged. There were, however, changes in the NMR spectrum of the C-2 to C-4 positions, and only one acetyl group was present. (The ^{1}H NMR spectrum of 6-0-acetylmorphine is quite sensitive to the presence of impurities such as ammonium acetate or acetic acid obtained from buffer salts used in the purification process. Only when these were removed by adsorbing the compound on a C-18 SepPak column, washing with water, and eluting with organic solvent could we obtain consistent proton NMR spectra identical with those of genuine 6-0-acetylmorphine.)

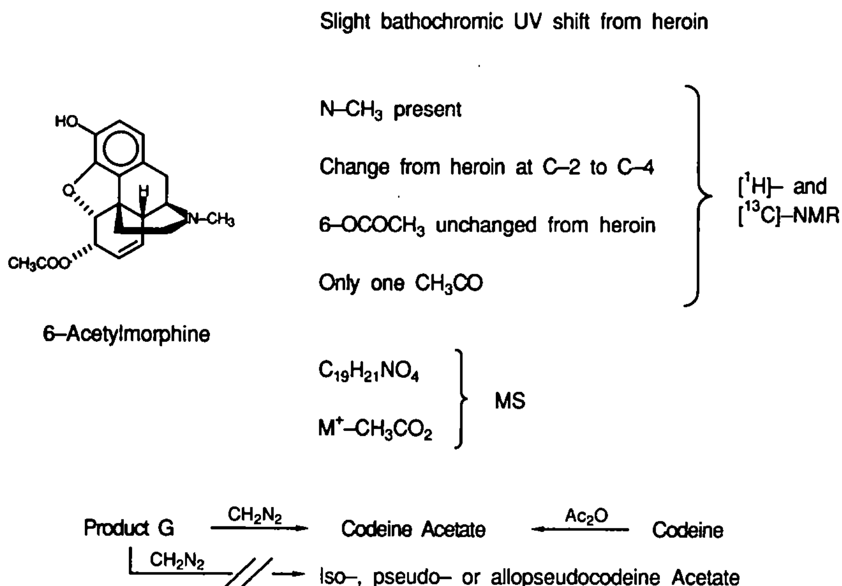


FIGURE 2. ***Identification of heroin pyrolysis product G as 6-0-acetylmorphine***

High-resolution mass spectrometry showed a molecular formula identical to that of 6-0-acetylmorphine and the loss of a fragment corresponding to acetate (or loss of acetic acid from a protonated molecular ion). Treatment of the compound with diazomethane yielded a product identical with that obtained by acetylation of codeine but differing in properties from iso-, pseudo-, or allopseudocodeine acetate.

Pyrolysis product C (figure 3) was identified as N-acetylnorheroin resulting from formal elimination of the N-methyl group and acetylation of the resulting secondary amine. Its UV spectrum closely resembled that of heroin. Proton and ^{13}C NMR spectra showed the absence of the N-methyl group of heroin and the presence of three acetyl groups. Both spectra showed little change from that of heroin for carbons 1 to 8, thus indicating that this portion of the molecule was essentially unchanged. The mass spectrum showed the addition of one carbon and one oxygen atom to heroin and the loss of various combinations of ketene and acetic acid on fragmentation. All of these data pointed to N-acetylnorheroin, and, when not-morphine was treated with acetic anhydride, product C was obtained.

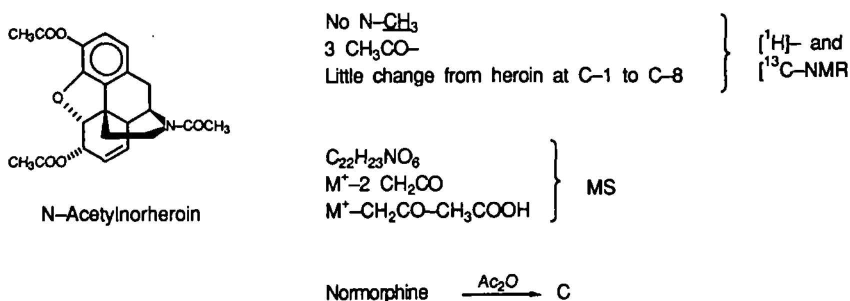


FIGURE 3. *Identification of heroin pyrolysis product C as N-acetylnorheroin*

As figure 4 shows, pyrolysis product D was identified as N, 6-0-diacetylnormorphine, the 3-desacetyl analog of product C. As was the case with 6-0-acetylmorphine, there was a small bathochromic UV shift from that of heroin. NMR spectra of product D, however, showed no N-methyl group. The presence of two acetyl groups was observed from the ^{13}C and proton NMR, and the region from C-l to C-8 was very similar in its NMR properties to that of 6-O-acetylmorphine. Mass spectrometry indicated the formal loss of CH_2 from heroin, and the fragmentation pattern showed loss of ketene from the molecular ion. These properties again are consistent with N, 6-0-diacetylnormorphine, and acetylation of D gave the same product as that obtained when normorphine was acetylated.

Thus the three major products were conclusively identified. Product A (figure 5) was obtained in much smaller quantities. It exhibited a strong *UV* chromophore. Its high-resolution mass spectrum showed there was no

Small Bathochromic UV shift from heroin

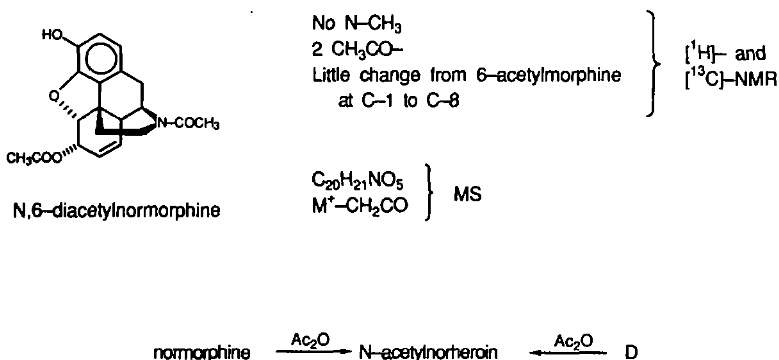


FIGURE 4. *Identification of heroin pyrolysis product D as N, 6-0-diacetylnormorphine*

nitrogen in the molecular ion and indicated a formula of $C_{18}H_{14}O_4$. The mass spectrum also indicated fragmentation involving the loss of two CH_2CO residues, consistent with the presence of two acetate functions. The degree *of* unsaturation revealed by mass spectrometry could be accommodated by three aromatic rings together with two acetoxy groups, leading us to tentatively identify this product as 3, 4-diacetoxyphenanthrene. A search of the literature showed that this compound had been reported in 1886 by Fischer and Gerichten from heating of heroin methiodide with silver acetate in acetic acid; and it appears that this indeed is product A, although an authentic sample was not available for comparison. (Dr. Fischer, unfortunately, was not in a position to provide us with such a sample.)

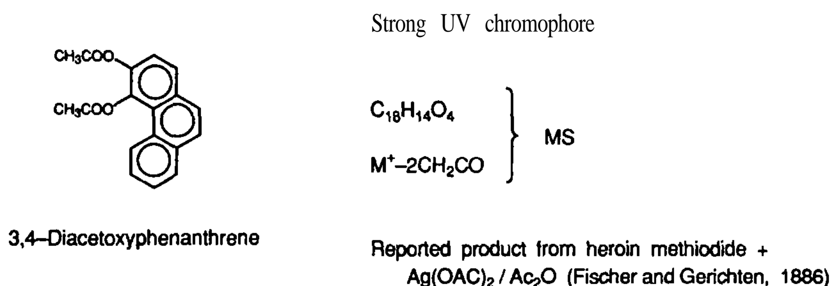


FIGURE 5. *Tentative identification of heroin pyrolysis product A as 3,4-diacetoxphenanthrene*

The pryrolysis products identified are consistent with anticipated pyrolytic mechanisms shown in figure 6. In this figure, we see the initial formation of a quaternary N-acetyl intermediate by interaction of two molecules of heroin. Attack of a nucleophile is most likely to occur on the N-methyl group leading to its loss and the generation of the N-acetyl compound. Although nucleophilic attack could also take place on the other carbon substituents of the nitrogen, it is less likely to occur there for steric reasons. Alternatively, removal of a proton in the position ß to the nitrogen would lead to formation of a double bond and opening of the piperidine ring. Alternatively, in the case of heroin hydrochloride, nucleophilic attack on the methyl group of the protonated amine would lead to the formation of norheroin, which could undergo acetylation by reaction with other molecules of heroin.

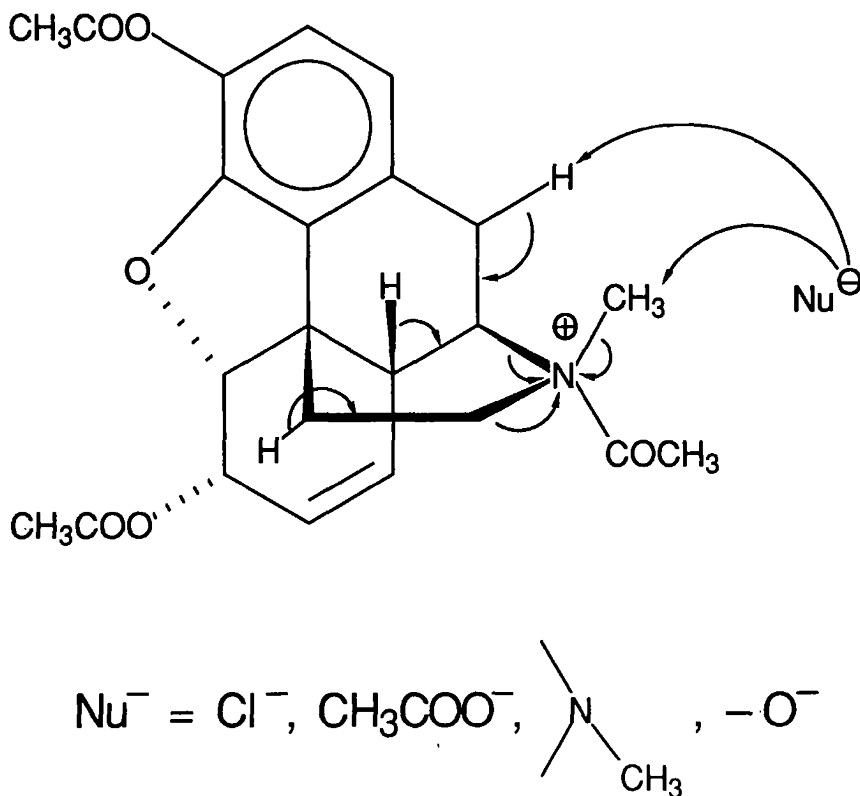
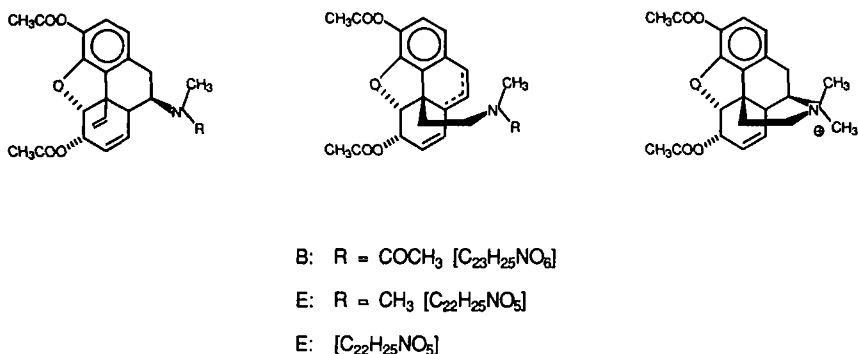


FIGURE 6. *Possible pyrolytic mechanisms for heroin*

That some of the reactions involving ß elimination are also occurring is indicated by the data for pyrolysis products B and E. Product E had a formula indicating formal addition of CH_2, to heroin. The structures of figure 7, where $R=CH_3$, would fit this molecular formula. Such structures could derive from N-methylation followed by olefin formation. Since product E did not have a styrene nor a conjugated diene chromophore, opening of the piperidine ring to give the nonconjugated diene appears the most likely explanation at this time. Pyrolysis product B has a molecular ion at m/z 411, which is consistent with the N-acetyl N-methyl formulas shown in figure 7.



B: $R = COCH_3$ $[C_{23}H_{25}NO_6]$

E: $R = CH_3$ $[C_{22}H_{25}NO_5]$

E: $[C_{22}H_{25}NO_5]$

FIGURE 7. *Possible structures for heroin pyrolysis products B and E*

Products involving rearrangements or inversions of the allylic acetate system did not appear to play a major role in the pyrolysis, although such compounds could certainly exist as some of the minor products shown in the HPLC chromatogram. Finally, a large amount of unidentified tarry material was produced, which could have resulted from further reaction of some of the initially formed pyrolysis products.

The pyrolysis of heroin hydrochloride and heroin free base was studied at several temperatures ranging from 200 to 800 °C (Jones and Davis, personal communication). As figure 8 shows, decomposition was much more extensive with the hydrochloride salt. Less than 10 percent of the heroin remained as the parent compound after 10 minutes at 300 "C. At this time and temperature, almost two-thirds of the heroin free base was recovered as the parent compound. 6-0-Acetylmorphine was the major product at 250 °C in the pyrolysis of heroin hydrochloride. About 20 percent of the original heroin was converted to this product, together with 14 percent of N-acetylnorheroin and about 6 percent of N,6-0-diacetylnormorphine. Pyrolysis of heroin free base at somewhat higher temperatures yielded similar patterns and amounts of these products.

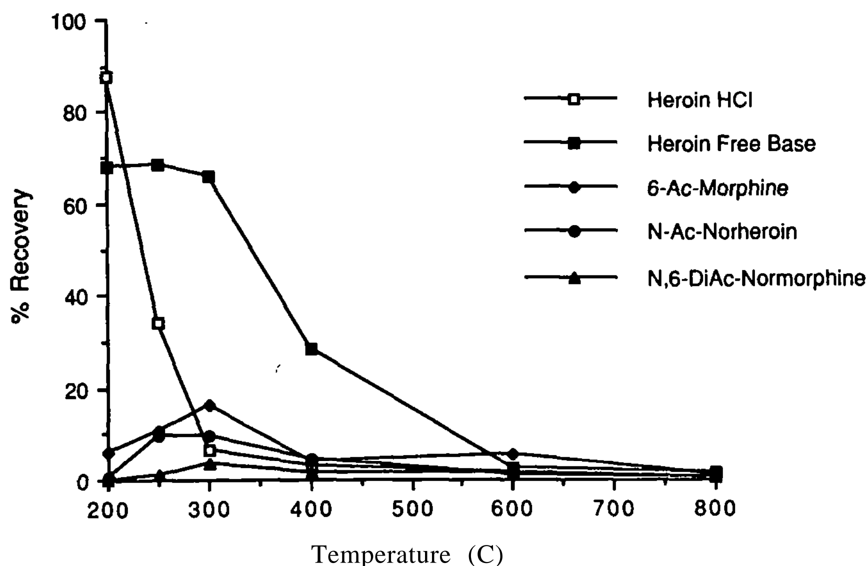


FIGURE 8. *Products from pyrolysis of heroin and heroin hydrochloride at various temperatures*

NOTE Data on breakdown products arc from pyrolysis of the hydrochloride. (Unpublished data obtained from K. Davis and L. Jones; mean of two experiments.)

Huizer (1987) has reported on the decomposition of heroin at varying temperatures and in the presence of a number of possible diluents. Some of these, such as caffeine and barbital, enhance the recovery of heroin from the hydrochloride under the conditions of "chasing the dragon," whereas others, such as procaine hydrochloride, decreased the yield of heroin in the pyrolysis condensate. Most of the diluents decreased the recovery of heroin base, and none actually enhanced its recovery. Thus Huizer concluded that the available yield of heroin is strongly dependent upon the presence of contaminants or diluents and the nature of these substances.

In summary, extensive degradation occurs at temperatures that might be involved in inhalation of heroin by smoking. Even heroin free base undergoes significant decomposition under these conditions. 6-0-Acetylmorphine, which is formed on smoking and is also a metabolite of heroin, has narcotic activity and would be expected to contribute to the pharmacological effect. N-Acetylnormorphine derivatives, also formed in significant amounts, are probably unlikely to have intrinsic opiatelike activity but could have other pharmacological effects or perhaps be metabolically converted to normorphine. Since a large number of other compounds were apparently produced

in minor amounts by heating of heroin, and since diluents used in street samples may also undergo pyrolysis to potentially toxic products, it appears that the smoking of heroin could have significant toxicological consequences. Further information on the pharmacological and toxicological implications of this type of heroin administration is needed.

PYROLYSIS STUDIES OF COCAINE

The smoking of cocaine free base has become an increasingly popular method of self-administration. The ready availability of crack (a form of cocaine free base) in particular has raised major concerns about this route of administration. Since esters undergo pyrolytic elimination reactions, it seemed plausible to expect cocaine to be degraded by heat. We, therefore, undertook a study to identify the pyrolysis products of this compound (Cook et al. 1985; Jeffcoat et al. 1985).

Materials and Methods

Unlabeled cocaine and cocaine containing tritium in the N-methyl position or on the C-4 methylene group were obtained from the National Institute on Drug Abuse and diluted with unlabeled cocaine. The radiochemical purity was greater than 92 percent by HPLC analysis.

Pyrolysis. Tritiated cocaine in quantities of 25 to 200 mg was pyrolyzed by an apparatus similar to that used for the pyrolysis of morphine. Pyrolysis products in the quartz tube and those collected in the U-tube were recovered by dissolving them in acetonitrile.

Human Studies. Cocaine was administered to human subjects by the intravenous, smoking, and nasal insufflation routes. The clinical and analytical procedures have been described (Jeffcoat et al. 1989; Perez-Reyes et al. 1982).

Results and Discussion

Cocaine hydrochloride is almost completely destroyed at 800 °C (the temperature of the glowing end of a tobacco cigarette) and cocaine itself is extensively degraded with only 16 percent of the pyrolysis product being intact cocaine. At lower temperatures, however, cocaine is much more stable, although the percentage of intact cocaine recovered appears to be strongly dependent on exact experimental conditions. Separation of pyrolysis product mixtures by HPLC showed four major peaks of radiolabeled material. The third of these peaks had the same retention time as cocaine, and its mass spectrum was identical with authentic cocaine.

