

other than nicotine that may contribute to behavioral effects of cigarette smoking.

The toxicity of nicotine is discussed in detail in Appendix B.

Nicotine and Other Alkaloids in Various Tobacco Products

Nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring (Figure 1). Nicotine may exist in two different three-dimensionally structured shapes, called stereoisomers. Tobacco contains only (S)-nicotine (also called l-nicotine), which is the most pharmacologically active form. Tobacco smoke also contains the less potent (R)-nicotine (also called d-nicotine) in quantities up to 10 percent of the total nicotine present (Pool, Godin, Crooks 1985). Presumably some racemization occurs during the combustion process.

The nicotine yield of cigarettes, as determined by standardized smoking machine tests, is available for most brands. However, the amount of nicotine in cigarettes or other tobacco products is not specified by manufacturers. Because tobacco is a plant product, there are differences in the amount of nicotine among and within different types and strains of tobacco, including variations in different parts of the plant, as well as differences related to growing conditions. Table 1 shows concentrations of nicotine and other alkaloids in several different tobacco leaves used in making commercial tobacco products. Within a tobacco plant, leaves harvested from higher stalk positions have higher concentrations of nicotine than from lower stalk positions; ribs and stems of the leaves have the least (Rathkamp, Tso, Hoffmann 1973). Combining different varieties of tobacco and different parts of the plant is a way to change the nicotine concentration of commercial tobacco.

In a study of amounts of nicotine in the tobacco of 15 American cigarette brands of differing machine-determined yields (Benowitz, Hall et al. 1983), tobacco contained on average 1.5 percent nicotine by weight. Nicotine yield of the cigarettes, as defined by Federal Trade Commission smoking machine tests, was correlated inversely with nicotine concentrations in the tobacco. Thus, tobacco of lower-yield cigarettes tended to have higher concentrations of nicotine than did tobacco of higher-yield cigarettes. However, lower-yield cigarettes also contained less tobacco per cigarette, so the total amount of nicotine contained per cigarette, averaging 8.4 mg, was similar in different brands. Thus, low-yield cigarettes are low yield not because of lower concentrations of nicotine in the tobacco, but because they contain less tobacco and have characteristics which remove tar and nicotine by filtration or dilution of smoke with air. Concentrations of nicotine in commercial tobacco products are summarized in Table 2.

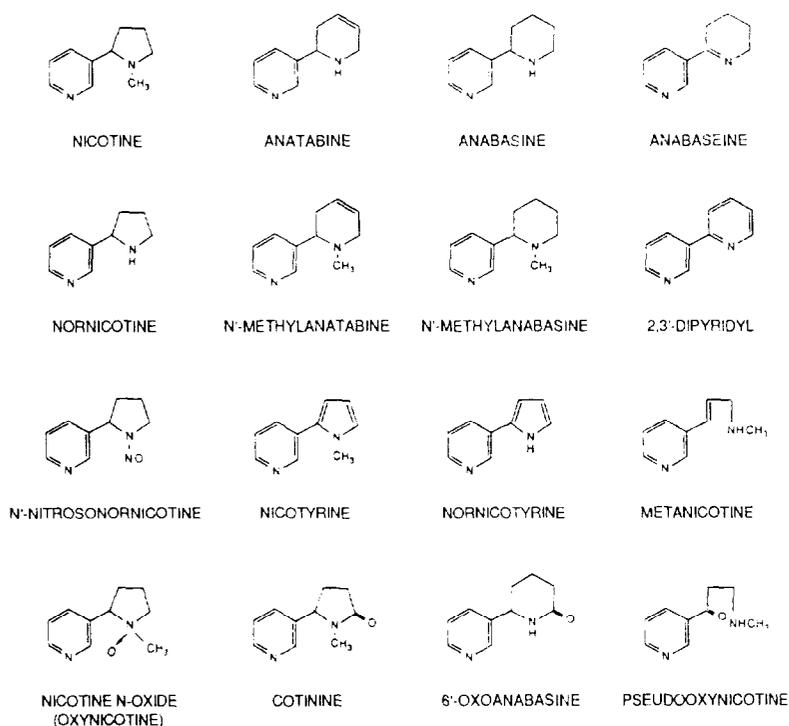


FIGURE 1.—Chemical structures of nicotine and minor tobacco alkaloids

SOURCE: Lete (1983).

Although the major alkaloid in tobacco is nicotine, there are other alkaloids in tobacco which may be of pharmacologic importance. These include nornicotine, anabasine, myosmine, nicotyrine, and anatabine (Figure 1). These substances make up 8 to 12 percent of the total alkaloid content of tobacco products (Table 1) (Piade and Hoffmann 1980). In some varieties of tobacco, nornicotine concentrations exceed those of nicotine (Schmeltz and Hoffmann 1977).

Typical quantities of the minor alkaloids in the smoke of one cigarette are: nornicotine (27 to 88 μg), cotinine (9 to 50 μg), anabasine (3 to 12 μg), anatabine (4 to 14 μg), myosmine (9 μg), and 2,3' dipyridyl (7 to 27 μg). N'-methylanabasine, nicotyrine, nornicotyrine, and nicotine-N'-oxide have also been identified in cigarette smoke (Schmeltz and Hoffmann 1977). Puffing characteristics, especially puff frequency, influence the delivery of the component alkaloids (Bush, Grünwald, Davis 1972).