Since the materials in the first two peaks were too volatile to permit their isolation from the HPLC solvents, the crude pyrolysis product mixture was

examined by combined capillary gas chromatography/mass spectrometry (GC/MS). Three major types of pyrolysis products in addition to cocaine were observed. One set of three peaks with similar retention times relative to cocaine had almost identical mass spectra, which were also identical to the published spectra of methyl ecgonidine (Lowry et al. 1979; Lukaszewski and Jeffery 1980). Although this compound is reported to be formed when cocaine hydrochloride is introduced into a heated injection port of a gas chromatograph, we found no such occurrence when cocaine free base was injected at the temperature used in this work The presence of three gas chromatography peaks indicates that other isomeric products are formed in addition to methyl ecgonidine. One of these could be the ß, y isomer. Internal ring opening could also lead to compounds isomeric with methyl ecgonidine (Cook et al. 1985).

A second set of four peaks with similar but not equivalent mass spectra was also observed. The presence of molecular ions at m/z 150 was consistent with identification of these as the doublebond isomers of methylcycloheptatrienecarboxylate. This assignment was further supported by a base peak at m/z 91 corresponding to benzyl or cycloheptatrienyl ion with loss of carboxymethyl residue and transfer of a hydrogen. The compounds also underwent loss of methyl and methoxy residues (Cook et al. 1985).

In addition to these major peaks, significant amounts of methyl benzoate, benzoic acid, and N-methylbenzamide were also observed (Cook et al. 1985). These products are explained by elimination of benzoic acid and by mechanisms involving acylation of the tertiary nitrogen of cocaine to form intermediates, which undergo nucleophilic attack much as was described in the case of morphine.

The effectiveness of smoking as a route of administration is readily seen by comparison of plasma concentrations of cocaine in human subjects who either smoked the drug or were given an intravenous injection of cocaine hydrochloride (Jeffcoat et al. 1989). The who curves are essentially comparable, and it was observed that the plasma levels of cocaine had reached a maximum by approximately 6 minutes after the beginning of smoking (figure 9). Table 1 shows calculations of pharmacokinetic parameters and bioavailability calculated after different routes of administration in studies from our laboratory (Jeffcoat et al. 1989). Average elimination half-lives ranged from 56 minutes for the subjects who smoked cocaine to 80 minutes for those who snorted the drug. Bioavailability based on the amount of radiolabeled material actually inhaled showed a range of 32 to 77 percent. Since simulated smoking studies indicated that only about 44 percent of the material inhaled was cocaine, this suggests that most of the cocaine actually inhaled reaches the circulation unchanged. However, marked interindividual variation in the amount of cocaine obtained by smoking is likely.

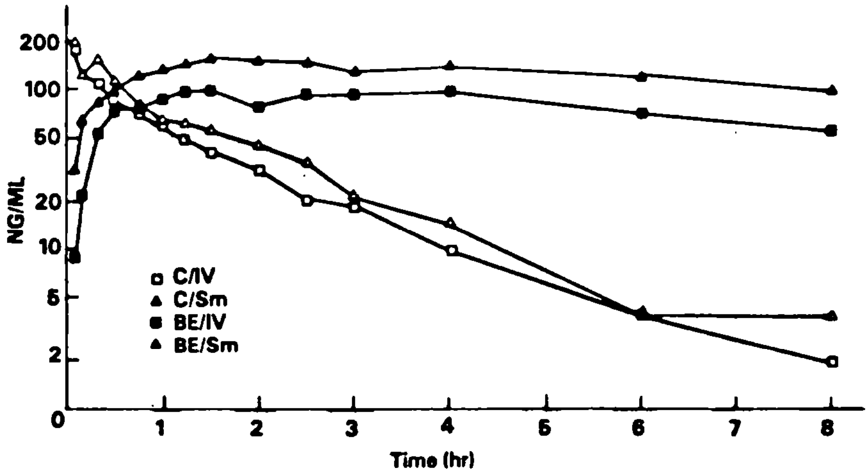


FIGURE 9. *Cocaine and benzoylecgonine concentrations in the plasma of subjects who received cocaine by smoking or intravenous injection*

SOURCE Cook et al. 1985, copyright 1985, Biomedical publications.

A somewhat smaller proportion of the inhaled cocaine was excreted in the urine than was the case for intravenous cocaine. This suggests that some of the pyrolysis products are eliminated in the bile and feces. Examination of the urinary excretion of cocaine metabolites (figure 10) showed little difference in the average amount of ecgonine methyl ester excreted whether the drug was given intravenously, by smoke inhalation, or by snorting; but the amount of benzoyl ecgonine appeared significantly less in the case of the smoking route. Figure 11 shows the ratio of benxoyl ecgonine to ecgonine methyl ester in the urine. During the period 0 to 24 hours, there is a significantly ($p<.05$) lower average ratio for the smoking route than for the other two routes of administration. This difference is even more pronounced in the period from 24 to 72 hours after administration. However, little (about 14 percent) of the total urinary excretion occurred during this latter period, with 86 percent of the excretion occurring in the 0- to 24-hour period. Furthermore, there was considerable overlap in the ranges in the 0- to 24-hour period. Data in a recent paper by Ambre et al. (1988) show a range of ratios of benzoylecgonine to ecgonine methyl ester excretion of 0.8 to 8.7 after intravenous administration. Thus, although an average difference in metabolism based on route appeared to be present in our population of cocaine users, this ratio could not be used as a means of distinguishing whether a given individual had smoked or snorted cocaine.

TABLE 1. ***Pharmacokinetic analysis of cocaine in plasma***

Route	Number	a (hr^{-1})	a (hr^{-1})	B (hr^{-1})	Bioavailability Percent
IV	4	—	3.71[a]±1.73	0.532±0.127	100
Snorting	6	3.54±2.12	—	0.523±0.113	80±13 68-103[b]
Snorting[c]	6	2.28±0.39[d]	—	0.535±0.04[d]	
Snorting[c]	6	3.92±0.38[d]	—	0.465±0.017[d]	
Smoking	6	38.O±16.0	3.14[f]	0.740±0.274	47±19[8] (32-77)[b,g]
Smoking[c]	6	31.0±8.3[d]	—	0.718±0.083[d]	
Overall	16	—	—	0.607±0.210	

KEY: [a]Three subjects; [b]range; [c]simultaneous fit of all data; [d]SE; [e]fit of mean plasma data; [f]one subject only; [g]based on inhaled radiolabeled material.

NOTE Unless otherwise noted, date are unweighted means ± SD of Individual subjects.

SOURCE: Based on data in Jeffcoat et al, 1989.

METABOLISM OF 1-PHENYLCYCLOHEXENE—A PYROLYSIS PRODUCT OF PCP

The increase in popularity of PCP as a drug of abuse has been credited to a shift in its mode of use from oral ingestion to smoking (Stillman et at 1980). As a tertiary benzylamine, PCP is a likely candidate for elimination reactions under pyrolytic conditions, and in 1980 Martin and colleagues reported that in a burning cigarette 1-phenylcyclohexene (PC) is a major product from PCP (Martin et al. 1980; Freeman and Martin 1981). The production, absorption, pharmacokinetics, and metabolic fate of this compound are thus of interest.

Materials and Methods

Pyrolysis apparatus and conditions have been described (Cook et al. 1981), as have the in vitro metabolism of 1-phenylcyclohexene and synthesis of metabolites (Cook and Brine 1984). Details of the clinical and analytical portion of studies involving smoking of PCP have also been published (Cook et al. 1982). Pharmacokinetic parameters were fit to the mean PC concentrations in plasma by the nonlinear least squares regression procedure

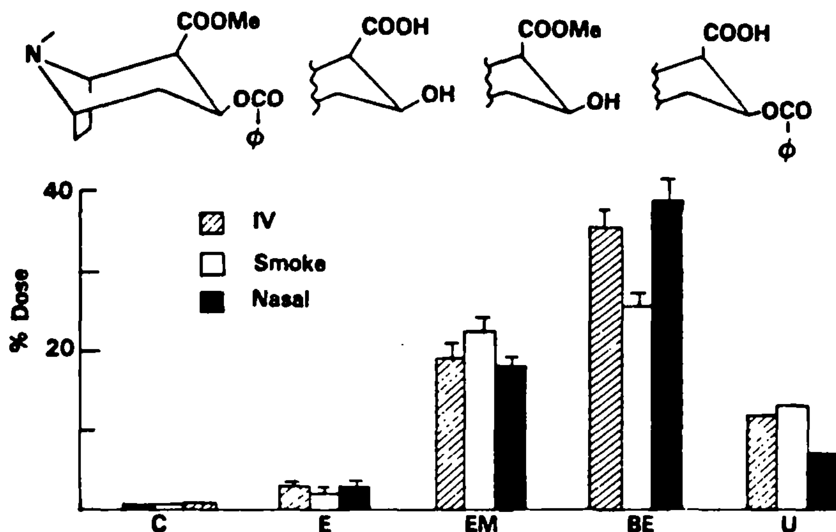


FIGURE 10. *Percent of dose of cocaine excreted as parent drug or metabolites in urine as a function of route of administration (average 3-day excretion)*

SOURCE: Cook et al. 1985, copyright 1985, Biomedical Publications.

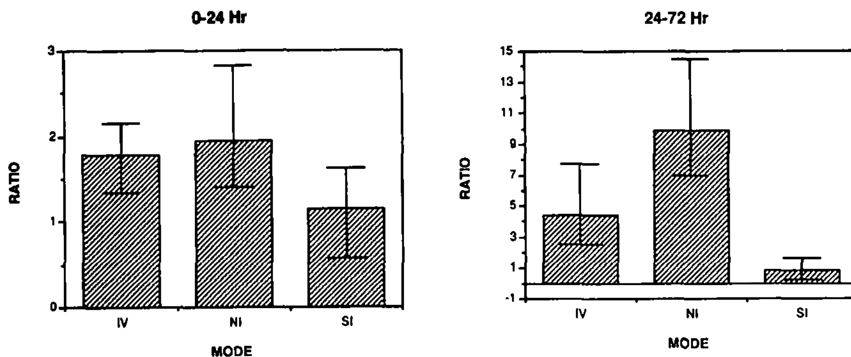


FIGURE 11. *Ratio of benzoylecgonine to ecgonine methyl ester excreted in urine as a function of route of administration and time from drug administration*

NOTE: Error bars show ranges. Number of subjects four for intravenous injection (IV), six each for nasal insufflation (NI) and smoking (SI).

in the program RS/l (Bolt, Beranek, and Newman, Cambridge, MA). Points were weighted as the reciprocal of variance.

Results and Discussion

When heated either as the free base or its hydrochloride salt, PCP is converted to 1-phenylcyclohexene (Martin et al. 1980; Freeman and Martin 1981; Cook et at 1981). Even at the relatively low temperature of 300 °C, the hydrochloride salt is converted in 93 percent yield to l-phenylcyclohexene and piperidine hydrochloride. These products were identified by ^{1}H and ^{13}C NMR analysis, and the structures were confirmed by GC/MS analysis of 1-phenylcyclohexene and of piperidine as its benzoyl derivative (Cook et al. 1981). The free base of PCP undergoes considerably less de composition at 300 "C, and more than half of the material is recovered as PCP. In contrast to simple pyrolytic conditions, both the hydrochloride salt and free base give similar yields of PCP and PC when smoked in a parsley cigarette, with almost 1.5 times as much PCP as PC being found in the smoke under simulated smoking conditions (Cook et al. 1981).

When human subjects, using an apparatus that permitted the collection of sidestream smoke and exhalant, smoked parsley cigarettes containing 100 µg of tritium-labeled PCP hydrochloride, we could estimate the dose of PCP and PC actually inhaled by the subjects by measuring the radioactivity not inhaled and using the results of the simulated smoking studies (figure 12) (Cook et al. 1981; Cook et al. 1982; Cook et al. 1983).

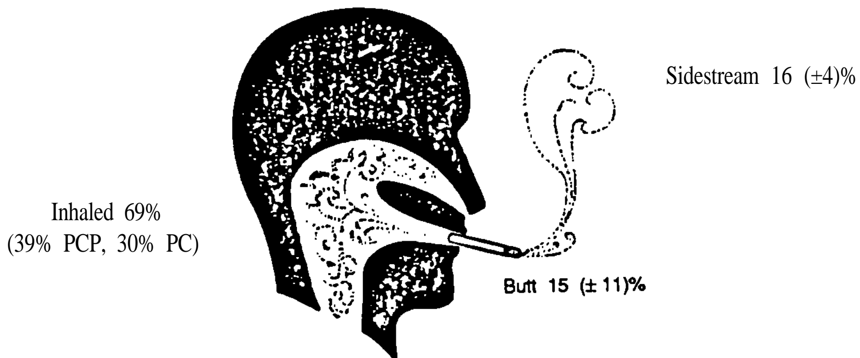


FIGURE 12. *Fate of PCP hydrochloride on parsley cigarettes smoked by human volunteers*

NOTE: Values in mode % of PCP on cigarette. Inhaled percentage based on simulated smoking studies.

SOURCE Cook et al. 1985, copyright 1985. Biomedical publications.

High-performance liquid radiochromatography demonstrated that both PCP and PC were present in plasma. Our calculations showed that PCP teaching the lungs was well absorbed with excellent bioavailability and that, in general, the pharmacokinetics and metabolism of PCP were similar to those observed after intravenous administration (Cook et al. 1982). The terminal half-life was 24±7 (SE) hours in five individuals who smoked PCP and 16±2 (SE) hours in six who were given the drug intravenously. If we estimated the dose of PCP actually inhaled as described above, then the calculated bioavailability averaged 110±47 percent. This suggests both good absorption of unchanged PCP and considerable variability in individual smoking (table 2).

TABLE 2. *Pharmacokinetics and bioavailability of phencyclidine by intravenous, oral, and smoking routes*

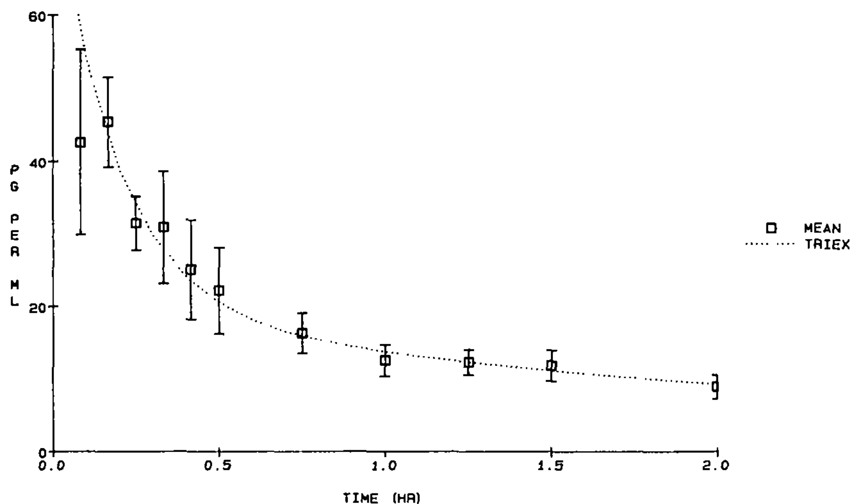
Compound	Route (Number)	Dose (μg/kg)	Terminal (hr)	Total Clearance (liter/min)	Renal Clearance (liter/min)	Volume of Distribution (V_{48}) (liter/kg)	Bioavailability (Percent)
PCP	IV (6)	1.2-12	16 (2)	0.38 (0.08)	0.033 (0.008)	6.2 (0.3)	100
PCP	Oral (5)	14 (1)	27	—	0.037 (0.016)	—	72 (8)
PCP	Smoke (5)	0.43 (0.02)	24 (7)	—	0.033 (0.007)	—	110 (17)
PCP	All (16)	0.4-14	22 (3)	—	0.034 (0.006)	—	—
PC	Smoke	0.20 (0.02)	15 (3)	2.0* (0.4)	<0.003	35*	—

*Apparent value.

NOTE: Data are mean (SE). Renal clearance is pH-dependent; average urine pH was -65.

SOURCE: Bused on data In Jeffcoat et al. 1989.

As figure 13 indicates, PC was also rapidly absorbed. The average time to peak was 8 minutes. The subjects actually showed the highest concentration of PC at 5 minutes after beginning of smoking, the earliest time point for which plasma samples were available. Thus, it was not possible to determine absorption half-life, but it is very rapid. A standard multiexponential pharmacokinetic equation was fit to the data by means of nonlinear regression analysis. Where necessary, points before the peak plasma concentration were ignored in this analysis. In three subjects, a biexponential equation best fit the data, whereas a triexponential equation gave a better fit for two of the subjects (Cook et al. 1982). When the mean plasma data



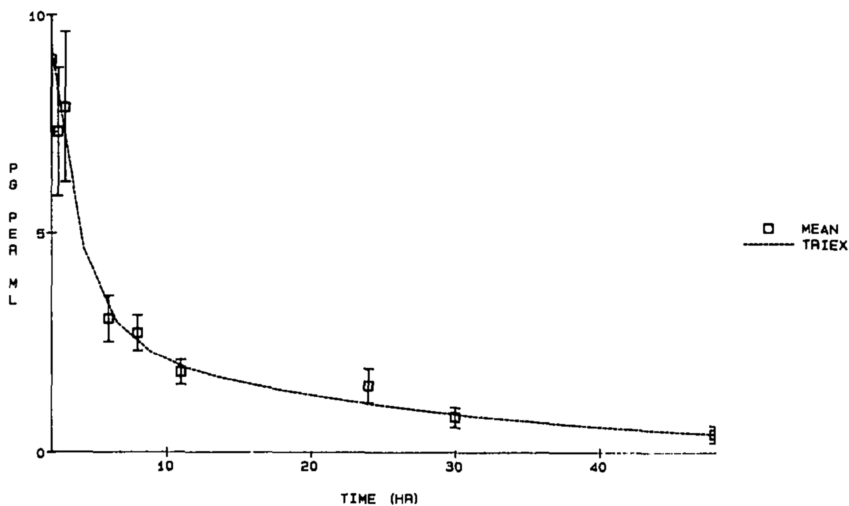


FIGURE 13. *Mean plasma concentrations (±SEM) of PC in five subjects who smoked parsley cigarettes containing 100 µg of PCP hydrochloride*

NOTE: Line is beat fit of a triexponential equation to data points from 10 minutes to 72 hours. Top panal shows from 0 to 2 hours, bottom panel from 2 to 48 hours.

were used (figure 14), the data points from 10 minutes to 72 hours after smoking began were best fit by a triexponential equation (F test, $p<.01$)

with half-lives of 0.131, 1.44, and 17.0 hours for the three phases, respectively.

α	$5.30 \pm 1.56\ h^{-1}$	$t_{1/2}(\alpha)$	=	0.13 h
β	$0.481 \pm 0.075\ h^{-1}$	$t_{1/2}(\beta)$	=	1.4 h
γ	$0.0408 \pm 0.0041\ h^{-1}$	$t_{1/2}(\gamma)$	=	17 h
Estimated Dose	$16 \pm 1\ \mu g$	Cl_T	=	131 L/h*
$V_{d,\gamma}$	= 3210 L*	Cl_R	=	<0.14 L/h

FIGURE 14. *Pharmacokinetic parameters calculated from mean of plasma concentrations of PC*

*Apparent value.

NOTE: Dose was calculated from percent of dose inhaled and fraction of PC from simulated smoking studies. Data represent mean ± SE (n=5). a ß, and Y derived from nonlinear regression analysis of mean data.

A word of caution is in order with regard to the pharmacokinetic calculations for PC. Because of the very low concentrations, long counting times had to be used, and, even with analysis of 3 ml of plasma, the mean value at 72 hours represents only about 3 decompositions per minute above background. The standard errors of the parameters were quite reasonable, however, and the calculated values of the rate constants were changed less than 10 percent if the data points only to 30 hours were used in the calculation. We also converted the picograms per milliliter values to percent of dose per liter of plasma (based on the individual estimates of dose inhaled), normalized to an 80-kg person. This had almost no effect on the variance of the values or the fitted rate constants.

PC had a very large apparent volume of distribution (approximately 35 L/kg) if it is assumed that all of the estimated dose of PC reached the circulation. Renal clearance of PC was extremely low in comparison with total clearance, indicating that extensive metabolism occurred before excretion. Fecal excretion may also play a major role in the elimination of PC metabolites, since the amount of PC metabolites identifiable in urine constituted less than 10 percent of the estimated dose of PC (Cook et al. 1982). In many respects, the pharmacokinetics of PC thus resembles that of the very lipophilic compound Δ^9-tetrahydrocannabinol (Agurell et al. 1986;

Chiang and Barnett 1984; Wall et al. 1983; Hunt and Jones 1980; Barnett et al. 1982)—perhaps not a surprising conclusion.

The small fraction of PC metabolites excreted in urine and the low doses used made identification of metabolites in humans very difficult. We therefore made an initial study of the in vitro metabolism of this compound by rat liver. Our results (Cook et al. 1984) confirmed and extended the results of Martin et al. (1982) on metabolism by mouse liver. A large number of metabolites were identified by comparison of physical data with those of standards whose structures were established by synthesis and rigorous physical methods (Cook et al. 1984). These studies showed that PCP undergoes oxidative metabolism at multiple sites on the molecule and that multiple oxidations also occur (figure 15). The double bond may be epoxidized and hydrolyzed to a diol. Oxidation may occur at the 3 position to yield an alcohol or ketone. Further hydroxylation may occur at the 4 position, and oxidation may also occur at the 6 position. In several of the metabolites, an asymmetric center is present, thus raising the possibility of stereoselective metabolism. Further research is needed on this feature of PC metabolism.

Good separation of many of these metabolites may be achieved by HPLC on a Pattisil-PAC column, and reasonable separations are also achieved on a reverse phase chromatography system.

Extraction experiments showed that PC metabolites in urine were highly polar and, therefore, likely consisted of conjugated substances. Thus, for identification of metabolites, urine samples were incubated with a mixture of B-glucuronidase and sulfatase. Extraction with butyl chloride then gave an organic-soluble phase from which acid-soluble PCP metabolites could be extracted with aqueous HCl. A subsequent extraction with aqueous sodium hydroxide removed basesoluble PC metabolites, accompanied by some of the more water-soluble neutral metabolites. We found little evidence for the presence of phenolic metabolites of PC. The neutral metabolites were purified by small-scale preparative HPLC, generally on a reverse-phase column. Plasma extracts were also examined by HPLC in an attempt to identify PC metabolites present in plasma. Metabolites were designated a, ß, **γ, δ, ε** in order of their polarity. Little, if any, of metabolite a was present in urine.

Only small amounts (generally less than a microgram) of these metabolites could be isolated from urine of a single subject. Therefore, we relied on techniques such as derivatization and isotope dilution experiments to identify the metabolites. Thus, metabolite ß was suspected to be l-phenylcyclohexen-3-o1, based on its retention time on HPLC. To confirm this, the isolated metabolite was mixed with a known amount of standard compound, and the specific activity was determined by HPLC analysis. The weight of the material in the HPLC peak was determined by comparison of UV

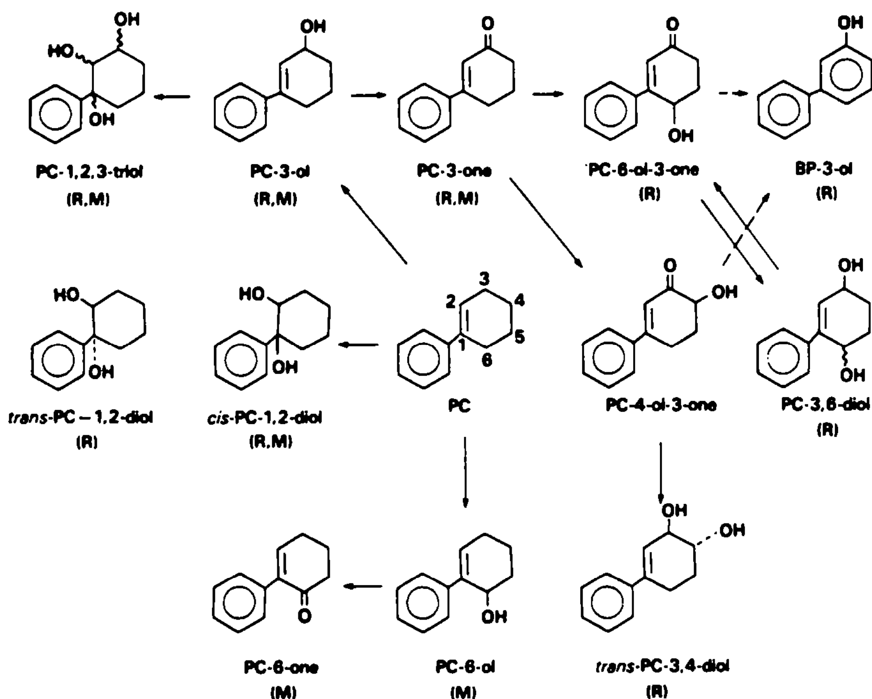


FIGURE 15. *In vitro metobolites of phenylcyclohexene*

NOTE: Arrows represent chemically reasonable, but speculative, metabolic pathways. Broken and solid lines show relative stereoechemical relationships, not absolute configuration. The position of the hydroxyl at C-3 In PC-1,2,3-triol and BP-3-ol was not conclusively established. M designates metabolites Identified from mouse (Martin et al. 1982); *R* designates rat metabolites.

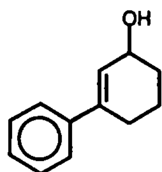
SOURCE Cook et al. 1984, copyright 1984, American Society for Pharmacology and Experimental Therapeutics.

absorption with a standard curve, and the radioactivity was measured by liquid scintillation spectrometry. Other aliquots of the mixture were derivatized and subjected to this same procedure. As derivatives, we chose the acetyl and *p*-nitrobenzoyl esters, to achieve a range of retention times on HPLC, thus maximizing the probability of separating closely related substances. Since the specific activity of the two derivatives coincided very closely with that of the parent metabolite, we concluded that metabolite ß is indeed 1-phenylcyclohexen-3-o1.

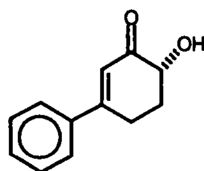
Metabolite y on treatment with methoxylamine underwent a shift in retention time on HPLC, thus indicating the presence of a keto group. Benzoylation also resulted in a change in retention and suggested the presence of a

hydroxyl group. Since the metabolite had the same retention time as 4-hydroxy-1-phenylcyclohexen-3-one, we suspected that it was this compound. To confirm this, a mixture of isolated metabolite and standard was reduced with sodium borohydride to give a mixture of diols separable by chromatography. The major product, the trans-3,4-diol, was then recystallized to constant specific activity. Very little of the radioactivity associated with metabolite was lost during recystallization, thus confirming that this metabolite was 4-hydroxy-1-phenylcyclohexen-3-one.

Figure 16 summarizes the basis for the firm identification of two metabolites of PC (ß and y) in human urine. Evidence for the identity of four other metabolites was also obtained (figure 17). l-Phenylclohexen-3-one cochromatographed with a major metabolite (a) in plasma and is a reasonable candidate for a. Metabolite o from urine was separated into two components with the retention times of cis- and trans-1-phenylcyclohexene-3,4-diol. Only one third to one-half of the radioactivity associated with these metabolites was found when they were converted to ester derivatives; we regard this identification as less conclusive than the ones above. 1-Phenylcyclohexene-3,6-diol cochromatographed with radioactivity from the metabolite mixture, and this compound may also be present in hydrolyzed urine.



Isotope dilution / HPLC of parent + 2 esters



Reacts with methoxyamine / HPLC
Reacts with benzoyl chloride / HPLC
Isotope dilution / recrystallization of $NaBH_4$ product

FIGURE 16. *Summary of evidence for identification of PC metabolites* **ß** *and* **γ** *from human urine*

CONCLUSION

It has been shown that the drugs PCP, heroin, and cocaine undergo fairly extensive decomposition when heated. A variety of pyrolytic products have been isolated and identified. These compounds may exert their own

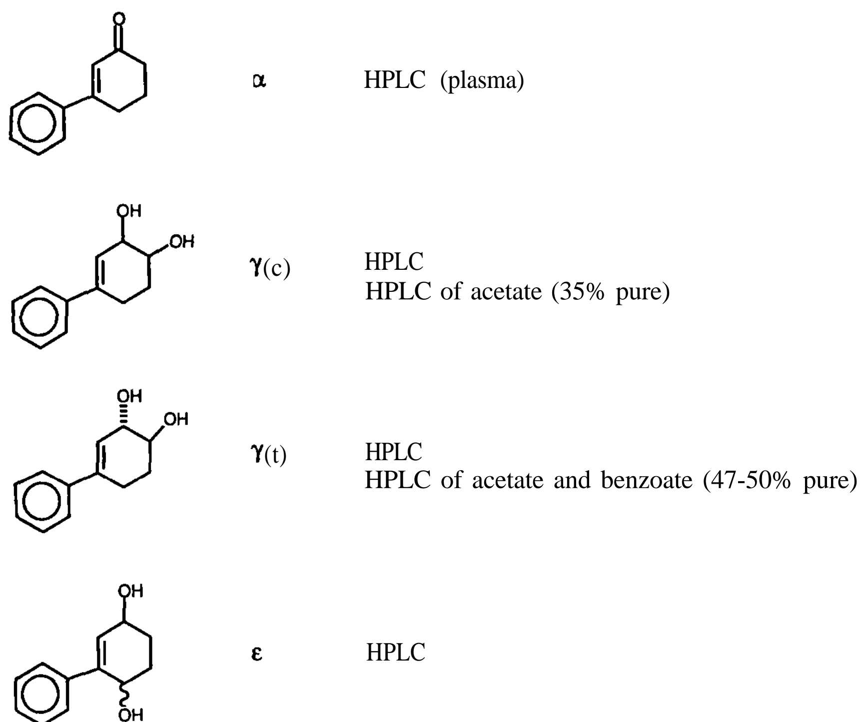


FIGURE 17. Summary of evidence for tentative identification of PC metabolites a, o, and E in human subjects

pharmacological activity or may prove to have toxic effects. Much yet remains to be learned about these potential hazards of smoking of abused drugs.

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Chemical and Biological Analysis of Marijuana Smoke Condensate

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INTRODUCTION

The various products derived from Cannabis sativa L., generally lumped together under the descriptive name "marijuana," are ingested for recreational purposes by a sizeable portion of the population. Most popularly, the leaves and flowering parts of the plant are smoked, producing a variety of physical and psychomimetic effects commonly referred to as being "high." Owing to the large number of people using marijuana, an intensive effort has been under way since about 1968 to define its chemistry, the metabolism of its major active ingredients, and the possible health effects of its use. Although much knowledge has been gained about the chemistry, pharmacology, metabolism, and pharmacokinetics of pure compounds from Cannabis, an insufficient amount is known about these subjects as they relate to marijuana smoke because of its complex chemistry.

The general goal of our work was the development of methodology for the comprehensive analysis of the biologically active fractions of marijuana smoke condensate (MSC). Biological activity was assessed through the use of the Ames Salmonella mutagenesis assay (Maron and Ames 1983). This "mutagenesis directed" research was designed to focus the analytical effort on the most biologically active fraction(s) to increase the possibility of identifying potential mutagens or carcinogens in marijuana smoke condensate. Chemical and biological screening of smoke condensate fractions was conducted for both marijuana and tobacco to allow for comparisons.

The complex chemistry of marijuana smoke involves not only the major cannabinoids, but also a large number of combustion and pyrolytic products. Many compounds found in marijuana smoke are known to have adverse biological properties. For example, one study (Leuchtenbeyer et al. 1973) showed that exposure of human lung explants to fresh marijuana smoke resulted in alterations in DNA and chromosome complement. But-stein

et al. (1973; Burstein et a1. 1975) have presented evidence that certain phenolic components present in the extracts of Cannabis sativa L. are strong inhibitors of prostaglandin biosynthesis. Polycyclic aromatic hydrocarbons (PAH) are formed during any organic combustion process and have been isolated from MSC and tobacco smoke condensate (TSC) (Lee et al. 1976). These compounds are among the most studied of any class with regard to their mutagenic and carcinogenic properties. Novotny et al. (1980), Maskarinec et al. (1976), and Merli et al. (1981) have identified many toxicologically important species in various MSC fractions and have, in some cases, compared these data with similar information from TSC factors. Some studies on the mutagenicity of MSC have been reported (Busch et al. 1979; Wehner et al. 1980), but no research has yet combined mutagenic studies with a concerted, comprehensive effort to identify specific compounds responsible for the biological activity.

Historically, a tremendous amount of work has been done to determine the composition and pharmacological activity of tobacco smoke. Parameters (puff frequency, volume, duration, and holding time) governing the generation of TSC are well documented in the literature (Ogg 1964). Although it is easy to assume that such methodology is directly applicable to the marijuana situation, this assumption in fact is not correct (Peterson 1979). Information found in the literature partially describing the physicodynamics of marijuana smoking and the observation of research subjects smoking marijuana cigarettes indicate that people smoking marijuana tend to do so in a much more vigorous and aggressive manner than is pursued with tobacco. While this information illustrates that a single set of parameters describing the physicodynamics of marijuana smoking cannot be defined easily, it clearly shows that marijuana smoking parameters are more rigorous than those describing the tobacco smoking process.

GENERATION OF SMOKE CONDENSATES

The particular smoking parameters used in governing the production of marijuana smoke are important since the composition of the resulting MSC will vary, depending on the conditions under which it is produced. In a previous study performed at Research Triangle Institute (Davis et al. 1984), the burning temperature and other values associated with the collection of MSC in a constant draft apparatus operating at varying flow rates were determined. These data demonstrated that the total mass of smoke condensate produced, as well as the recovery of Δ^9-THC, decreases with increasing flow rate. The cannabinoid profile, however, remained relatively constant, and the burning temperature of the marijuana did not appear to be greatly dependent on flow rate. Also, the burning temperature of standard National Institute on Drug Abuse cigarettes being smoked by human subjects was measured. A range of burning temperatures of 730 to 800 °C was determined in these experiments.

Methods and Results

Selection of Marijuana Samples for Study. Several distinctly different types of marijuana are available on the illicit market and thus were potential subjects for study under this program. With respect to Δ^9-THC potency, these range from essentially nonpotent hemp-type marijuana, which is found on rare occasions, to the common Colombian or Mexican marijuana of 3 to 4 percent Δ^9-THC content, to extremely potent sinsemilla of Colombian, Hawaiian, or Californian origin containing 6 to 12 percent Δ^9-THC. Governed by the practical limitations of this program, we chose to study two of these types, producing MSC from them and carrying out biological testing and analysis as described later. Although a number of pairs could be chosen from among the types listed above, we felt that samples of Mexican marijuana containing, respectively, 1.3 percent Δ^9-THC (low dose) and 4.4 percent Δ^9-THC (high dose) represented the most practical choices, bracketing most marijuana available on the street in this country. For comparison, a typical cigarette tobacco was studied along with the two marijuana samples. This tobacco was obtained as reference cigarettes from the Tobacco and Health Research Institute (University of Kentucky, Lexington, KY).

Constant Draft and Intermittent Puff Smoking Systems. Realizing that there is no smoking system available that exactly duplicates the way humans smoke marijuana, we felt that the best approach to the production of MSC was to use two types of smoking systems: first, the constant draft apparatus to produce smoke condensate representative of the total smoke produced at the burning temperature of marijuana cigarettes being smoked by human subjects; and, second, intermittent puff smoking systems operating under the less rigorous tobacco smoking parameters, equivalent to the least stringent conditions under which marijuana might be smoked by humans. By using each extreme of actual smoke conditions, the evaluation of smoke condensates from these two systems could provide a qualitative and quantitative range within which the various components of the marijuana smoke actually experienced by human smokers might be found.

Cigarettes of 70 mm length were prepared from high-potency marijuana and low-porosity "street" cigarette papers with a manual cigarette-rolling machine. Low-potency marijuana and research tobacco cigarettes were machine rolled, cut to 70 mm length, and rerolled in low-porosity papers. Each of the three cigarette types was cornbusted under puff and constant draft modes on a 30-port cigarette smoking machine (figure 1) to produce six different smoke condensate types. The cigarette smoking was conducted at flow rates of 1,200 mL per minute for all constant draft combustion runs, 40 mL per 2-second puff (1 puff per minute) for puff mode combustion runs with marijuana, and 35 mL per 2-second puff (1 puff per minute) for puff mode combustion runs with tobacco cigarettes. Mainstream smoke was collected in a three-element trapping system consisting of an ethanol

bubbler, a packed sand trap, and an impinger trap arranged in series. The volume of each smoke condensate was reduced in *vacuo* at room temperature to less than 200 mL. At least 10 grams dry weight of each type of smoke condensate were produced. Six smoke condensates were thus produced: MSC-low potency by puff and by constant draft mode, MSC-high potency by puff and by constant draft, and TSC by puff and by constant draft.