TABLE 1.—Alkaloid content of various tobaccos (mg/kg, dry basis)

Alkaloid	Dark commercial tobacco			
	A	B	Burley	Bright
Nicotine	11,500	10,000	15,400	12,900
Nornicotine	550	200	630	210
Anatabine	360	380	570	600
Anabasine	140	150	99	150
Cotinine	195	140	90	40
Myosmine	45	50	60	30
2,3-Dipyridyl	100	110	30	10
N'-Formyl-nornicotine	175	210	140	40

SOURCE: Pade and Hoffmann (1980).

TABLE 2.—Nicotine content of various tobacco products

Product	Number of brands tested	Concentration of nicotine (mg/g tobacco)	Typical single dose ¹ (g tobacco)	Nicotine in single dose ¹ (mg)	Nicotine in dose typically consumed in a day
Cigarettes ¹	15	15.7 (13.3-26.9)	0.54	8.4	168 mg/20 cigs
Moist snuff ²	8	10.5 (6.1-16.6)	1.4	14.5	157 mg/15 g
Chewing tobacco ^{3,4}	2	16.8 (9.1-24.5)	7.9	133	1,176 mg/70 g

¹Single dose refers to a cigarette or an amount of smokeless tobacco placed in the mouth. Range.

SOURCE: ¹Benowitz, Hall et al. (1983); ²Kozłowski et al. (1982); ³Gritz et al. (1981); ⁴Benowitz, Porchet et al. (in press).

Nornicotine and anabasine have pharmacologic activity qualitatively similar to that of nicotine, with potencies of 20 to 75 percent compared with that of nicotine, depending on the test system and the animal (Clark, Rand, Vanov 1965). In addition to direct activity, some of the minor alkaloids may influence the effects of nicotine. For example, nicotyrine inhibits the metabolism of nicotine in animals (Stalhandske and Slanina 1982).

The pharmacology of the minor tobacco alkaloids is discussed in more detail in the last section of this Chapter.

Pharmacokinetics and Metabolism of Nicotine

Absorption of Nicotine

Nicotine is distilled from burning tobacco and is carried proximally on tar droplets (mass median diameter 0.3 to 0.5 μm) and probably also in the vapor phase (Eudy et al. 1985), which are inhaled. Absorption of nicotine across biological membranes depends on pH (Armitage and Turner 1970; Schievelbein et al. 1973). Nicotine is a weak base with a pKa (index of ionic dissociation) of 8.0 (aqueous solution, 25°C). This means that at pH 8.0, 50 percent of nicotine is ionized and 50 percent is nonionized. In its ionized state, such as in acidic environments, nicotine does not rapidly cross membranes.

The pH of tobacco smoke is important in determining absorption of nicotine from different sites within the body. The pH of individual puffs of cigarettes made of flue-cured tobacco, the predominant tobacco in most American cigarettes, is acidic and decreases progressively with sequential puffs from pH 6.0 to 5.5 (Brunnemann and Hoffmann 1974). At these pHs, the nicotine is almost completely ionized. As a consequence, there is little buccal absorption of nicotine from cigarette smoke, even when it is held in the mouth (Gori, Benowitz, Lynch 1986). The smoke from air-cured tobaccos, the predominant tobacco in pipes, cigars, and in a few European cigarettes, is alkaline with progressive puffs increasing its pH from 6.5 to 7.5 or higher (Brunneman and Hoffmann 1974). At alkaline pH, nicotine is largely nonionized and readily crosses membranes. Nicotine from products delivering smoke of alkaline pH is well absorbed through the mouth (Armitage et al. 1978; Russell, Raw, Jarvis 1980).

When tobacco smoke reaches the small airways and alveoli of the lung, the nicotine is rapidly absorbed. The rapid absorption of nicotine from cigarette smoke through the lung occurs because of the huge surface area of the alveoli and small airways and because of dissolution of nicotine at physiological pH (approximately 7.4), which facilitates transfer across cell membranes. Concentrations of nicotine in blood rise quickly during cigarette smoking and peak at its completion (Figure 2). Armitage and coworkers (1975), measuring exhalation of radiolabeled nicotine, found that four cigarette smokers absorbed 82 to 92 percent of the nicotine in mainstream smoke, another smoker presumed to be a noninhaler absorbed 29 percent, and three nonsmokers (who were instructed to smoke as deeply as possible) absorbed 30 to 66 percent.

Chewing tobacco, snuff, and nicotine polacrilex gum are of alkaline pH as a result of tobacco selection and/or buffering with additives by the manufacturer. The alkaline pH facilitates absorption of nicotine through mucous membranes. The rate of nicotine absorption from smokeless tobacco depends on the product and the

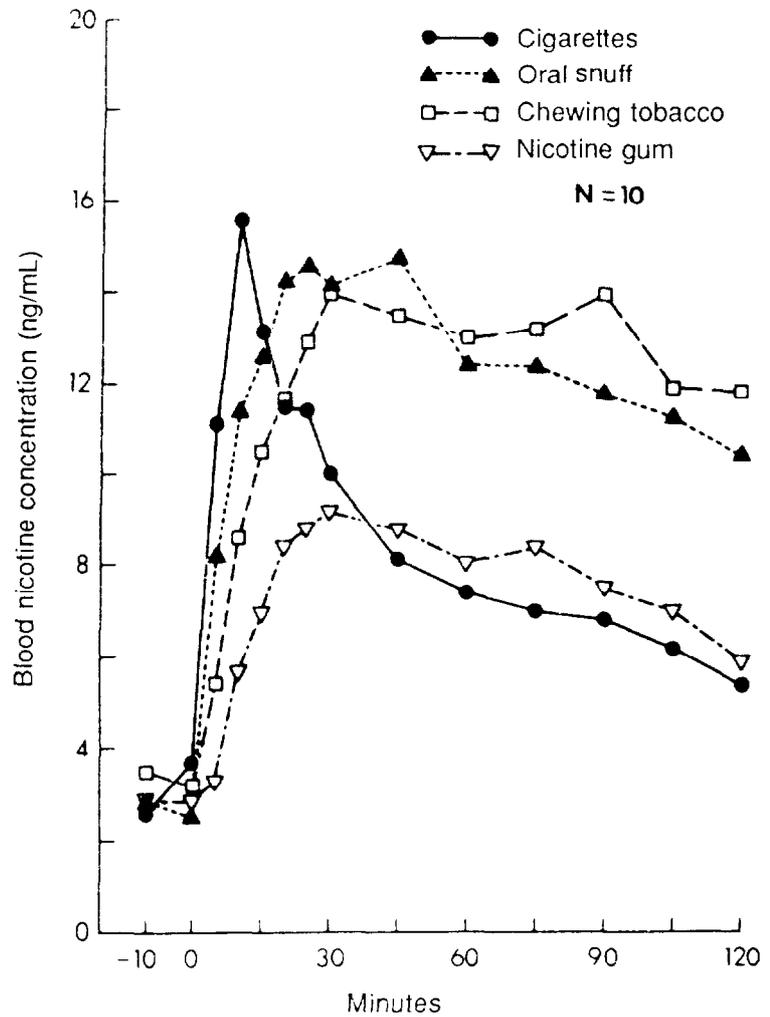


FIGURE 2.—Blood nicotine concentrations during and after smoking cigarettes (1 1/3 cigarettes), using oral snuff (2.5 g), using chewing tobacco (average, 7.9 g), and chewing nicotine gum (two 2-mg pieces)

SOURCE: Benowitz, Porchet et al., 1988.

route of administration. With fine-ground nasal snuff, blood levels of nicotine rise almost as fast as those observed after cigarette smoking

(Russell et al. 1981). The rate of nicotine absorption with the use of oral snuff and chewing tobacco is more gradual. Nicotine is poorly absorbed from the stomach due to the acidity of gastric fluid (Travell 1960), but is well absorbed in the small intestine (Jenner, Gorrod, Beckett 1973), which has a more alkaline pH and a large surface area. Bioavailability of nicotine from the gastrointestinal tract (that is, swallowed nicotine) is incomplete because of presystemic (first pass) metabolism, whereby, after absorption into the portal venous circulation, nicotine is metabolized by the liver before it reaches the systemic venous circulation. This is in contrast to nicotine absorbed through the lungs or oral/nasal mucosa, which reaches the systemic circulation without first passing through the liver. Nicotine base can be absorbed through the skin, and there have been cases of poisoning after skin contact with pesticides containing nicotine (Faulkner 1933; Benowitz, Lake et al. 1987; Saxena and Scheman 1985). Likewise, there is evidence of cutaneous absorption of and toxicity from nicotine in tobacco field workers (Gehlbach et al. 1975).

Because of the complexity of cigarette smoking processes and use of smokeless tobacco products, the dose of nicotine cannot be predicted from the nicotine content of the tobacco or its absorption characteristics. To determine the dose, one needs to measure blood levels and know how fast the individual eliminates nicotine. This topic, estimation of systemic doses of nicotine consumed from various tobacco products, will be considered in a later section after discussion of relevant pharmacokinetic issues.

Distribution of Nicotine in Body Tissues

After absorption into the blood, which is at pH 7.4, about 69 percent of the nicotine is ionized and 31 percent nonionized. Binding to plasma proteins is less than 5 percent (Benowitz, Jacob et al. 1982). The drug is distributed extensively to body tissues with a steady state volume of distribution averaging 180 liters (2.6 times body weight (in kilograms)) (Table 3). This means that when nicotine concentrations have fully equilibrated, the amount of nicotine in the body tissues is 2.6 times the amount predicted by the product of blood concentration and body weight. The pattern of tissue uptake cannot be studied in humans, but it has been examined in tissues of rabbits by measuring concentrations of nicotine in various tissues after infusion of nicotine to steady state (Table 4). Spleen, liver, lungs, and brain have high affinity for nicotine, whereas the affinity of adipose tissue is relatively low.

After rapid intravenous (i.v.) injection, concentrations of nicotine decline rapidly because of tissue uptake of the drug. Shortly after i.v. injection, concentrations in arterial blood, lung, and brain are high, while concentrations in tissues such as muscle and adipose (major storage tissues at steady state) are low. The consequence of this

TABLE 3.—Human pharmacokinetics of nicotine and cotinine

	Nicotine	Cotinine
Half-life	120 min	18 hr
Volume of distribution	180 L	88 L
Total clearance	1,300 mL/min	72 mL/min
Renal clearance	200 mL/min (acid urine)	12 mL/min
Nonrenal clearance	1,100 mL/min	60 mL/min

SOURCE: Average values based on data from Benowitz, Jacob et al. (1982) and Benowitz, Kuyt et al. (1983).