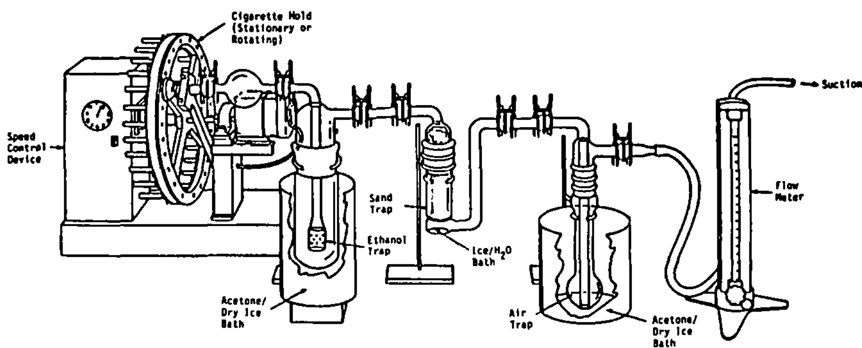

FIGURE 1. *Cigarette smoking machine for generation of smoke condensates*

FRACTIONATION OF SMOKE CONDENSATES

MSC and TSC are known to be highly complex matrices containing up to several thousand compounds at levels that may vary over several orders of magnitude (Gudzinowicz and Gudzinowicz 1980). Adequate characterization (chemical and biological) of these components requires prefractionation to (1) increase the relative mass of particular components and thereby the sensitivity of biological and analytical techniques for characterization, and (2) allow accurate assessment of biological and chemical characterization for significant constituents with minimum interference from other components in the matrix. The latter becomes a particularly important consideration with respect to the testing of mutagenic activity, since toxicity resulting from a few specific components in complex sample mixtures often becomes the limiting factor.

Methods and Results

Fractionation of smoke condensates (MSC and TSC) was accomplished using an acid-base liquid to liquid partition sequence, Similar sequences have been used in many studies of smoke condensates (Merli et al. 1980;

Merli et al. 1981) as part of the fractionation methodology due to the facile separation of major classes. Liquid-liquid partition provides a good means of dealing with the relatively large mass of crude smoke condensate that must he used to obtain adequate levels of individual constituents for later characterization.

The acid-base. partition scheme used in the fractionation of smoke condensates is shown schematically in figure 2. The procedure is based on that of Petersen et al. (1982) as applied to diesel soot extract.

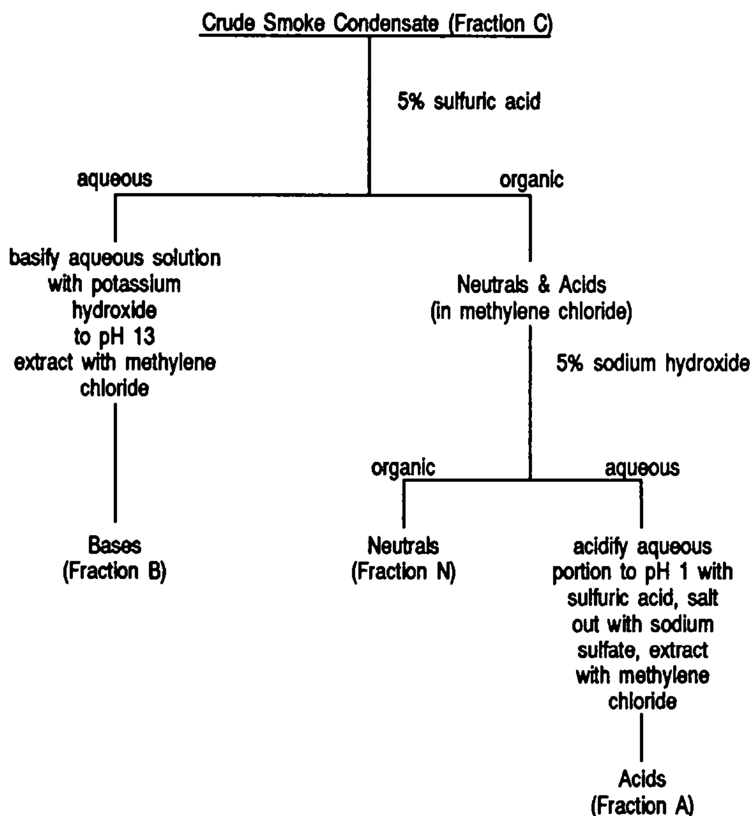

FIGURE 2. *Acid-base partition scheme*

In summary, the smoke condensates were taken to dryness and redissolved in methylene chloride (100 mL). Aliquots were removed for total mass determination. Portions of the condensate solution were then extracted in a

separatory funnel three times with 5 percent aqueous sulfuric acid. The three combined aqueous fractions were back-extracted once with 20 mL of methylene chloride. This methylene chloride was returned to the original smoke condensate solution.

To recover bases from the aqueous phases, the pH of the aqueous phase was adjusted to 13 with 40 percent aqueous potassium hydroxide. This aqueous phase was then extracted in a separatory funnel three times with methylene chloride. The base fraction (methylene chloride) was dried with sodium sulfate.

Next, acids were recovered from the original condensate solution (now in methylene chloride) by extraction with 5 percent aqueous sodium hydroxide. The combined aqueous base solutions were back-extracted with one aliquot of methylene chloride and the aliquot returned to the original methylene chloride smoke condensate. The acids were recovered by adjusting the pH of the sodium hydroxide solution to acidic values and extracting the solution with methylene chloride. Thus, methylene chloride solutions were obtained for the crudes (C), neutrals (N), acids (A), and bases (B). Aliquots were removed from all phases for total mass determination.

The neutral fraction (N) was further subfractionated by silica gel column chromatography. Prior to subfractionation, a portion of the neutral fraction was solventexchanged into hexane. A 20 g silica gel column was used. The neutral fraction was introduced at the top of the column and then eluted. Four eluting solvents were used to collect four fractions. Fraction 1 was eluted with 60 mL of hexane, fraction 2 with 100 mL of 1:l toluene and hexane, fraction 3 with 100 mL of methylene chloride, and fraction 4 with 200 mL of methanol. Each fraction was collected separately; further fractionation has not been conducted.

The base fractions (B) of the smoke condensates were further fractionated by normal phase, gradient HPLC. A cyanopropyl column with 5-micron spherical packing was used. Three solvent mixes were used:

Solvent A: 0.05 percent methanol/hexane
Solvent B: 0.05 percent methanol/methylene chloride
Solvent C: 20 percent methanol/methylene chloride

The gradient was as follows:

100 percent solvent A for 5 minutes
100 percent solvent A to 100 percent solvent B over 20 minutes
100 percent solvent B for 8.5 minutes
100 percent solvent C for 6 minutes

The flow rate was maintained at 4.0 mL per minute. Seven subfractions were collected based on the time elapsed from the start of the run. The cut points were selected arbitrarily, based on the appearance of the chromatogram. Fractions were collected as follows:

Fraction 1: 0 to 5 minutes
Fraction 2: 5 to 9 minutes
Fraction 3: 9 to 12 minutes
Fraction 4: 12 to 15.5 minutes
Fraction 5: 15.5 to 23.5 minutes
Fraction 6: 23.5 to 33 minutes
Fraction 7: 33 to 39 minutes

Multiple runs were performed and like fractions from each run were combined.

CHARACTERIZATION OF SMOKE CONDENSATES

Biological Characterization

The objectives of the bioassay testing were (1) to determine and compare the mutagenic activity of the chemical class fractions from TSC and MSC in the Ames *Salmonella* mutagenicity assay (Maron and Ames 1983), (2) to determine the effect of smoking mode (puff vs. constant draft) and marijuana batch on mutagenicity, and (3) to identify the most mutagenic fractions so that effective analytical chemical characterization techniques could be developed for identification of mutagenic and carcinogenic compounds.

The mutagenic potential of the various fractions was evaluated using the preincubation modification of the standard plate incorporation technique of the Ames *Salmonella* assay. Fractions were tested for mutagenicity under the following conditions: *Salmonella typhimurium* strains TA98 and TAlOO at five dose levels (10 to 2.50 µg per plate), triplicate plates per dose, with and without Aroclor 1254-induced hamster liver S9. Preincubation was performed for 20 minutes at 37 °C with shaking.

The following fractions were tested: crude (C), neutral (N) acid (A), and base (B) fractions of the six smoke condensates, and the seven subfractions (base fractions 1 to 7) of the base fraction of the high-dose MSC generated by constant draft (see above). Appropriate solvent and negative controls were conducted concurrently with each ‘assay. Bioassay results were analyzed for mutagenic activity by slope analysis modeling. Slopes are reported as revertants per µg of sample. Slope values were given a ranking for mutagenic activity based on slope analysis of an historical data base of mutagens and nonmutagens (table 1). The higher the slope value, the more mutagenic the fraction was. Slope analysis for crude and chemical class

TABLE 1. *Ranking of slope values from mutagenic fractions*

Slope* (Revertants/µg)	Mutagenic Ranking in Ames System
0.01 to 0.1	negative/equivocal
0.1 to 1.0	weak
1.0 to 5.0	moderate
above 5.0	strong

* Nonlinear regression analysis of Myers et al. (1981).

fractions (A, B, N) of marijuana and tobacco smoke condensates is provided in table 2. Slope analysis of HPLC subfractions of the basic fraction is shown in table 3.

Chemical Characterization

Mass Determinations. Dry weight determinations were made on aliquots of the various chemical class fractions and HPLC fractions of the crude condensates. All weight determinations were made in triplicate by withdrawing 100 µL aliquots from the previously measured total volume of the fraction in solvent. These aliquots were placed in a tared container and taken to dryness in a drying oven (50 “C). Each container was alternately weighed and dried until a constant weight was obtained.

Table 4 shows the mass distribution obtained from the chemical class fractions (A, B, N) of the crude condensates. Most of the material was found in the neutral fraction.

Table 5 shows the mass distribution of the HPLC fractions of the base fraction of three of the crude condensates. As can be seen from the table, the bulk of the material was found in the last three fractions.

Thermogravimetric Analysis. The base fractions of the crude smoke condensates were subjected to thermogravimetric analysis. In this technique, the weight of a sample is continuously recorded while the sample is heated using a specific temperature program. The rate and extent of physical and chemical changes that are accompanied by an increase or decrease in mass can be determined in this manner. Weight losses can be attributed to drying, volatilization, or combustion. Weight losses occurring below 100 °C are generally due to drying.

Samples of 20 to 50 mg were heated, from ambient temperature, at 2 °C per minute to approximately 600 °C at atmospheric pressure. A

TABLE 2. *Slope analysis of chemical class fractions from tobacco and marijuana smoke condensate in the Ames Assay*

Fraction	Slope (Revertants/μg) TAlOO	TA98	Mutagenic Ranking*
TSC-Puff-Crude	1.39	0.66	weak
TSC-Puff-Neutrals	0.03	0.17	weak
TSC-Puff-Acids	0.00	0.31	weak
TSC-Puff-Bases	0.00	0.92	weak
TSC-Constant-Crude	0.14	0.53	weak
TSC-Constant-Neutrals	0.68	0.11	weak
TSC-Ccnstant-Acids	0.04	0.08	equivocal
TSC-Ccnstant-Bases	1.16	2.08	moderate
MSC-Low-Puff-Crude	0.16		weak
MSC-Low-Puff-Neutrals	0.21	A E	weak
MSG-Low-Puff-Acids	0.12	0.23	weak
MSG-Low-Puff-Bases	0.19	0.89,	weak
MSG-Low-Constant-Crude	0.17	0.25	weak
MSG-Low-Constant-Neutrals	0.52	0.09	equivocal
MSG-Low-Constant-Acids	0.10	0.09	equivocal
MSG-Low-Constant-Bases	1.54	3.79	moderate
MSG-High-Puff-Crude	0.28	138	moderate
MSG-High-Puff-Neutrals	0.15	0.36	weak
MSG-High-Puff-Acids	0.00	0.46	weak
MSG-High-Puff-Bases	1.92	7.04	strong
MSG-High-Constant-Crude	0.11	0.60	weak
MSC-High-Constant-Neutrals	0.14	0.61	weak
MSG-High-Constant-Acids	0.05	0.18	weak
MSG-High-Constant-Bases	1.54	7.16	strong

*see table 1.

KEY: TSC=tobacco smoke condensate.
MSC=marijuana smoke condensate.
Puff/Constant smoking mode used in condensate generation.

NOTE: Salmonella typhimurium TA98 and TAl00 with Aroclor-induced hamster liver S9, five dose levels, triplicate plates per dose. preincubation with shaking for 20 minutes at 37 °C

thermogravimetric analyzer coupled to a data analyzer was used. A flow of 50 mL per minute of inert gas was maintained through the sample chamber throughout the test.

TABLE 3. *Slope analysis of HPLC subfractions of base fractions from tobacco and marijuana smoke condensate in the Ames assay*

Fraction	Slope (Revertants/µg)
TSC-Constant-Base Fraction 1	0.75
TSC-Constant-Base Fraction 2	0.84
TSC-Constant-Base Fraction 3	0.37
TSC-Constant-Base Fraction 4	1.28
TSC-Constant-Base Fraction 5	4.81
TSC-Constant-Base Fraction 6	1.95
TSC-Constant-Base Fraction 7	4.00
MSC-Puff-Base Fraction 1	1.89
MSC-Puff-Base Fraction 2	3.36
MSC-Puff-Base Fraction 3	
MSC-Puff-Base Fraction 4	12.89
MSC-Puff-Base Fraction 5	14.98
MSC-Puff-Base Fraction 6	16.48
MSC-Puff-Base Fraction 7	18.68
MSC-Constant-Base Fraction 1	1.01
MSC-Constant-Base Fraction 2	3.52
MSC-Constant-Base Fraction 3	4.04
MSC-Constant-Base Fraction 4	9.68
MSC-Constant-Base Fraction 5	13.38
MSC-Constant-Base Fraction 6	14.23
MSC-Constant-Base Fraction 7	11.43

KEY: TSC=tobacco, smoke, and condensate.
MSC=marijuana, smoke, condensate, high dose.
Puff vs. Constant=smoking mode used in condensate generation.

NOTE: Salmonella typhimurium TA98 with Aroclor-induced hams&r lives S9, five dose levels, triplicate plates per dose, preincubation with shaking for 20 minutes at 37 °C.

Table 6 shows the results from this experiment. Minor weight losses due to drying were observed in all samples. Most of the sample material was volatilized at temperatures below 300 °C. This indicates that the bulk of the material is amenable to gas chromatography (GC) or gas chromatography/mass spectrometry (GC/MS) analysis.

TABLE 4. *Weight percents of chemical class fractions*

Condensate	Fraction (Percent by Weight)				Total Recovery
	Acid	Base	Neutral	Insoluble	
MSC-Low-Puff	5.5	7.2	38.0	2.9	54
MSC-Low-Constant	9.5	4.0	22.5	9.5	47
MSC-High-Puff	5.8	5.7	48.8	2.3	63
MSC-High-Constant	6 3	6.9	51.7	2.5	67
TSC-Puff	7.4	11.9	54.7	5.7	80
TSC-Constant Draft	8.2	6.6	34.9	7.3	51

KEY: MSC=marijuana smoke condensate.
TSC=tobacco smoke condensate.
Insoluble=emulsion formed during solvent partition.
Puff vs. Constant=smoking mode used in condensate generation.

TABLE 5. *Weight percents of HPLC fractions of bases*

Condensate	HPLC Fraction	Percent by Weight
MSC-Low-Puff	1	5.2
	2	2.6
	3	5.6
	4	8.4
	5	25.4
	6	13.1
	7	39.6
MSC-Low-Constant	1	9.7
	2	2.3
	3	5.0
	4	9.9
	5	31.1
	6	10.7
	7	31 3
TSC-Constant	1	30.0
	2	3.5
	3	12.2
	4	9.9
	5	16.9
	6	8.4
	7	19.1

KEY: MSC=marijuana smoke condensate.
TSC=tobacco smoke condensate.
Puff vs. Constant=smoking mode used in condensate generation.

TABLE 6. *Thermogravimetric analysis of marijuana and tobacco smoke condensate bases*

Condensate	Percent Volatile (°C)								
	70	110	150	190	230	270	310	350	600
MSC-Low-Puff	a	53	66	71	75	78	79	80	82
MSC-Low-Constant	3	13	22	37	51	63	71	75	83
MSG-High-Puff	2	10	25	44	59	68	74	18	82
MSC-High-Constant	5	14	26	44	62	76	135	89	94
TSC-PUff	3	7	30	68	79	86	91	93	97
TSC-Constant		11	37	66	76	84	87	90	93

KEY: MSC=Marijuana smoke condensate.
TSC=Tobacco smoke condensate.
Puff vs. Constant=smoking mode used in condensate generation.

Elemental Analysis. All crude smoke condensates were submitted to Galbraith Laboratories, Inc., Knoxville, TN, for elemental analysis. Elements determined were carbon, hydrogen, nitrogen, sulfur, and oxygen. Results are presented in table 7 as percent of total. No significant differences were found between any of the condensates analyzed. The atomic carbon/hydrogen ratios for all samples were low (~0.6), indicating a "paraffinic" sample, rather than an "aromatic" one (C/H ratios for aromatic systems are typically greater than 1.0).

TABLE 7. *Elemental analysis results*

Condensate	%C	%H	%N	%S	%O
MSC-High-Puff	6653	8.43	6.48	033	17.84
MSC-High-Constant	64.92	8.71	6.82	0.40	18.42
MSC-Low-Puff	63.18	9.14	4.17	0.26	20.65
MSC-Low-Constant	61.78	8.37	4.29	031	23.77
Tobacco-Puff	63.11	8.33	4.70	0.25	19.48
Tobacco-Constant	63.70	854	4.46	0.18	18.74

KEY: MSC=Marijuana smoke condensate.
TSC=Tobacco smoke condensate.
Puff vs. Constant=smoking mode used in condensate generation.

Gas Chromatography/Mass Spectrometry of Base HPLC Fractions. The base fraction of high-dose marijuana smoke condensate (constant draft) was analyzed by GC/MS using electron impact ionization. A 60 m DB-1701

capillary column was used with the following program: 40 °C for 5 minutes, then 2 °C per minute to 265 °C, then 265 °C for 5 minutes. The source temperature was held at 180 °C. A skilled MS interpreter analyzed the resulting spectra and identified the compounds listed in table 8. The table also shows the HPLC fraction in which those compounds appeared.

TABLE 8. *GC/MS compound identification for HPLC fractions 2 to 7 for the base fraction of MSC batch B (constant draft)*

Compound Identification	Fraction
propionamide	6
butyroamide	5,7
cyclopentadiene	3
phenol	3,4
methylpyrazine	3,4,5
1-methyl imidazole	5,6
2-vinyl pyridine	7
dimethyltrisulfide	7
3,3dimethyloxetase	3
3,3-dimethylcyclobutanecarbonitrile	
methyl ethyl pyrrole	2,3
dimethylpiperazine	
1,2-dimethylimidazole	6,7
alkyl amide	
N-methyl-2-pyridinamine	3,4,5
1,3,5-trimethylpytazole	
dimethylethylpyrrole	2,3,7
valeramide	5,7
methyl pyrimidine	6
3-acetylpyridine	3
2-methoxy-3-methylpyrazine or $C_6H_8N_2O$	6
dimethylethanamine imidazole	6,697
tropolone	4
nitropicoline	3,4
C_7 alkyl amine	3
dimethylphenol	4
G_3-alkyl pyrazole	6
dimethylpyrimidone	4,6,7
methyl acetyl pyrrole	
1,4-benzoquinone	2,3
$C_9H_{14}O$ isomer	4,5,7
alkyl amide	5
m-aminophenol	5

NOTE: Relatively high levels of compounds are indicated by repeated fraction numbers.

TABLE 8. (continued)

Compound Identification	Fraction
$C_8H_{12}O$ isomer	5
C_3-alkyl pyrazole	6
1H-indazole, 4,5,6,7-tetrahydro or ($C_7H_{10}N_2$) isomer	3,5,6
1-butoxy-2-propanol	3
methylpropionyl furan	4,5
benzimidazole	3,4
$C_7H_6N_2$ isomer	4,4
3-methyl-5-triazolo (4,3-a)pyrazine	2
N-(a-picolidene)-n-propylamine	3
5-hydroxyindole	2
C_8-alkyl amine	3
quinoline	2
dimethyl tetrazine	7
C_4-alkyl pyrazole isomer	4,6,6,6,7,7,7
C_9-alkyl amine	5,7
C_5-alkyl pyrazole isomer	5,6,6,6,6,6,7,7,7
3-methyl-4-ethylpyrrole	4
$C_9H_{12}O$	4
$C_9H_{14}O$	5,7
$C_{10}H_{14}O$	4
$C_8H_{12}O$	7
$C_8H_{10}O$	4
methyl ethyl pyrazine	5,7
phenoxy ethanol	3,4,5
nicotine	4,5
aminobenzamide	4
phenyl urea	4
methyl benzimidazole	2,2,3,3,4,4,6
methylthiopyridine	7
methylquinoline or C_{10}HgN isomer	2,2,2,2,6
C_6,-alkyl pyrazole	6
dimethylbenzimidazole or ethylbenzimidazole isomer	2,2,2,2,3,3,3,4,4,4,6,6
methoxybenzaldehyde	3,3,6
4-methyl carbostyril	2
C_4,-alkyl pyrazine or $C_8H_{12}N_2$	5,6,6,6,6,7,7,7,7
($C_{10}H_{14}O_2$) propyl methoxy phenol isomer	5,5
$C_nH_{2n+1}OH$ (alcohol)	5
3-methyl-l,8-naphthyridine or isomer	2
pyridine carboxylic acid, methyl	3,4
benzoic acid, 3-methyl	3

NOTE: Relatively high levels of compounds are indicated by repeated fraction numbers.

TABLE 8. (continued)

Compound Identification	Fraction
phenyl pyrazoline	4
3,4-dimethylbenzoic acid	3
benzyl acetate	4
$C_{10}H_{16}O$	7
1,2-dihydro-3-isobutyl-l-methylpyrazin-2-one	3,5
ethyl hydroxy acetophenone	4
2,4-dimethylquinazoline	2,3
phenyl methyl urea	3
phenyl pyridine	5 5 3
propylbenzimidazole	4
aminoquinoline or $C_9H_8N_2$,	3
dimethyl naphthyridine	4
N-phenyl acrylamide	3
methoxy propyl pyrazine	4,5
phenyl alcohol	3
ethoxy benzaldehyde	4,5
tolyl azide	3
phenyl methyl quanidine	5
C_6,-alkyl phenol	3,4
C_3,-alkyl benzimidazole	4
1-decanol	4,5,6,7
C_5,-alkyl pyrazine	7
alkyl amide	5,6,6
dimethylbenzimidazone isomer	5
trimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine	7
dimethoxybenzene isomer	5
aminodimethylpyrimidine	7
hydroxymethylquinoline	7
methylbenzoxazole	7,7
tert-butyl-parahydroxybenzoate	4
$C_{10}H_{12}0_2$ (ester)	7
methyl-n(pyrid-2-yl)dihydropyrrole	3
$C_{12}H_{18}O$	3,4
methylaminonaphthyridine	3
diphenylamine	2,2,2,3
$C_9H_{10}O_3$	4,5
tert-butylparahydroxybenzoate	5
ethoxyquinazoline or isomer	3
diethylphenylene diamine	7
$C_5H_5N_5$ isomer	4
N,N-dimethyl-N-(p-methoxyphenyl) formamide	4

NOTE: Relatively high levels of compounds are indicated by repeated fraction numbers.

TABLE 8. (continued)

Compound Identification	Fraction
nitroacetanilide	4
2,2,4-trimethylpenta-1,3-diol di-isobutyrate	2,3,4,5,6,7
$C_{11}H_{16}O$ (alcohol)	3,5,7
($C_{12}H_{20}N_2$) N,N'-dimethyl-N,N'-diethyl-p-phenylene diamine	3,4
dimethylbenzimidazole	6
diethyl biphenyl	2
N-benzy 1-4-aminobutyronitrile ($C_{11}H_{14}N_2$)	2
N-methyl diphenylamine	2
1-undecanol	2,3,4,5,6,7
dimethylnaphthyridine or $C_{10}H_{10}N_2$, isomer	6,6
trimethylnaphthyridine or $C_{11}H_{12}N_2$ isomer	4,5,5,6,6
alkyl amide	4
hexanenitrile 3(pyrrolidinylmethylene) or ($C_{11}H_{18}N_2$) isomers	3,494
aminodiphenylene oxide	4
methyl-pteridinone isomer	5
alkyl nitrile	4
alcohol	5,6
2-(propylamino)benzothiazole	4
$C_{13}H_{22}N_2$ isomer	3,3
phenyl benzothiazole	4
aminomethylquinoline	4
tetramethylcyclopentane dione	5
1-methyl-dihydro-ß-carboline	2
alkyl amine	4,6,6,6
alkylthiopyridine	6
lystrin	3
N,N-dicyano-4-methylphenylene diamine	4
alkyl thiopyridine	7
7,8-benzoquinoline	2
tetramethylcyclopentanedione	4
5,5-diphenylimidazolid-4-one or $C_{15}H_{14}ON_2$	2
1-methylphenazine	2
aliphatic alcohol (n-dodecanol?)	5,6,7
alkyl amide	5,5,7
alkyl amine	7
methyl palmitate	7
dimethylnaphtho (2,3,6-) thiophene	4
homologous aliphatic alcohol (n-tridecanol?)	7
1-methyl-ß-carboline	3,4,5,6

NOTE: Relatively high levels of compounds are indicated by repeated fraction number.

TABLE 8. (continued)

Compound Identification	Fraction
ß-carboline	5,6,7
p-cumyl phenol	3
dibutyl phthalate	2,3,7
benzyl acetophenone	3
homologous aliphatic alcohol (n-tetradecanol?)	6,7
diphenylpyridine isomer	2
alkyl ester	2
dihydroxymethyl phenyl quinazoline	4
ditolyl ethane	3
1-azido naphthalene	6
1-phenyl decane	6
dimethyl-ß-carboline isomer	5,5,6
alkyl amide	6
phenylbenzimidazole	3
2,6-diterbutylnaphthalene or isomer	3
$C_{14}H_{10}O_3$ isomer	3,4
homologous aliphatic alcohol (n-tetradecanol?)	3,5,6,7
methylthiazolopyrimidine	2,3
8-acetoxy-pyrazolobenzo-as-triazine or $C_{11}H_8N_2O_4$	2,3,6
methyl stearate	3,7
methyl phenyl cinnoline or $C_{15}H_{12}N_2$, isomer	3
2-thiocyanatodiphenylamine	4
methylpyriloindole	4
alcohol (n-pentadecanol?)	5,6
naptho-sydinone	6
n -hexadecanol	6
n-$C_{22}H_{46}$ (docosane)	3,5,7
alkyl amine	5,7
$C_{12}H_{10}N_2O_4$ isomer	3,3,4
methylphenyl quinoxaline or $C_{15}H_{12}N_2$	3
n-$C_{23}H_{48}$, tricosane	3,4,5,6,7
homologous aliphatic alcohol (n-heptadecanol?)	5
n-$C_{24}H_{50}$ (tetracosane)	3,4,5,6,7
DL-cannabichromene	2,3
n-$C_{25}H_{52}$ (pentacosane)	3,4,5,6,7
3-*n*-pentyl-delta-9-tetrahydrocannabinol	2,3,7
dioctyl phthalate	3,4,5,6,7
n-$C_{26}H_{54}$ (hexacosane)	4,5,7
3-*n*-pentyl cannabinol	2,3,4,6,7
n-$C_{27}H_{56}$ (heptacosane)	4,5,6,7
alkyl amide	3,4,5,6,7

NOTE: Relatively high levels of compounds are indicated by repeated fraction numbers.

TABLE 8. (continued)

Compound Identification	Fraction
n-$C_{28}H_{58}$ (octacosane)	3,4,5,7
saturated hydrocarbon	5,5
n-$C_{29}H_{60}$ (nonacosane)	2,5,7
alkyl phthalate	6
saturated hydrocarbons	5,5,6
n-$C_{30}H_{62}$	4,5,6

NOTE: Relatively high levels of compounds are indicated by repeated fraction numbers.

CONCLUSION

Bioassay results showed that (1) the crude fractions were weakly mutagenic, (2) the acidic fractions were not significantly mutagenic, (3) the neutral fractions were weakly mutagenic, (4) the base fractions were significantly mutagenic, (5) the marijuana high-dose base fraction was sevenfold more mutagenic than either the tobacco or the low-dose marijuana base fraction, (6) constant draft base fractions were more mutagenic than puff mode basic fractions for both marijuana and tobacco, and (7) the more polar subfractions (numbers 4 to 7) of the base fraction were more mutagenic than the less polar subfractions.

Thermogravimetric analysis was performed on the base fractions of the crude smoke condensates to provide an indication of the applicability of GC or GC/MS analysis to these fractions. Most of the material was found to volatilize at temperatures below 300 “C, indicating GC as an appropriate technique for analysis of these fractions.

Elemental analysis of the crude smoke condensates for carbon, hydrogen, nitrogen, sulfur, and oxygen revealed no significant differences between the various condensates. The carbon/hydrogen ratios indicated that the crude samples were “nonaromatic.”

Approximately 200 compounds were tentatively identified by mass spectrometry. Of this total, about half were amines, with about half of these being aromatic amines. Pyrazines, pyrimidines, pyrroles, pyridines, and isoxazoles were the predominant compound classes. Some alkylated pyrazoles and pyrazines were detected in very large amounts, as was an alkylated benzimidazole. Some THC derivatives were also detected. It should be noted that the mutagenicity of the vast majority of these compounds has not been determined.

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Pyrolysis and Inhalation Studies With Phencyclidine and Cocaine

Billy R. Martin and Joseph Boni

INTRODUCTION

Smoking has been the major mode of use for many substances (including tobacco and marijuana) for centuries. In the past decade, the list of substances has been enlarged to include phencyclidine (PCP) and cocaine free base. A particularly important reason for the surge in smoking of these substances is the rapid absorption that occurs with inhalation, which results in a rapid onset of behavioral effects that are deemed pleasurable. In the case of PCP, it has been suggested that this rapid onset of effects allows the user to titrate his drug and therefore minimize serious side effects. However, smoking or inhalation of abused substances creates a spectrum of problems from minor behavioral problems to death. Despite the fact that many substances are abused either by smoking or inhalation, they are frequently administered by other routes of administration when they are characterized pharmacologically. Obviously, smoking or inhalation studies are fraught with difficulties that can be avoided by simply injecting the drug. The question then arises as to what factors must be considered when extrapolating from the drug injection studies to the human abuse situation.

It is essential to understand the fate of abused substances during the smoking process as well as the absorption, biodisposition, and metabolism of these substances by the inhalation route. Moreover, we must be able to determine whether the pharmacological effects of the drugs are quantitatively and qualitatively similar after different routes of administration. Our strategy has been to develop an apparatus and procedures to (1) produce smoke or drug vapor in a way that mimics the drug abuser's behavior, and (2) expose animals to this smoke or vapor in sufficient quantities to produce a pharmacological effect. Initially, we determine the extent of pyrolysis that occurs and identify the pyrolysis products. Then, conditions are established for exposing animals to the smoke or vapor, followed by biodisposition studies of the parent compound and pyrolysis products. Finally, behavioral studies are conducted to assess the correlation with the biodisposition

studies. Difficulties that are unique for each drug arise when conducting inhalation and smoking studies, although some of the problems are common to all drug studies. One common difficulty is establishing dosimetry in inhalation and smoking experiments. This difficulty can be overcome by employing radiolabeled drug and subsequent quantitation of total body radioactivity in the animal after drug exposure.

PYROLYSIS OF PHENCYCLIDINE

PCP has been abused by several routes of administration, which include smoking, snorting, oral ingestion, and intravenous (IV) injection. However, it is thought that smoking became very popular because this route permitted a precise control of dosage and thus reduced the probability of overdose. PCP is frequently applied to parsley or marijuana dry leaf material to be smoked, possibly the most common method of abuse. The quantity of drug present in mainstream smoke and pyrolysis products is an important factor in determining pharmacological effects. That PCP was heat labile was shown by the earlier work of Lin and colleagues (1975), who reported the formation of 1-phenyl-1-cyclohexene when PCP was analyzed by gas-liquid chromatography. Given the fact that the tip of a burning cigarette can reach temperatures of 600 it °C was predicted that PCP would be extensively pyrolyzed when smoked.

To investigate the pyrolysis of PCP, an alcoholic solution of ^{3}H-PCP-HCI (radiolabeled in the phenyl ring) was injected into cigarettes made of commercially processed parsley leaves, along the longitudinal axis (Freeman and Martin 1981). The ethanol was allowed to evaporate by air drying at room temperature. The cigarettes, which contained 50 mg of ^{3}H-PCP-HCl in most experiments, were smoked in an apparatus designed to mimic human smoking conditions. The apparatus, depicted in figure 1, consisted of a cigarette holder connected to a series of traps to collect the constituents of the mainstream smoke. Air was drawn through the entire apparatus by a water aspirator or vacuum pump. In addition, puffing was simulated by a valve that opened and closed the system at designated intervals. Air flowed only when the valve was closed. An air flow regulator, located between the last trap and the switching valve, was set to deliver 45 ml of smoke during a 7-second puff duration. At the end of the pyrolysis experiment, the entire apparatus was disassembled and washed with methanol. Radio activity was quantitated in these washes of the apparatus and glass wool trap, as well as the liquid traps. A cigarette requires approximately 6 to 8 minutes to bum under these conditions. The major portion of radioactivity was collected on the glass wool trap, and only trace quantities of radioactivity were found in the cigarette butt and the remainder of the apparatus. The glass wool trap has been found to be the most effective means of collection of PCP and other drug vapors, including the organophosphates (Scimeca et al. 1985) and cocaine. Using liquid nitrogen traps and bubbling the vapor or smoke through methanol traps proved to be less

effective. Analysis of the glass wool traps by thin-layer chromatography and gas chromatography/mass spectrometry (GC/MS) revealed that the PCP was readily converted to 1-phenyl-1-cyclohexene (PC). Actually, comparable quantities of PCP and PC were delivered in mainstream smoke, and little material was lost in the side-stream smoke (Freeman and Martin 1981). It was assumed that each mole of PCP that was cleaved to form PC resulted in the formation of an equal quantity of piperidine. However, the volatile nature of piperidine and the fact that this portion of the PCP molecule was not radiolabeled made quantitation difficult. Therefore, studies were conducted with ^{3}H-PCP-HCl that was radiolabeled in the piperidine ring. Analysis by both thin-layer chromatography and GC/MS of the glass wool extracts revealed that only 6 percent of the starting material was recovered as ^{3}H-pipetidine. As in the previous experiments, ^{3}H-PCP and PC accounted for the major portion of the material, while almost 30 percent of the starting material could not be identified (Martin and Freeman 1983).

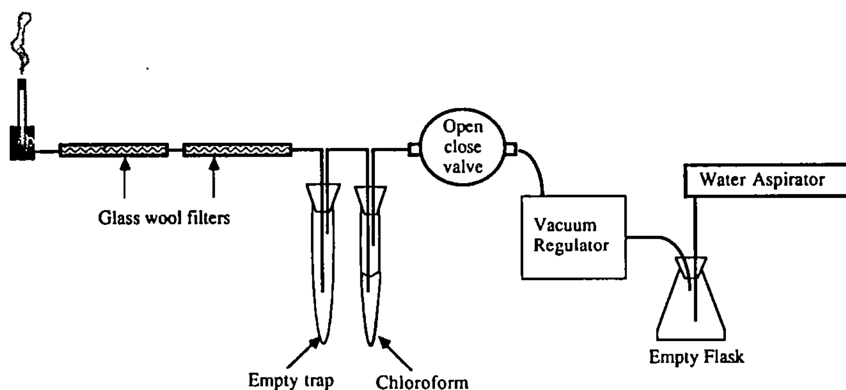

FIGURE 1. *First generation PCP pyrolysis apparatus*

One of the important questions was whether there was a constant delivery of PCP during the smoking of a cigarette through which PCP was evenly distributed. To assess the rate of delivery, the glass wool traps were changed as each third of the cigarette was smoked. It was clear from these studies that the major portion of the PCP was delivered during the smoking of the last third of the cigarette, which suggested that the PCP was condensing in the cigarette (Freeman and Martin 1981). Although we verified that the burning tip of the cigarettes reached temperatures in excess of 600 °C (Lue et al. 1986), it was thought that PCP was most likely being vaporized before the burning tip of the cigarette reached it (figure 2). Cook et al. (1985) had demonstrated that PCP was completely destroyed when

heated to 600 °C in a quartz oven. Therefore, it appeared that PCP was being volatilized at a temperature lower than that of the burning tip, since PCP is effectively delivered in mainstream smoke.