TABLE 4.—Steady state distribution of nicotine

Tissue	Tissue to blood ratio
Blood	1.0
Brain	3.0
Heart	3.7
Muscle	2.0
Adipose	0.5
Kidney	21.6
Liver	3.7
Lung	2.0
Gastrointestinal	3.5

NOTE: Tissue to blood nicotine concentration ratios based on 24-hr constant i.v. infusion of nicotine in rabbits.
SOURCE: Benowitz (1986b).

distribution pattern is that uptake into the brain is rapid, occurring within 1 or 2 min, and blood levels fall because of peripheral tissue uptake for 20 or 30 min after administration. Thereafter, blood concentrations decline more slowly, as determined by rates of elimination and rates of distribution out of storage tissues.

Rapid nicotine uptake into the brain has been demonstrated in animal studies. Oldendorf (1974) showed a high degree of nicotine uptake from blood in the first pass through the brains of rats. Schmitterlöv and colleagues (1967) showed by autoradiographic techniques that high levels of nicotine were present in the brain 5 min after i.v. injections in mice and that most nicotine had been

cleared from the brain by 30 min. Stalhandske (1970) showed that intravenously injected ^{14}C -nicotine is immediately taken up in the brains of mice, reaching a maximum concentration within 1 min after injection. Similar findings based on positron emission tomography of the brain were seen after injection of ^{11}C -nicotine in monkeys (Mazière et al. 1976).

Nicotine inhaled in tobacco smoke enters the blood almost as rapidly as after rapid i.v. injection except that the entry point into the circulation is pulmonary rather than systemic venous. Because of delivery into the lung, peak nicotine levels may be higher and lag time between smoking and entry into the brain shorter than after i.v. injection. After smoking, the action of nicotine on the brain is expected to occur quickly. Rapid onset of effects after a puff is believed to provide optimal reinforcement for the development of drug dependence. The effect of nicotine declines as it is distributed to other tissues. The distribution half-life, which describes the movement of nicotine from the blood and other rapidly perfused tissues, such as the brain, to other body tissues, is about 9 min (Feyerabend et al. 1985). Distribution kinetics, rather than elimination kinetics (half-life, about 2 hr), determine the time course of central nervous system (CNS) actions of nicotine after smoking a single cigarette.

Nicotine is secreted into saliva (Russell and Feyerabend 1978). Passage of saliva containing nicotine into the stomach, combined with the trapping of nicotine in the acidic gastric fluid and reabsorption from the small bowel, provides a potential route for enteric nicotine recirculation. This recirculation may account for some of the oscillations in the terminal decline phase of nicotine blood levels after i.v. nicotine infusion or cessation of smoking (Russell 1976).

Nicotine freely crosses the placenta and has been found in amniotic fluid and the umbilical cord blood of neonates (Hibberd, O'Connor, Gorrod 1978; Luck et al. 1982; Van Vunakis, Langone, Milunsky 1974). Nicotine is found in breast milk and the breast fluid of nonlactating women (Petrakis et al. 1978; Hill and Wynder 1979) and in cervical mucous secretions (Sasson et al. 1985). Nicotine is also found in the freshly shampooed hair of smokers and of nonsmokers environmentally exposed to tobacco smoke (Haley and Hoffmann 1985).

Elimination of Nicotine

Nicotine is extensively metabolized, primarily in the liver, but also to a small extent in the lung (Turner et al. 1975). Renal excretion of unchanged nicotine depends on urinary pH and urine flow, and may range from 2 to 35 percent, but typically accounts for 5 to 10 percent of total elimination (Benowitz, Kuyt et al. 1983; Rosenberg et al. 1980).

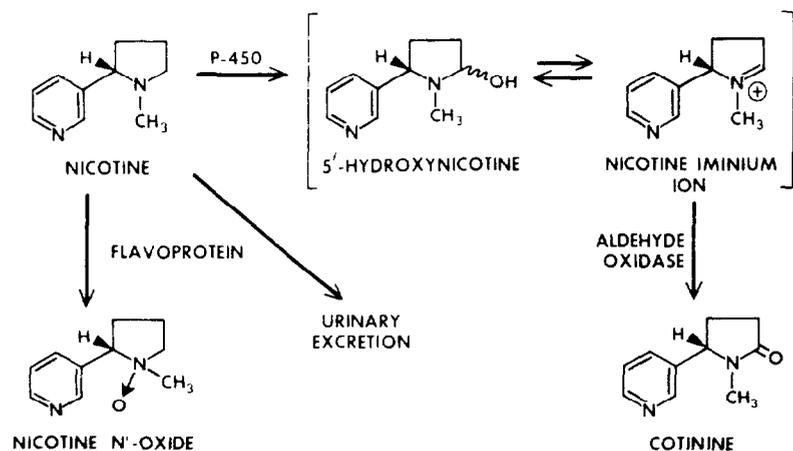


FIGURE 3.—Major pathways of nicotine metabolism

Pathways of Nicotine Metabolism

The primary metabolites of nicotine are cotinine and nicotine-N'-oxide (Figure 3). Cotinine is formed in the liver in a two-step process, the first of which involves oxidation of position 5 of the pyrrolidine ring in a cytochrome P-450-mediated process to nicotine- Δ^{15} -iminium ion (Peterson, Trevor, Castagnoli 1987). In the second step the iminium ion is metabolized by a cytoplasmic aldehyde oxidase to cotinine (Hibberd and Gorrod 1983).