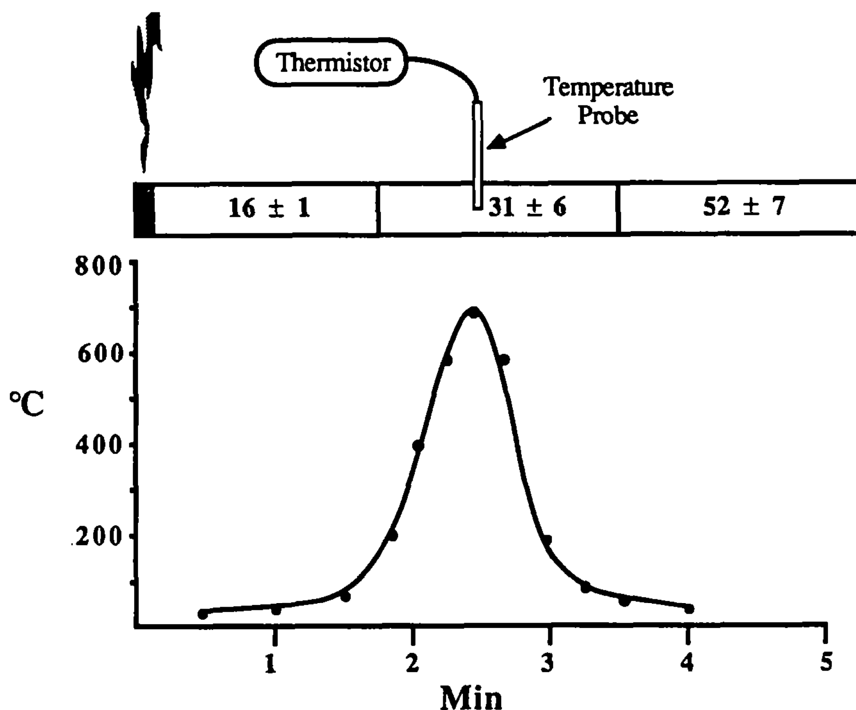

FIGURE 2. *Recovery of PCP from a burning cigarette*

In the above studies, the emphasis was on the delivery of PCP during the smoking process and the formation of PC Questions remained regarding the fate of the piperidine moiety. Beaver and Jones (1984) later reported that the pyrolysis of PCP in a quartz oven at high temperatures resulted in the formation of significant quantities of polynuclear aromatics, which included styrene, a-methyl styrene, naphthalene, 2-methyl naphthalene, l-methyl naphthalene, biphenyl, cyclohexylbenzene, acenaphthene, phenanthrene, and anthracene. The pyrolysis of PCP was evaluated further to determine whether these polynuclear aromatics, in addition to other pyrolysis products, were formed during smoking (Lue et al. 1936). For these studies, marijuana placebo cigarettes (easier to use than parsley cigarettes) were impregnated with 100 mg of PCP-HCI along with 0.5 to 5.0 µCi of phenyl-[^{3}H]PCP and piperidino-[^{3}H]PCP and burned under the conditions described

above. Mainstream smoke was passed through glass wool filters as well as through acidic and basic traps. Greater than 90 percent of the starting material could be accounted for, with the major portion (77 percent) in the first glass wool trap.

Methanolic extracts of the glass wool traps were analyzed by both high pressure liquid chromatography (HPLC) and GC/MS. For quantitative GC/MS analysis, concentration curves were obtained for 1, 5, 7.5, and 10 ng of each polynuclear aromatic standard using single-ion monitoring analysis. The limit of detectability for each of the standards was 0.5 ng per injection or 1 ug/10 ml of methanol extract. Because of the possibility that the polynuclear aromatics could be formed during smoking but might not be collected on the glass wool filters, control experiments were conducted in which the marijuana placebo cigarettes were treated with 4.8 mg of ^{3}H-PCP-HCl with and without 0.1 mg of each of the polynuclear aromatic standards. The methanol extracts of the glass wool filter condensates from these cigarettes were analyzed by GC/MS using single-ion monitoring. The recoveries of the polynuclear aromatic standards ranged from 33 to 100 percent. None of the polynuclear aromatics was detected in the condensates from cigarettes impregnated with ^{3}H-PCP-HCl alone. It appears that these compounds are formed from PCP only under conditions that do not mimic smoking.

In addition to single-ion monitoring analysis, total ion chromatograms were also obtained for all of the methanol extracts. Four peaks were observed in the condensates from the ^{3}H-PCP-HCl-spiked cigarettes that were not evident in those from the placebo cigarettes. The spectra and retention times of these compounds were coincident with those of PCP, l-phenyl-l-cyclohexene, piperidine, and N-acetylpiperidine, which represented 39, 46, 7, and 9 percent, respectively, of the starting material (figure 3). These results suggest that PCP is not exposed to temperatures during smoking that are high enough to produce polynuclear aromatics.

PYROLYSIS OF 1-(1-PIPERIDINO) CYCLOHEXANECARBONITRILE (PCC)

PCC is a major contaminant in illicit PCP because the most common clandestine synthesis of PCP employs PCC as the intermediate; the PCC is not always removed from the final product (Helisten and Shulgin 19776; Giles et al. 1977). The presence of PCC in PCP represents a potential health risk given the fact that it is considerably more toxic than PCP (Baker 1982). PCC is readily degraded to 1-(1-piperidino)-l-cyclohexene, piperidine, and cyclohexanone during either GC or thin-layer chromatogmphic (TLC) analysis or on standing in solution (Helisten and Shulgin 1976; Giles et al. 1977). As the stability of PCC during smoking had not been investigated, despite the potential contribution its pyrolysis products may make to the pharmacology and toxicology of illicit PCP, a study was conducted to

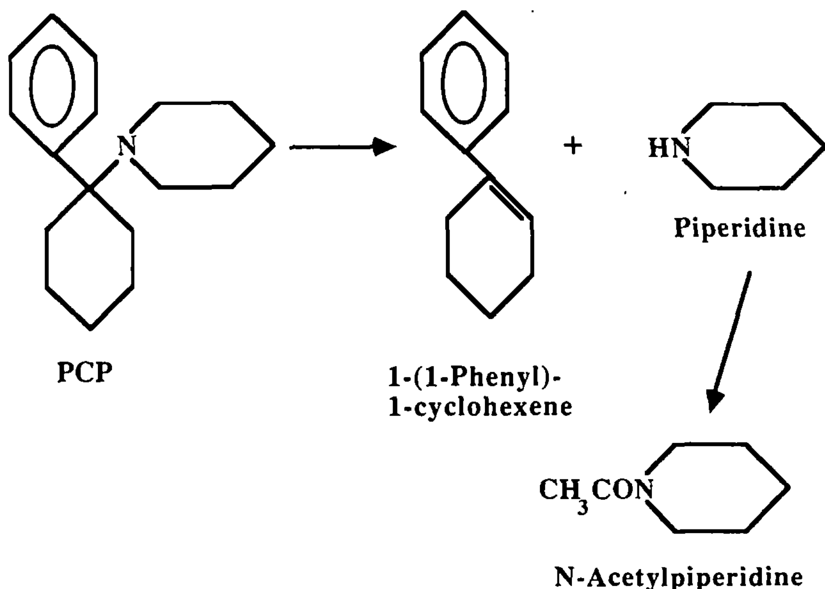

FIGURE 3. *Pyrolytic fate of PCP during smoking*

determine the quantity of PCC that is delivered in mainstream smoke and to identify and quantify pyrolysis products (Lue et al. 1988).

[^{3}H]Piperidino-[^{14}C]cyano-PCC was synthesized to facilitate the quantitation of PCC in mainstream smoke and the identification of pyrolysis products. Marijuana placebo cigarettes were impregnated with this double-labeled PCC and smoked with the apparatus depicted in figure 1. The mainstream smoke was passed through the glass wool, acidic, and basic traps. Approximately 75 percent of the tritium was collected in these traps, while 46, 11, and 5 percent of the starting ^{14}C was found in the glass wool, H_2SO_4 and NaOH traps, respectively. The constituents of the glass wool trap were analyzed by GC/MS, which revealed the presence of l-(l-piperidino)-1-cyclohexene, PCC, piperidine, and N-acetylpiperidine. Cyanide ion was detected in the glass wool, acidic, and basic traps by a titration procedure. It appeared that approximately 47 percent of the PCC was delivered intact in mainstream smoke. Approximately 58 percent was cleaved to form cyanide and 1-(1-piperidino)-1-cyclohexene. The latter was further broken down to cyclohexanone (representing 21 percent of the starting material), piperidine (29 percent), and N-acetylpiperidine (7 percent); about 2 percent remained intact. The entire pyrolytic profile is shown in figure 4.

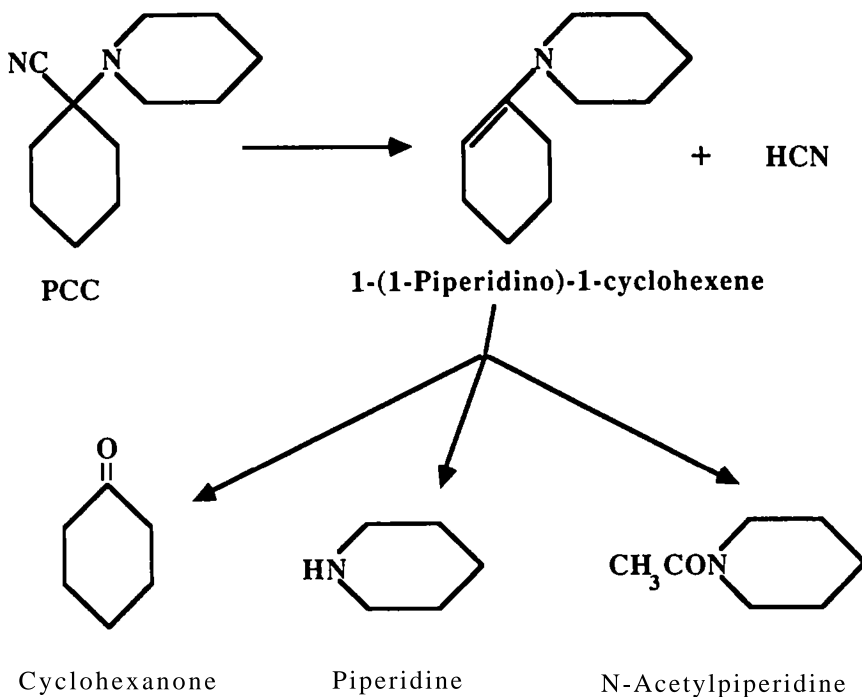

FIGURE 4. *Pyrolytic fate of PCC during smoking*

PYROLYSIS OF COCAINE

Although the abuse of cocaine has generated considerable attention in the past few years, cocaine has been used for religious, medicinal, and recreational purposes for thousands of years. Inhalation of burning coca leaves or smoking coca evolved gradually and remained major modes of medicinal and recreational use until enactment of the Pure Food and Drug Ad of 1906 and the Harrison Act of 1914, which curtailed the distribution and use of coca leaves as well as coca products. It is ironic that these laws probably contributed to the diversion of cocaine use from coca smoking to intranasal and IV administration of cocaine-HC1, because the latter product became more readily available. Cocaine-HCl has been smoked with marijuana, tobacco, or herbs either as a cigarette or in a pipe. The relatively modest effects that were derived from smoking the salt soon led to smoking coca paste (crude extract containing free alkaloids) and finally to "free base," which is cocaine-HCl that has been converted to the free base (Jeri et al. 1978; Siegel 1985). The physical properties of the different preparations have dictated their mode of use. Cocaine-HCl has a melting point of 195 °C, is not very volatile, and decomposes with heat, but it is highly water soluble, therefore readily absorbed via the mucous membranes. Conversely, cocaine free base has a melting point of 95 °C making it readily volatile. As a result of these properties, cocaine-HCl is usually administered IV or intranasally, whereas cocaine free base is volatilized and

absorbed through the large surface area of the lungs after inhalation. One of the greatest problems facing us, as summarized by Siegel (1985), is the emergence of new patterns of use, such as the inhalation or smoking of free base, which has increased the risks of dependency and toxicity.

The toxicity of cocaine depends upon several factors: the route of administration, dose, drug combinations, etc. (Cohen 1985). The general features of acute cocaine intoxication include extensive central nervous system (CNS) stimulation progressing to convulsions followed by cardiovascular and respiratory failure (Jones 1984). Although cocaine has long been considered a potentially dangerous drug of abuse, the popularity of free basing has led to the realization of these dangers. The recent upsurge of "crack" has increased the availability and decreased the cost of cocaine. Additionally catastrophic is the fact that "crack" is a rapidly addicting form of cocaine that easily results in lethal overdose. At present, the underlying factors for this increased toxicity resulting from free basing are unclear. The influence of route of administration on addictive potential and lethality of cocaine undoubtedly involves pharmacokinetic differences. However, there may be other contributory factors. For example, Pearman (1979) suggested that factors that increase the absorption of cocaine may be more important considerations for toxicity than simply overdose. Several investigators have found that the bioavailability and intensity of many cocaine effects are similar after oral and nasal cocaine (Wilkinson et al. 1980; Van Dyke et al. 1978; Jones 1984), while the profiles of cocaine effects are similar after IV and smoked routes. Bioavailability by the nasal route was found to be 30 to 40 percent (Jones 1984) and 80 percent (Cook et al. 1985) of that after IV. Biodegradation of cocaine (through oral (PO), IV, and intranasal routes) has been reported to form benzoylecgonine and ecgonine methyl ester (methylecgonine) (Lukaszewski and Jeffery 1980; Chinn et al. 1980; Wallace et al. 19776; Wallace et al. 1977; Jindal and Vestergaard 1978).

Although previously published studies have dealt with the pharmacological and pharmacodynamic properties of cocaine abuse, little attention has been paid to the volatilized products of free basing cocaine or crack (Gawin 1980; Isner et al. 1986; Novak and Salemink 1984). Some of the important aspects of cocaine inhalation include the purity of street cocaine, imprecision of dosing by variability in volatilization and pyrolysis of cocaine, smoking technique, rate of inhalation, naive vs. chronic use, target tissue, and underlying pathology. Previous studies have shown that cocaine can be thermally degraded to benzoic acid and methylecgonidine (Cook and Jeffcoat 1986; Cook et al. 1985) as well as methyl-4-(3-pytidinyl)butyrate (Novak and Salemink 1984). The species and quantities of volatilized products of cocaine formed under conditions simulating free basing have not been thoroughly investigated, however, despite their pharmacological and toxicological importance. Therefore, the present study was conducted to identity and quantify the delivery of cocaine and to investigate the formation of pyrolysis products during volatilization (Martin et al. 1989).

Protocol

The method of volatilizing ^{3}Hcocaine (levo-(-)-[benzoyl-3,4-^{3}H(N)]-cocaine) represented a modification of the procedure described above for PCP. A U-shaped pipe was constructed from glass tubing (6 mm inside diameter and 24 cm in length) and placed in a Lindberg Crucible Furnace filled with glass beads, as depicted in the bottom of figure 5. After the pipe had reached the desired temperature, 50 mg of cocaine base containing 1 uCi of [^{3}H-benzoyl]-cocaine was inserted into the pipe with the cocaine delivery system. Cocaine was placed between two glass wool plugs in a glass tube, which was inserted into the glass pipe at the start of the experiment. The cocaine and glass wool plugs were pushed out of the tubing and into the pipe. Vapor was drawn through the volatilization apparatus at 8-second puff intervals with intermittent 4-second rest periods (no flow). Cocaine and its pyrolysis products were recovered from the pipe with ethanol (10 ml), and they were extracted from the glass wool with three l0-ml portions of dichloromethane. The pH of the acid trap was adjusted to approximately 8.5 using sodium bicarbonate prior to extraction with three l0-ml portions of dichloromethane. The pH of the alkaline trap was adjusted to 4.5 to 5.0 prior to extraction with three l0-ml portions of dichloromethane. The multiple extractions were combined and concentrated to 10 ml under a gentle stream of nitrogen at room temperature. In some experiments, the vapor was sampled repetitively using l-ml air-tight syringes. The vapor was added to ethanol, and the ethanol was analyzed by liquid scintillation spectrometry and by GC/MS.

Air Flow Rate

The rate of air flow through the system had a dramatic effect on volatilization of the cocaine. At a flow rate of 100 ml per minute, only 7 percent of the starting material was recovered in the glass wool trap, and only trace quantities were found in the other traps after a 5-minute volatilization at 260 °C temperature. When the flow rate was increased to 400 ml per minute, approximately 40 percent of the radioactivity remained in the pipe and approximately 40 percent was recovered in the glass wool trap. As with the lower flow rate, only trace quantities of radioactivity were observed in the remaining traps. The contents in the pipe, glass wool, and traps were analyzed by mass spectrometry either by acquiring the total ion chromatogram or by selected-ion monitoring. Both procedures revealed the presence of only cocaine, methylecgonidine, and benzoic acid (figure 6). There was considerable pyrolysis of cocaine at the low flow rate, so only small quantities of cocaine remained in the pipe and in the glass wool trap. The major component was methylecgonidine in both the pipe and the glass wool. At the higher flow rate of 400 ml per minute, there was little pyrolysis, as evidenced by the fact that high concentrations of cocaine were found in the pipe as well as in the glass wool trap.

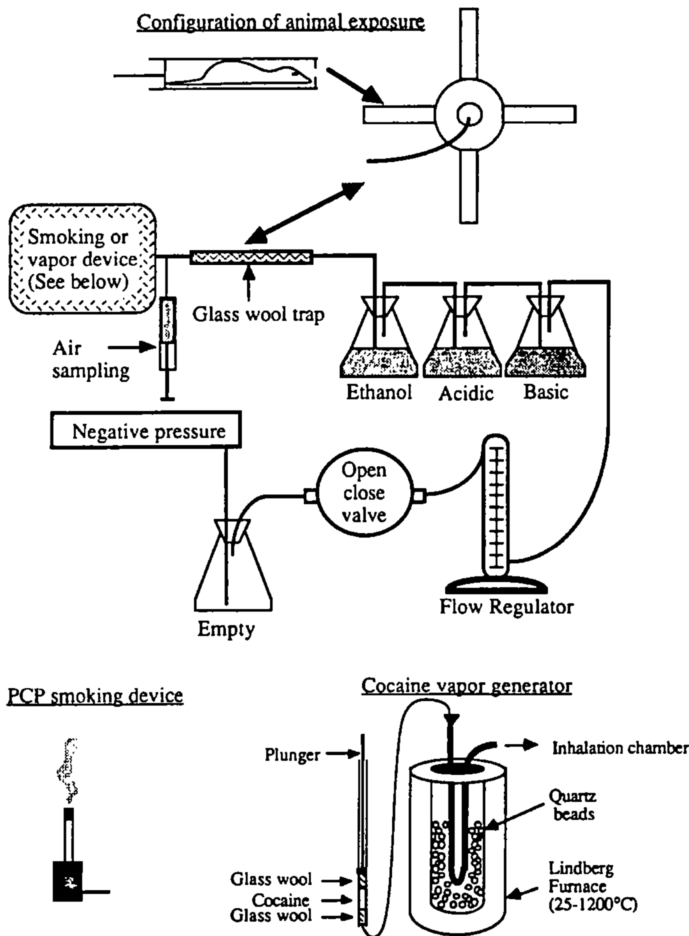

FIGURE 5. *Smoking-exposure apparatus for PCP and cocaine*

Temperature

The effect of temperature on pyrolysis was examined by maintaining the flow rate of 400 ml per minute during a IO-minute volatilization. Increasing the temperature from 260 °C to 400 °C produced a linear increase in the formation of methylecgonidine and benzoic acid. In the temperature range of 350 to 400 °C, approximately 50 percent of the cocaine was de graded, whereas only trace quantities of cocaine remained intact at 650 °C

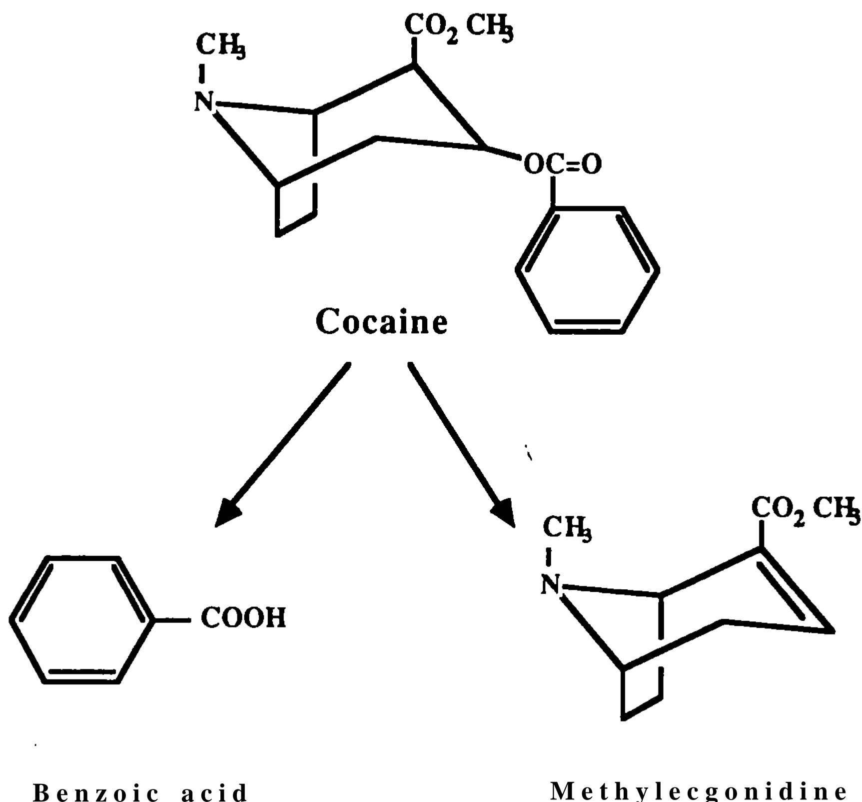

FIGURE 6. *Pyrolytic profile of cocaine*

Because pyrolysis and volatilization of cocaine are highly dependent upon the experimental condition, studies were conducted to determine optimal conditions for delivery of a constant concentration of cocaine for subsequent animal studies. The results, shown in figure 7, reveal the time course of radioactivity in the air stream as the air flow rate is altered. Most of the radioactivity is delivered in the first minute at a flow rate of 400 ml per minute, whereas the delivery period is extended to approximately 3 minutes when the flow rate is reduced to 100 ml per minute. In a separate series of experiments, the flow rate was held constant at 400 ml per minute, and the temperatures were varied between 200 and 260 °C. A relatively constant cocaine delivery of 5 minutes duration was obtained at 200 °C. At higher temperatures, the duration of a plateau phase was shortened to the point where maximal cocaine delivery was observed within a l-minute time frame (figure 8).

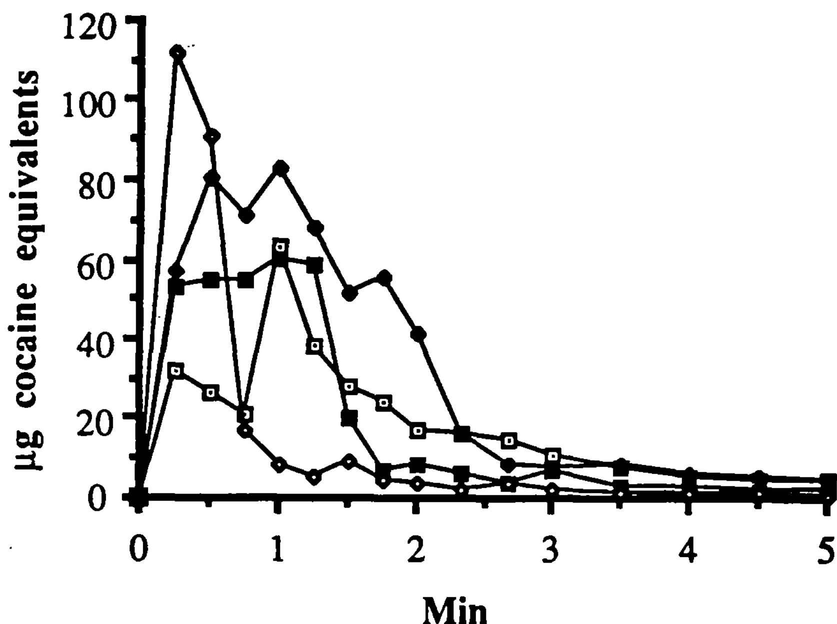

FIGURE 7. *The effect of flow rate on the delivery of W-cocaine*

NOTE The results are presented as means from three experiments in which the flow rates were 100 (□), 200 (◆), 300 (■), or 400 (◇) ml per minute.

It is clear that the experimental conditions are an important determinant in the volatilization of cocaine as well as in its degradation. The toxicity associated with inhalation of cocaine remains to be determined, but it may well depend upon the conditions under which cocaine is vaporized. It is important that future inhalation studies with either humans or animals be conducted under carefully controlled conditions, so that the delivery of cocaine, as well as the extent of degradation, can be determined (Snyder et al. 1988).

PHARMACOLOGICAL CONSEQUENCES OF INHALATION OF DRUGS OF ABUSE

One of the major concerns regarding the smoking of drugs of abuse is whether the pharmacological and toxicological properties of the drug are similar to those found after other routes of administration. One important aspect of smoking drugs is that the smoker is exposed to the parent compound as well as to pyrolysis products. In addition, the metabolism and biodisposition of the drugs may well be different following inhalation.

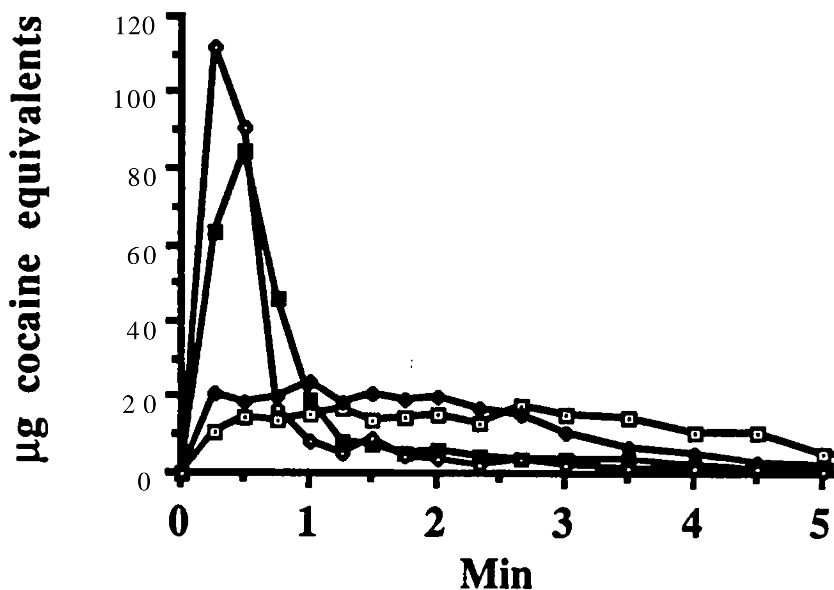

FIGURE 8. *The effect of temperature on the delivery of ^{3}H-cocaine*

NOTE: The results are presented as means from three experiments in which the themperatures were 200(□), 220(◆), 240(■), or 260 °C.

Studies conducted in our laboratories have shown that 1-^{3}H-pheny1-1-cyclohexene (^{3}H-PC), the major pyrolysis product of PCP, is metabolized extensively when incubated in vitro with crude microsomal membranes from mouse livers (Martin et al. 1982). The major routes of metabolism were allylic hydroxylation, oxidation of the allytic alcohol, and epoxidation-hydrolysis. The following metabolites were identified: l-phenyl-1-cyclohexen-3-o1 (major metabolite), 1-phenyl-1-cyclohexen-3-one (PC-3-one) (major), 1-phenyl-1-cyclohexen-6-o1 (minor), l-phenyl-l-cyclohexen-6-one (minor), and l-phenylcyclohexane-1,2-diol (PC-1,2-diol) (minor). Pharmacological evaluation of PC clearly showed that it produced less behavioral effect and was less toxic than PCP (Holsapple et al. 1982). The ED, and confidence limits for PCP in the inverted-screen test were 2.5 (1.6 to 2.9) umol/kg, with a duration of action of approximately 30 minutes after IV administration. PC's effects lasted less than 5 minutes after IV administration, and the ED, (confidence limits) was 325 (294 to 360) μmol/kg. The behavioral activity of PC-3-ol, PC-3-one, and PC-1,2-diol (PC-6-ol and PC-6-one were not synthesized in quantities sufficient for testing) also lasted less than 5 minutes (Martin et al. 1982). The diol was considerably less active than PC, and PC-3-o1 and PC-3-one were somewhat mote active than

PC. Nonetheless, all the metabolites were considerably less active than PCP and undoubtedly contributed little to the latter's behavioral activity.

To determine whether the behavioral properties of the drug are the same after smoking and other routes of administration, studies were carried out in our laboratories. Male Sprague-Dawley rats were trained to discriminate intraperitoneal (IP) injections of 3.0 mg/kg PCP from saline under a 2-lever fixed-ratio 32 schedule of food presentation (Wessinger et al. 1985). After reliable discriminative control of lever choice was established, a dose-dependent increase in PCP-lever selection and dose-dependent decreases in rates of responding were determined after IP injections. When doses of PCP were administered by exposure to smoke from cigarettes containing PCP, a dose-dependent increase in PCP-lever responding was also observed. Δ^9- Tetrahydrocannabinol (THC) administered via smoke exposure, up to doses that markedly suppressed response rates, did not result in PCP-appropriate responding, demonstrating the specificity of the PCP stimulus by the inhalation route. Brain levels and distribution of ^{3}H-PCP were determined in rats administered doses calculated to result in 50 percent generalization by the IP injection or smoke inhalation route. By both routes of administration, roughly equivalent brain levels were attained, and the distribution was relatively even across the seven brain areas analyzed.

CO-ABUSE OF MARIJUANA AND PCP

There is also the possibility of drug interactions by the smoking route. One obvious interaction is PCP and Δ^9-THC, since PCP is sometimes smoked in marijuana cigarettes. The behavioral and pharmacological interactions between Δ^9-THC and PCP were studied following coadministration of the drugs in smoke to mice (Freeman and Martin 1983). Although Δ^9-THC (25, 50, or 100 mg per cigarette) had little effect on spontaneous motor activity, all doses attenuated the hyperactivity elicited by PCP•HCL (25 and 50 mg per cigarette). Δ^9-THC produced a dose-related hypothermia, whereas PCP•HCL (50 mg per cigarette) had no effect on body temperature. However, PCP enhanced the hypothermia produced by smoke from cigarettes containing 25 mg of Δ^9-THC. Δ^9-THC (100 mg per cigarette) had no effect on the biodisposition of ^{3}H-PCP and its pyrolytic product, ^{3}H-phenylcyclohexene (^{3}H-PC), when examined immediately after ^{3}H-PCP•HCl (50 mg per cigarette) exposure. At 30 minutes, however, brain, liver, lung, and plasma contained higher concentrations of ‘H-PC, and fat and plasma contained lower concentrations of ^{3}H-PCP in the mice exposed to both drugs, compared to those that were exposed to ^{3}H-PCPmHCl alone. It appears, therefore, that Δ^9-THC has the potential for altering the behavioral, pharmacological, and pharmacokinetic sequelae of PCP abuse.

CO-ABUSE OF COCAINE AND PCP

Chitwood (1985) reported that most cocaine users are polydrug users who frequently use cocaine in conjunction with CNS depressants. There is also

co-abuse with heroin, commonly known as "speedball," which represents a highly toxic combination (Finkle and McCloskey 1978). There appears to be less co-abuse with stimulants. In one report, cocaine users reported simultaneous use of PCP and cocaine, albeit infrequently (Chitwood 1985). There have been recent reports in the media of intoxication with "space base," which is a combination of PCP and cocaine. The behavioral and pharmacological profiles of cocaine and PCP are similar in many respects, as discussed by Cunningham and Appel (1982) and Bagachi (1985). They also exert similar effects on the sympathomimetic and serotonergic systems, which may or may not suggest a common mechanism of action (Cunningham and Appel 1982). It is noteworthy that Colpaert et al. (1979) found cocaine and PCP to have similar discriminative properties. Cunningham and Appel (1982) found PCP to partially substitute for cocaine in cocaine-treated animals. More important, Thompson and Winsauer (1985) found that cocaine potentiates the disruptive effects of PCP on complex operant behavior in monkeys. Although the similarities and dissimilarities of cocaine and PCP remain to be established unequivocally, it is becoming evident that co-abuse of cocaine and PCP could have dire consequences. An understanding of the pharmacokinetics and pharmacological effects of combinations of these agents following inhalation would provide not only insight into the potential dangers of such co-abuse but a basis for devising treatment strategies.

CONCLUSION

Inhalation represents one of the most effective modes of self-administration of drugs that are relatively easily volatilized. In some cases, inhalation represents a relatively safe way to use some drugs, such as PCP, in that the user is better able to titrate the dosage to achieve a desired behavioral effect. Certainly fewer deaths are reported for smoking than for oral administration of PCP. Also, the user does not have to resort to IV injection to achieve a rapid onset. Nonetheless, inhalation presents the user with dangers that are not associated with other modes of administration. Usually the drug must be volatilized by heating, which can result in degradation of the drug to products that may be dangerous. Inhalation that is achieved by smoking leafy material results in the introduction of foreign material into the lungs, the health consequences of which are well documented. A major concern is that the pharmacological profile of the drug obtained after smoking or inhalation may be different from that produced after other routes of administration. There is no doubt that far greater addiction is produced when cocaine is smoked than when coca leaves are chewed. The greater pharmacological effects that are associated with inhalation of cocaine are accompanied by greater toxicity. It has been well established that smoking and inhalation represent effective means of self-administering PCP and cocaine. It remains to be determined whether drug users will attempt to use other drugs by this mode of administration.

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Animal Models of Drug Self-Administration by Smoking

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INTRODUCTION

Animal models of drug self-administration have made major contributions to understanding the behavioral and pharmacologic determinants of substance abuse practices. Intravenous (IV) administration has been the predominant route employed in such studies; the limited self-administration literature on organic solvents and volatile anesthetics demonstrates that the inhalation route is useful and practical. The literature on animal models of smoking is less persuasive because the experimental designs have been limited and because the technical challenges offered by these experiments can be substantial. Description of the dose delivered by smoking may be complex because of the number of compounds present and the differences in deposition and absorption associated with gases and particulates. The pattern of particulate deposition is species and particle-size dependent. Combustion products may be aversive because of eye or airway stimulation; behavior of the user may minimize aversive stimulation. In some species, the trigeminal reflex limits exposure to acutely aversive atmospheres. Combustion or pyrolysis need not occur if cocaine base is heated gently; the smoke observed under these conditions is a condensation aerosol that may not be aversive. Special generation techniques will be required to generate: (1) reliable exposure concentrations with stable particle size distributions; (2) test atmospheres of low irritancy that are appropriate for the species under study; and (3) exposures tailored specifically for studies of self-administration, behavioral and physiological effects, pharmacokinetics, or acute or chronic toxicity.

The chemicals subject to abuse by inhalation may be divided into two classes, those that are volatile at room temperature (inhalants) and those that require heat for self-administration, i.e., smoking. This chapter will examine the commonalities and differences between these substance abuse practices in a search for the major determinants of the process and for the implications for developing useful animal models of the process.

The inhalation route offers a number of advantages; only the simplest equipment is needed, no needles are required, and the rapid onset of effects is unmatched. A wide variety of chemicals are self-administered by inhalation. Inhalant abuse continues as an unrelenting substance abuse problem that is global in scope; organic solvents, fuels, gases, and volatile anesthetics continue to be abused to such an extent that the lifetime incidence of inhalant abuse among high school seniors ranks fifth behind alcohol, tobacco, marijuana, and stimulants. Smoked compounds include tobacco products (cigars, cigarettes, pipes), narcotics (opium, heroin), cannabis products (marijuana leaf, hashish, extracts), hallucinogens (dimethyltryptamine, the arylcyclohexylamines), and cocaine base (crack, freebase). A drug or chemical can be "smoked" if it displays substantial vapor pressure below temperatures at which pyrolysis occurs.

Forcible exposure to combustion products by chamber or tracheostomy are useful for some toxicologic purposes, but they yield no information on self-administration, and they may vary in their ability to provide adequate identification or characterization of the health consequences of smoking. The test atmospheres generated in such studies do not necessarily resemble the products generated by human smoking topographies, because the products of a burning cigarette are complexly determined, as are the properties of the aerosol (Davies 1988). In such studies, animals are exposed either continuously or during a fixed proportion of breaths, and the spectrum of toxic effects has been observed to differ from that in humans (Larson and Silvette 1971; Auerbach 1967; Hammond et al. 1970).

There are many anecdotal reports of animals that smoke. Darwin (1892) reports

> Many kinds of monkeys will, as I have myself seen, smoke tobacco with pleasure . . . The same tastes are common to some animals lower in the scale. Mr. A Nichols informs that he kept in Queensland in Australia three individuals of the Phaseolarstus cinereus [koala] that . . . acquired a strong taste for rum and smoking tobacco. (Darwin 1892, p. 7)

Tracheostomized dogs have been used extensively in studies of lung diseases induced by cigarette smoke, and the animals seem to adapt to these procedures quite well:

> After a few weeks, the [tracheostomized] dogs became habituated to cigarette smoking and seemed to enjoy it as indicated by tail wagging and jumping into the 'smoking box' voluntarily. Thereafter, the use of the pump was discontinued and the dogs voluntarily inhaled smoke by drawing on the cigarettes . . . (Hammond et al. 1970, p. 742)

> By and large as he becomes more used to the smoking, in humanoid fashion, he seems to relish the habit. (Cahan and Kirman 1968, p. 573)

The scientific literature on drug self-administration by smoking, however, is restricted and of limited value. There is no doubt that performances can be engendered that resemble human smoking topographies; monkeys can be trained to puff on cigarettes to obtain a liquid reinforcer, and this has been used as an exposure technique to study the various consequences of exposure to the test smoke atmosphere. Although these puffing performances can be characterized as responseproduced exposures, they are not necessarily demonstrations of self-administration, i.e., behavior maintained by the drug as a reinforcer. Few studies have conducted any of the control procedures necessary to document self-administration (Pickens and Thompson 1968; Wood 1979). These control procedures include

- acquisition of an arbitrary response with access to the agent as the reinforcer,
- decease in the frequency of the response when access to the purported reinforcer is discontinued;
- generation, by schedules of reinforcement, of typical patterns of behavior;
- maintenance by the agent of frequency of responding that is an inverted-U-shaped function of concentration; and
- discrimination by the subject between those responses that produce the agent and those that do not, that is, the behavior must not result from a general rate-increasing effect.