Cotinine itself is also extensively metabolized, with only about 17 percent excreted unchanged in the urine (Benowitz, Kuyt et al. 1983). Several metabolites of cotinine have been reported, including *trans*-3'-hydroxycotinine (McKennis, Turnbull et al. 1963), 5'-hydroxycotinine (Bowman and McKennis 1962), cotinine-N-oxide (Shulgin et al. 1987), and cotinine methonium ion (McKennis, Turnbull, Bowman 1963) (see Figure 4). Little is known about the quantitative importance of these metabolites. *Trans*-3'-hydroxycotinine appears to be a major metabolite (Jacob, Benowitz, Shulgin 1988; Neurath et al. 1987), with urinary concentrations exceeding cotinine concentrations by twofold to threefold. Cotinine N-oxide is a minor metabolite in humans, accounting for approximately 3 percent of ingested nicotine (Shulgin et al. 1987). Subsequent oxidative degradation of the pyrrolidine ring gives rise to 3-pyridylacetic acid. This compound has been identified in human urine (McKennis, Schwartz, Bowman 1964), but no quantitative data are available.

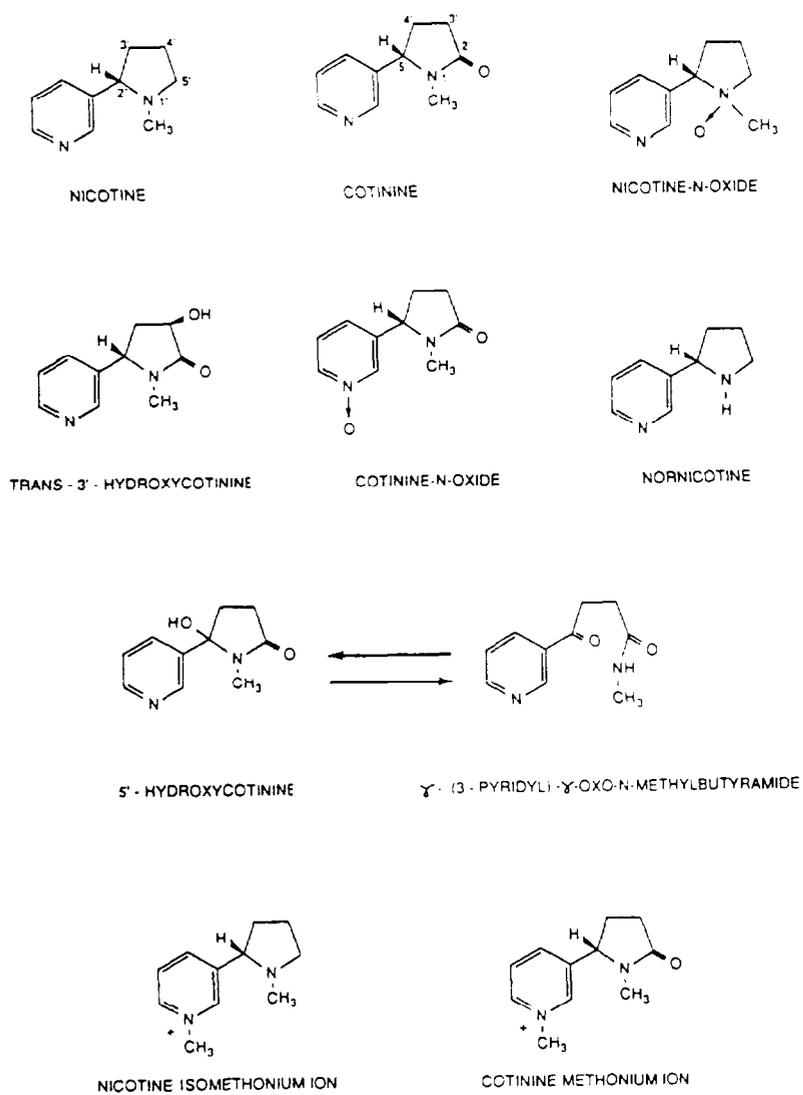


FIGURE 4.—Structures of nicotine and its major metabolites

SOURCE: P. Jacob III (with permission).

Nicotine-1'-N-oxide is quantitatively a minor metabolite of nicotine. Oxidation of the nitrogen atom of the pyrrolidine ring depends on a microsomal flavoprotein system and produces a mixture of the two diastereoisomers, 1'-(R)-2'-(S)-*cis*- and 1'-(S)-2'-(S)-*trans*-nicotine-1'-N'-oxide (Booth and Boyland 1970). After i.v. injection, 100 percent of nicotine-N'-oxide is excreted unchanged in the urine, indicating no further metabolism (Beckett, Gorrod, Jenner 1971a). However, after oral administration only 30 percent is recovered in the urine as nicotine-N'-oxide; the remainder is recovered as nicotine and its metabolites. To evaluate the possibility of reduction of nicotine-N'-oxide in the gastrointestinal tract, rectal administration of nicotine-N'-oxide was performed for experimental purposes. Less than 10 percent was recovered in the urine as nicotine-N'-oxide (Beckett, Gorrod, Jenner 1970). These findings indicate reduction of nicotine-N'-oxide back to nicotine within the human gastrointestinal tract, believed to be a consequence of bacterial action.

Experiments in rats indicate that significant amounts of nicotine-N'-oxide are converted to nicotine both in vitro and in vivo (Dajani, Gorrod, Beckett 1975a,b). Nicotine and cotinine have been measured in the blood of rats administered nicotine-N,N'-dioxide and nicotine-N'-oxide in drinking water (Sepkovic et al. 1984, 1986). Thus, while reduction of nicotine-N'-oxide to nicotine appears to be bacterial in humans, it may be mediated by endogenous enzymes in other species.

Quantitative aspects of the conversion of nicotine to its metabolites have not been well defined. Studies of cotinine excretion in urine collected for 24 hr after i.v. nicotine injection indicate less than 10 percent of nicotine is excreted as cotinine in nonsmokers compared with an average of 25 percent in smokers (Beckett, Gorrod, Jenner 1971b). Another study, comparing 24-hr urinary excretion of cotinine with nicotine content of cigarette butts after smoking, indicated 46 percent recovery as cotinine (Schievelbein 1982). However, both of these studies underestimate the conversion of nicotine to cotinine because the urine collection period was too short. In cigarette smokers, cotinine has a half-life averaging 18 to 20 hr (Benowitz, Kuyt et al. 1983), so that in 24 hr only a little more than half of cotinine is recovered. Urine collection for at least 72 hr is necessary to recover more than 90 percent of cotinine in most subjects. In addition, since only 17 percent of cotinine is excreted unchanged (Benowitz, Kuyt et al. 1983), urinary recovery analysis underestimates the cotinine generation rate.

At steady state, the rate of metabolite excretion reflects the rate at which the metabolites are generated. After i.v. dosing, 100 percent of nicotine-N'-oxide but only 17 percent of cotinine are excreted unchanged in the urine. Based on a ratio of urinary cotinine to nicotine-N'-oxide of 2.9 and based on excretion of that 17 percent of

cotinine and 100 percent of nicotine-N'-oxide unchanged in the urine, the relative generation rate of cotinine compared with that of nicotine-N'-oxide is calculated to be 17 to 1 (Benowitz 1986b). Because 4 percent of nicotine is excreted as nicotine-N'-oxide (Jacob et al. 1986; Beckett, Gorrod, Jenner 1971a), about 70 percent of nicotine appears to be converted to cotinine. Quantitative data on other metabolites that may have pharmacologic activity, such as nicotine isomethonium ion and nornicotine, are not available.

Rate of Nicotine Metabolism

The rate of nicotine metabolism can be determined by measuring blood levels after administration of a known nicotine dose. In one study, cigarette smokers were given i.v. infusions of nicotine for 30 to 60 min, and total and renal clearances were computed (Benowitz, Jacob et al. 1982). Total clearance (a term which describes the capacity to eliminate a drug) averaged 1,300 mL/min. Nonrenal clearance averaged 1,100 mL/min (Table 3), which represents about 70 percent of liver blood flow. Because nicotine is metabolized mainly by the liver (data in animals indicate only a small degree of metabolism by the lung) (Turner, Sillett, McNicol 1977), this means that about 70 percent of the drug is extracted from the blood in each pass through the liver. On the average, 85 or 90 percent of nicotine is metabolized by the liver.

Renal Excretion

Nicotine is excreted by glomerular filtration and tubular secretion within the kidney. Depending on urinary pH and urine flow rate, variable amounts of nicotine are reabsorbed by the kidney tubules. In acidic urine, where nicotine is mostly ionized and tubular reabsorption is minimized, renal clearance of nicotine may be as high as 600 mL/min (urinary pH 4.4) (Benowitz, Kuyt et al. 1983; Rosenberg et al. 1980). In alkaline urine, a larger fraction of nicotine is not ionized. Tubular reabsorption of nonionized nicotine results in lower rate of excretion and reduced renal clearances as low as 17 mL/min (urine pH 7.0). When urine pH is uncontrolled, averaging 5.8, renal clearance averages about 100 mL/min, accounting for the elimination of 10 to 15 percent of the daily nicotine intake.

Nicotine and Cotinine Blood Levels During Tobacco Use

Nicotine Levels

Plasma nicotine concentrations (or concentrations in blood, which are similar) sampled in the afternoon in smokers generally range from 10 to 50 ng/mL. 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 (Armitage et al. 1975;

Herning et al. 1983; Isaac and Rand 1972). Peak blood levels of nicotine are similar, although the rate of nicotine increase is slower for cigar smokers and snuff and chewing tobacco users compared with that for cigarette smokers (Armitage et al. 1978; Turner, Sillett, McNicol 1977; Gritz et al. 1981; Russell, Raw, Jarvis 1980; Russell et al. 1981) (Figure 2). Pipe smokers, particularly those who have previously smoked cigarettes and who inhale, may have blood and urine levels of nicotine as high as those of cigarette smokers (McCusker, McNabb, Bone 1982; Turner, Sillett, McNicol 1977; Wald et al. 1984).

The earliest published studies of nicotine elimination kinetics reported half-lives of 20 to 40 min (Armitage et al. 1975; Isaac and Rand 1972). In those studies, drug blood levels were followed only for 30 to 60 min, which is not long enough to determine the elimination half-life. Thus, half-lives were based on blood levels which included the distribution phase. When blood levels are followed for several hours after the end of nicotine infusion, a log-linear decline of blood levels with a half-life of about 2 hr is observed (Benowitz, Jacob et al. 1982; Feyerabend, Ings, Russell 1985).

The half-life of a drug is useful in predicting its accumulation rate in the body with repetitive doses and the time course of its decline after cessation of dosing. Assuming a half-life of 2 hr, one would predict nicotine to accumulate over 6 to 8 hr (3 to 4 half-lives) of regular smoking and persist at significant nicotine levels for 6 to 8 hr after cessation of smoking. If a smoker smokes until bedtime, significant nicotine levels should persist all night. Studies of blood levels in regular cigarette smokers confirm these predictions (Figure 5) (Russell and Feyerabend 1978; Benowitz, Kuyt, Jacob 1982). Peaks and troughs follow the use of each cigarette, but as the day progresses, trough levels rise and the influence of peak levels becomes less important. Thus, nicotine is not a drug to which people are exposed intermittently and that is eliminated rapidly from the body. To the contrary, smoking represents a multiple dosing situation with considerable accumulation during smoking and with persistent levels for 24 hr of each day.