The last control procedure is a critical requirement, since a response-produced exposure to a psychoactive substance may increase its own frequency of occurrence. For example, an animal may develop a stereotypy on or have a seizure near the response sensor; in the case of puffing, the drug exposure may alter the frequency of oral activities.

Further information supports inhalation, but not self-administration. Patent compounds or metabolites in body fluids, such as, in cigarette smoking, the presence of large amounts of cotinine or nicotine in the urine, indicates inhalation, because the acidic smoke is not absorbed through the oral mucosa (Armitage 1970). For puffing response topographies, the volume of smoke drawn past the subjects lips should be greater than that of the buccal cavity and/or typical puffing responses. Finally, exposure-related physiological changes support inhalation, but provide no evidence of self-adiministration.

TOBACCO SMOKING MODELS

Several investigators have produced smoke puffing by encouraging animals to drink through a straw, i.e., to pull a column of water up from a reservoir, intermittently switching from water to smoke and subsequently requiring puffing for liquid reinforcers. Ratner et al. (1974) generated puffing in a cebus monkey in this manner. Jarvik (1967) was able to train fewer than half of his monkeys to suck water through a tube. To improve this percentage, he obtained a group of maternally deprived and semi-isolated monkeys with a high rate of sucking their digits. They puffed cigarettes freely without any other reinforcing consequences; they also puffed on formaldehyde, glacial acetic acid, and isobutyric acid. These early studies relied on observation, lip contact, or water bubbling for response definition; the investigators occasionally observed nasal expulsion of smoke. The monkeys preferred cigarette smoke over hot air, tobacco smoke over tobacco vapor, and very fragrant pipe tobacco over cigarette smoke. No preference was demonstrated between high- and low-nicotine cigarettes, or between cigar and cigarette smoke; one would expect differential control by nicotine if it is the reinforcer maintaining the puffing behavior. In the case of cigar smoking, Armitage et al. (1970) demonstrated that nicotine in cigar smoke is readily absorbed through the oral mucosa, whereas it is not from cigarette smoke, and that this absorption was associated with the high pH of cigar smoke. Jarvik attributed the lack of preference to the duration of the drug effect, which may have made it difficult to discriminate between the consequences of the two smoke sources, assuming that nicotine was maintaining the puffing. Jarvik also demonstrated that water reinforcement could produce tenfold to twentyfold increases in the rate of puffing.

Glick et al. (1970) used lip contact on either of two mouthpieces as a measure of puffing and maintained this performance on a fixed ratio (up to 30 lip contacts per water reward); this baseline was used to evaluate the effects of drug treatment on puffing rates. Mecamylamine, a ganglionic blocking agent, irreversibly eliminated smoke puffing in two animals, had no effect on a third, and produced reduction followed by recovery of the smoke puffing preference in a fourth. The third and fourth animals were then administered varying doses of pentobarbital, hexamethonium, scopolamine, and amphetamine, in that order. Pentobarbital decreased the rate of puffing but did not alter the preference for smoke over air. Hexamethonium, another ganglionic blocking agent, reversed the preference while decreasing rate at large doses. Scopolamine and d-amphetamine reversed the preferences and greatly decreased the fixed ratio puffing rates. The pentobarbital effects indicated that preference reversal was not simply an artifact of rate reduction. A further experiment suggested that the scopolamine and amphetamine effects might be attributable to increased thirst, since water deprivation may increase the aversiveness of tobacco smoke. The hexamethonium and mecamylamine reversals of puffing preferences suggest that the reinforcing properties of cigarette smoke might represent pharmacologic effects other

than olfactory and gustatory effects. A subsequent study (Robinson et al. 1974) utilizing radioactive smoke indicated that some smoke was inhaled into the lungs with this training procedure.

Rucker (1970) maintained cigarette puffing in monkeys with a water reinforcer and examined the effects of fixed ratio size on puff volume, peak flow rate, and puff duration. An appendix to this thesis documents numerous attempts and strategies to produce inhalation. He instituted a progressive puff volume requirement that increased by 10 percent with each puff and decreased by 10 percent after four failures. He then changed the requirement to an increase equal to 10 percent of the amount by which the monkey exceeded the preceding requirement, with no decrement in the requirement. These procedures produced puffs that would not exceed 100 milliseconds in duration; the animals were sucking vigorously. Water deprivation was increased; attempts were made to reinforce blowing and, by manipulating the characteristics of the spout, to make puffing impossible. He differentially reinforced longer puff durations, which rarely exceeded 300 milliseconds and added resistance to the air flow, again without successful production of inhalation, as judged by the volume of the response. The monkeys failed to continue puffing in the absence of liquid reinforcement.

A baboon model of cigarette smoking also has been developed, again by using water as a reinforcer for maintaining a minimum negative pressure and duration on a mouthpiece (McGill et al. 1978). The criterion for smoke inhalation was elevated blood carbon monoxide levels in comparison to a presmoking baseline; CO levels in nonsmoking animals in the smoking room were not reported. Animals obtained their entire water ration in this manner, puffing on up to 48 cigarettes per day at spaced intervals throughout a 12-hour day. One attendant could maintain 20 animals on this regime, making Large chronic studies feasible.

This preparation has been used to examine the effects of nicotine content on puffing performance (Rogers et al. 1985), and to study bronchial reactivity following cigarette (Roehrs et al. 1981) or nicotine aerosol puffing (Wallis et al. 1982). The preparation has also been used for an extensive series of studies on thyroid hormone levels (Sepkovic et al. 1988), on atherosclerosis induction (Rogers et al. 1980, Rogers et al. 1988), on urinary mutagen formation (Marshall et al. 1983), on alveolar macrophage migration (Fine et al. 1981), and on bronchoalveolar lavage fluids (Rogers et al. 1981; Kolb et al. 1981; Radhakrishnamurthy et al. 1983).

Ando and Yanagita (1981) trained 14 rhesus monkeys to suck liquids through a tube; they then maintained air sucking at a tube using sweet fluids as a reinforcer at an adjacent spout. Animals were given prolonged periods of schedule-controlled puffing, where either a progressively increasing puff duration was required to produce liquid, or a tandem fixed-interval fixed-ratio schedule of puffing was in effect; in either circumstance, the

animals were given access to cigarettes in the afternoon without any other consequence for periods that were gradually lengthened to 20 hours. At the time of their report, only 2 of the 14 animals had developed sustained "voluntary smoking" that lasted for 2 or more years; in the face of great variability, the authors reported that low-nicotine cigarettes seemed to lead to decreases in smoking. In two animals that did not sustain voluntary smoking, schedule-controlled smoking was established using a sweet reinforcer, and control of the performance by the schedule of reinforcement was demonstrated. Schedule-controlled smoking was associated with elevated blood levels of nicotine. Yanagita et al. (1983) reported that 10 of the 14 monkeys continued to puff for a month or so after termination of the reinforcement contingency, and that the two chronic voluntary smokers continued to smoke from 1976 through the time of the later report.

Because the success rate was low with these procedures, Ando et al. (1986) tried two other techniques. In the first experiment, with two monkeys, smoking was established with a progressive puff duration requirement; the animals were then shifted to see if smoking could be maintained adjunctively by reinforcing nozzle licking at a separate tube with different values of a random interval schedule. One animal's puffing waned and did not recover; in the other animal, performance was maintained fairly well at short random-interval values. In the second experiment, four other monkeys were shifted from a random-interval smoking contingency with a 0.2 second minimum response duration to a modified Sidman avoidance schedule. When this performance stabilized at a low shock-delivery rate, the avoidance contingency was terminated; under these extinction conditions, two quit within 15 sessions, but the remaining two persisted for 50 and 80 sessions.

CANNABIS (MARIJUANA, DELTA-9-THC, AND HASHISH)

Cole et al. (1971) attempted to study the effects of delta-9-tetrahydrocannabinol (THC) on spaced responding performance by engendering smoking be havior in two chimpanzees and an orangutan. These great apes were reinforced for successively longer puff durations on unlighted cigarettes, the duration ranging from 0.2 to 5.4 seconds. Lights served as feedback for puffing, and M&M's were used as reinforcers. The reinforcer was presented at the time the criterion was exceeded (Pieper and Cole 1973). Criteria were increased between sessions in approximately 250 millisecond increments. When stable, long-duration puffing was established, smoke was gradually introduced into the air stream by reducing the number of holes punched at the base of the lit cigarette. The consistent increases in the rate of the spaced responding performance that occurred immediately following the smoking sessions with THC in the cigarette were consistent with drug intake, but the dose effect functions were equivocal. IV self-administration of delta-9-THC is difficult to engender as evidenced by the heroic but unsuccessful effort of Harris et al. (1974). Pickens et al. (1973) induced IV

delta-9-THC self-administration in animals with a recent history of phencyclidine self-administration.

Pickens and Thompson (1972) reported being able to obtain fixed-ratio schedule control of puffing, maintained by presentation of hashish smoke. Performance was not maintained by heated air. In disagreement with this early report, Pickens et al. (1973) subsequently reported being unable to maintain hashish smoking in the absence of food delivery. Hashish was removed from the burning chamber and the animal was placed on a concurrent (FR3 sucking) (FR5 lever pulling) schedule of food presentation. These values were selected because they produced approximately equal response frequencies in the absence of hashish. When the animal was given hashish for sucking, the frequency of sucking increased (resembling Jarvik's earlier finding with tobacco). An ADA reversal design was executed for two animals. In this design, hashish was available, removed, and made available again, demonstrating a reversal in preference; that is, in the absence of smoke, the animals preferred lever pressing maintained by food. The authors interpreted these finding cautiously, indicating that a decrease in the probability of lever pressing during hashish availability may have been an effect of hashish smoke on lever pressing, rather than a true preference; taste was also a complicating factor.

DlMETHYLTRYPTAMINE

Siegel and Jarvik (1980), using animals with tobacco- and cocaine-puffing histories, established puffing on lettuce cigarettes by requiring a l-second puff duration for access to 1.5 ml water, the monkeys earned all of their daily water in this manner in a 1-hour session. Termination of the water deprivation eliminated puffing. The subject was then. placed in a dark soundproof chamber with free access to food and water; this did not increase lettuce puffing. Animals were then removed from the chamber and puffed on dimethyltryptamine (DMT) cigarettes for access to water; " . . . after a few puffs on DMT cigarettes, monkeys frequently exhibited aggressive displays, threats, and barks directed at the smoking tube . . ." (Siegel and Jarvik 1980, p. 120). Termination of water deprivation eliminated DMT puffing. Animals were then placed on free access to food and water in the dark chamber, and DMT puffing increased "dramatically"; one animal puffed greatly during the first session, displayed convulsions and spasms, and did not approach the spout again. The other two animals gradually commenced puffing across several days, and clusters of puffs occurred at intervals of about 1 half-hour, approximating DMT's duration of action. Observers recorded increases in behaviors similar to those observed following injection of DMT to animals in darkened environments. Puffing produced some illumination of the chamber under both lettuce and DMT conditions, but was only sustained in the presence of DMT. The authors suggested that ". . . more complete tests of DMT's reinforcing properties in isolation sessions would require either challenges with forced injections of

DMT or choice trials with cigarettes containing short-acting nonhallucinogenic stimulants . . ." (Siegel and Jarvik 1980, p. 120). Since some puffing occurred spontaneously in the absence of drug or the water reinforcement contingency, DMT puffing may have been due to an agonistic property of the drug on puffing, rather than to a reinforcing property of the drug. If an arbitrary response had been required to produce the opportunity to puff on a lighted cigarette, then a comparison of the frequency of this response with a response with no drug consequence would argue against an agonistic effect of the drug. A subsequent reversal of which arbitrary operant was effective in producing drug access would demonstrate discriminative control of this behavior. A demonstration of control of the frequency of this responding by some parameter of delivered dose would then conclusively demonstrate reinforcing properties of DMT by inhalation. The truism that hallucinogens do not engender self-administration by laboratory animals apparently has arisen because nonarylcyclohexylamine hallucinogens have received limited. attention, and those that have been studied tend to have delayed onset or prolonged duration of action. Thus, studies of DMT self-administration by either the intravenous or inhalation route would be of interest.

COCAINE

Siegel at al. (1976) prepared lettuce cigarettes with cocaine base. Three monkeys were trained to puff as in the Jarvik (1967) study, and then were permitted for an hour to smoke lettuce or lettuce-cocaine base cigarettes while under water deprivation or not, and were finally given a choice between a lettuce or a lettuce-cocaine base cigarette under conditions of water deprivation. Although the authors did not perform the t-tests of most interest, under nonwater-deprived conditions the monkeys, by any measure, smoked more cocaine than lettuce cigarettes. Whether this performance was induced by a rate-increasing effect of cocaine or by a reinforcing effect of the drug is not known.

Siegel and Jarvik (1980) repeated these observations in a different order, finishing the experiment with nonwater-deprived cocaine smoking; this final condition provided 23-hour access to cocaine cigarettes rather than just 1 hour. Two monkeys tended to distribute puffing fairly evenly across the first few hours and then quit, only to resume after the daily l-hour break for service. The third animal displayed high rates of puffing that was not associated with increased urinary benzoylecgonine. The authors reported that the rate of puffing did not attenuate across this block of 20 sessions.

THE INFLUENCE OF AEROSOL PHENOMENA ON THE DESIGN OF SUITABLE ANIMAL MODELS

The pattern and efficiency of airway aerosol deposition are dependent on the size of the aerosol and the species exposed (Schlesinger 1985; Schlesinger 1988). We recently characterized the size of crack smoke

(cocaine base aerosol) to be about 2.3 microns with a geometric standard deviation ranging from 1.68 to 2.22 (Snyder et al. 1988). As one would expect from human experience, these particles are small enough to achieve alveolar deposition following oral inhalation. These observations on cocaine particle size have important implications for the development of animal models if comparable patterns of absorption are to be achieved in other species. Large particles impact on surfaces in the upper respiratory tract; because of their high velocity through and the tortuosity of the upper airways of the rodent, even relatively small particles impact in the noses of these species. The rat, for example, is an obligate nose breather, and to achieve penetration to the lung with this species will require the generation of cocaine aerosols with a much smaller particle size.

Smoke, as we think of it in this context, is a condensation aerosol of the drug of interest. As the drug is heated, it vaporizes, only to cool relatively rapidly; as it cools, the vapor condenses spontaneously or upon particles (condensation nuclei) too small to be seen with optical techniques. In the case of drug smoking using a torch and pipe, the torch provides very large numbers of these nuclei. Given a constant airborne drug concentration, the number of nuclei are the predominant determinant of the size of the resultant particles. At the butt of a tobacco cigarette, one might expect $3x10^9$ particles per cubic centimeter (Davies 1988). At such high particle numbers, the likelihood of particle collision and subsequent coagulation is great and proceeds very rapidly; Smoluchowski (1917) was the first to assert that the rate of particle coagulation is proportional to the square of the particle count. Thus, particles grow as the smoke ages, and the number of smaller particles decreases by as much as 40 percent in 1 second (Davies 1988).

It should be apparent that substantial technical problems are associated with the rapid generation and delivery of aerosols that are small enough to reach the lungs of small animals at the high air concentrations associated with drug smoking. The higher the desired concentration, the larger the particle tends to be. To decrease the size of the particle, the number of condensation nuclei can be increased; as the number of either condensation nuclei or condensed particles increases, the duration that the particle exists becomes dramatically shorter. Thus, there are practical and engineering limits to what can be achieved. Fortunately, a number of condensation aerosol generators have been designed since the initial LaMer-Sinclair generators of World War II (Muir 1965; Swift 1967). All consist of the same basic design: condensation nuclei flowing through a heated vapor generator into a reheating zone, which assures complete volatilization and subsequent uniform aerosol condensation in a downward flow chimney. Prodi (1972) demonstrated the feasibility of generating solid particles ranging in size from 0.22 to 2.8 microns from materials with melting points in the same range as cocaine base; his innovation provided independent control of vapor concentration and nucleus count. His condensation nucleus generator, however, is not capable of generating high enough counts to maintain these

small particle sixes at high airborne concentrations. Other condensation nucleus generation techniques may be suitable (Swift 1967).

Once such an atmosphere is made., characterizing the material can commence. Most continuous-particle-sizing instruments are designed for monitoring particles in the "clean" rooms of the semiconductor industry, and cannot accommodate the concentrations found at the end of a cigarette or in a burning building. Most such instruments rely on light scattering from single particles and require extraordinarily small measuring volumes and precise optics. Many have small physical paths that cannot accommodate high particle concentrations, bias particle measurement because of sampling artifacts (anisokinetic sampling), or may run at sufficiently high temperatures to alter the particle under measurement. Noncontinuous devices rely on particle impaction and subsequent recovery and quantitation of the impacted material. This process can be complicated by the presence of pyrolysis products; their presence may be difficult to accommodate without the use of sophisticated analytical devices, extensive control calibrations with the pyrolysate, or radioactive materials. The measurement of the air concentration of the chemical of interest poses the same challenge in addition to the special problems associated with trapping particles in contrast to vapors, e.g. cold fingers do not trap particulate materials. Thus, the initial generation and characterization of aerosol test atmospheres requires the scientific ingenuity of a multidisciplinary team; once this effort is under control, the pharmacology and toxicology can commence.

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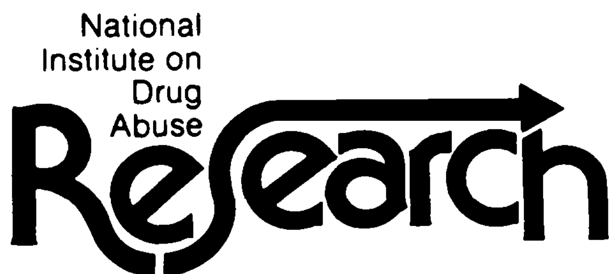

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