Cotinine Levels

Cotinine levels are of particular interest as qualitative markers of tobacco use and quantitative indicators of nicotine intake. Cotinine is present in the blood of smokers in much higher concentrations than nicotine. Cotinine blood levels average about 250 to 300 ng/mL in groups of cigarette smokers (Benowitz, Hall et al. 1983; Haley, Axelrad, Tilton 1983; Langone, Van Vunakis, Hill 1975; Zeidenberg et al. 1977). After stopping smoking, levels decline with a half-life averaging 18 to 20 hr (range 11 to 37 hr). But because of the long half-life, there is much less fluctuation in cotinine concentrations

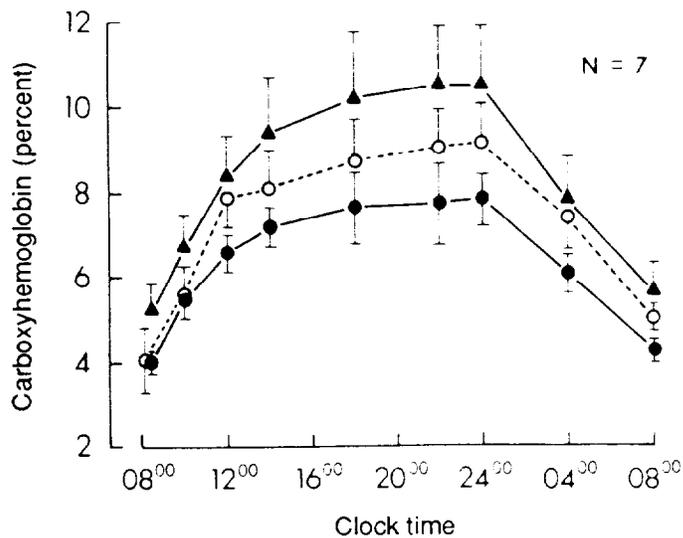
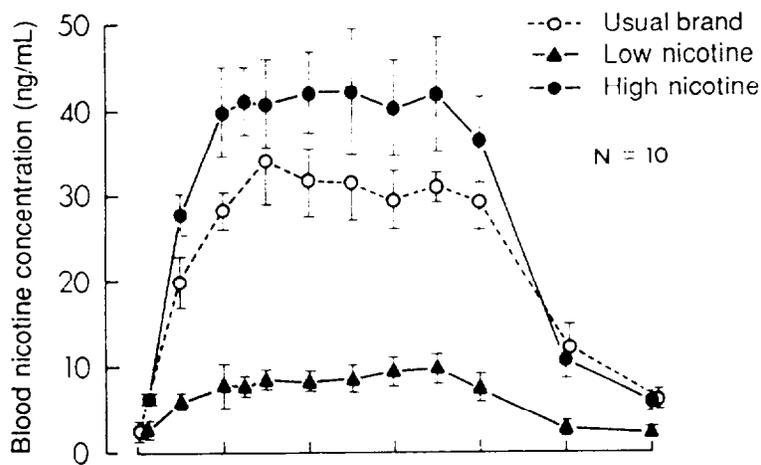


FIGURE 5.—Blood nicotine and carboxyhemoglobin concentrations in subjects smoking high nicotine (2.5 mg) and low nicotine (0.4 mg) Kentucky reference cigarettes and their usual brand (average nicotine yield, 1.2 mg) of cigarettes

NOTE: Subjects smoked on a fixed schedule of 1 cigarette every half hour from 8:30 a.m. to 11:00 p.m., for a total of 30 cigarettes/day; blood samples were collected just before the next scheduled cigarette.
 SOURCE: Benowitz, Kuyt, Jacob (1982).

throughout the day than in nicotine concentrations. As expected, there is a gradual increase in cotinine levels during the day, peaking at the end of smoking and persisting in high concentrations overnight.

Intake of Nicotine

Cigarette Smoking

Nicotine intake from single cigarettes has been measured by spiking cigarettes with ¹⁴C-labeled nicotine (Armitage et al. 1975). That study of eight subjects, each smoking a single filter-tipped cigarette, indicated an intake range of 0.36 to 2.62 mg. Intake was higher in smokers than in nonsmokers. Intake of nicotine from smoking a single cigarette or with daily cigarette smoking has been estimated by methods similar to those used in drug bioavailability studies (Benowitz and Jacob 1984; Feyerabend, Ings, Russell 1985). Metabolic clearance of nicotine was determined after i.v. injection. Metabolic clearance data were then used in conjunction with blood and urinary concentrations of nicotine measured during a period of smoking to determine the intake of nicotine. In five subjects, average intake of nicotine per cigarette was 1.06 mg (range, 0.58 to 1.49 mg) (Feyerabend, Ings, Russell 1985). In 22 cigarette smokers, 13 men and 9 women who smoked an average of 36 cigarettes/day (range 20 to 62), the average daily intake was 37.6 mg, with a range from 10.5 to 78.6 mg (Benowitz and Jacob 1984). Nicotine intake per cigarette averaged 1.0 mg (range 0.37 to 1.56 mg). Intake per cigarette did not correlate with yields obtained by smoking machine using standard Federal Trade Commission methods. This is because smoking machines smoke cigarettes in a uniform way, using a fixed puff volume (35 mL), flow rate (over 2 sec), and interval (every minute). Smokers smoke cigarettes differently, changing their puffing behavior to obtain the desired amount of tobacco smoke and nicotine.

Elimination Rate as a Determinant of Nicotine Intake by Cigarette Smoking

There is considerable evidence that smokers adjust their smoking behavior to try to regulate or maintain a particular level of nicotine in the body (Gritz 1980; Russell 1976). For example, when the availability of cigarettes is restricted, habitual smokers can increase intake of nicotine per cigarette 300 percent compared with the intake of unrestricted smoking (Benowitz, Jacob, Koslowski et al. 1986).

Techniques for measuring daily intake of nicotine (Benowitz and Jacob 1984) have been applied to study the influence of elimination on nicotine intake. The rate of renal elimination of nicotine was manipulated by administration of ammonium chloride or sodium

bicarbonate to acidify or alkalinize the urine, respectively (Benowitz and Jacob 1985). Compared with daily excretion during placebo treatment (3.9 mg nicotine/day), acid loading increased (to 12 mg/day) and alkaline loading decreased (to 0.9 mg/day) daily excretion of nicotine. The total intake of nicotine averaged 38 mg/day. Average blood nicotine concentrations were similar in placebo and bicarbonate treatment conditions but were 15 percent lower during ammonium chloride treatment. Daily intake of nicotine was 18 percent higher during acid loading, indicating compensation for increased urinary loss. The compensatory increase in nicotine consumption was only partial, replacing about half of the excess urinary nicotine loss. Bicarbonate treatment had no effect on nicotine consumption, consistent with the small magnitude of effect on excretions of nicotine in comparison to total daily intake.

These results seem compatible with the suggestion of Schachter (1978) that emotional stress, which results in more acidic urine, might accelerate nicotine elimination from the body and thereby increase cigarette smoking. But caution must be exercised in applying these findings to usual smoking situations. These studies were performed under conditions of extreme urinary acidification or alkalinization, so that the changes in renal clearance would be maximized. Even with extreme differences in urinary pH, differences in overall nicotine elimination rate and smoking behavior were modest. This is because renal excretion is a minor pathway for elimination of nicotine; most is metabolized. Smaller changes in urinary pH, such as occur spontaneously throughout the day or that might be related to stressful events, would not be expected to substantially influence nicotine elimination or smoking behavior.

Biochemical Markers of Nicotine Intake

Absorption of nicotine from tobacco smoke provides a means of verification and quantitation of tobacco consumption. The general strategy is to measure concentrations of nicotine, its metabolites (such as cotinine), or other chemicals associated with tobacco smoke in biological fluids such as blood, urine, or saliva. Different measures vary in sensitivity, specificity, and difficulty of analysis. Different investigators have used blood or urinary nicotine concentrations, blood or salivary or urinary cotinine concentrations, expired carbon monoxide or carboxyhemoglobin concentrations, or plasma or salivary thiocyanate (a metabolite of hydrogen cyanide, a vapor phase constituent) concentrations as measures of tobacco smoke consumption.

Relationships among daily intake of nicotine, daily exposure to nicotine (that is, blood concentrations of nicotine integrated over 24 hr), various parameters of cigarette consumption, and different measures of nicotine intake have been examined experimentally

during ad libitum cigarette smoking on a research ward (Benowitz and Jacob 1984). The best biochemical correlate to nicotine intake and exposure in this study was a random blood nicotine concentration measured at 4 p.m. This level did not depend on when the last cigarette was smoked. This finding is consistent with the observation that nicotine levels accumulate throughout the day and plateau in the early afternoon (see Figure 5). At steady state, with regular smoking throughout the day, there should be a reasonably good correlation between nicotine concentrations and daily intake. Carboxyhemoglobin (COHb) concentrations in the afternoon were the next best markers of nicotine intake. Also, morning (8 a.m.) levels of nicotine and COHb correlated with intake, presumably reflecting persistence of nicotine and COHb in the blood from exposure on the previous day.

Although cotinine is a highly specific marker for nicotine exposure, blood levels of cotinine across subjects in this study did not correlate as closely with nicotine intake as did blood levels of nicotine or COHb (Benowitz and Jacob 1984). This is probably due to individual variability in fractional conversion of nicotine to cotinine and in the elimination rate of cotinine itself.

Because of its relatively long half-life, cotinine levels are less sensitive than nicotine levels to smoking pattern, that is, when the last cigarette was smoked. For longitudinal within-subject studies, the cotinine level would be expected to be a good marker of changes in nicotine intake. Cotinine measurements have become the most widely accepted method for assessing the intake of nicotine in long-term studies of tobacco use (see also Chapter V).

As expected by the known variation in renal clearance due to effects of urinary flow and pH, urinary concentrations of nicotine did not correlate well with nicotine intake (Benowitz and Jacob 1984). In contrast, urinary cotinine, which is less influenced by urinary flow or pH, was as good a marker as blood cotinine concentration. Salivary and urinary cotinine concentrations correlate well ($r=0.8$ to 0.9) with blood cotinine concentrations (Haley, Axelrad, Tilton 1983; Jarvis et al. 1984). Therefore, salivary or urine cotinine concentrations should be almost as useful as blood levels in indicating nicotine intake.

Analytical Methods for Measuring Nicotine and Cotinine in Biological Fluids

Determination of nicotine concentrations in biological fluids requires a sensitive and specific method, because concentrations of nicotine in smokers' blood are generally in the low nanogram per milliliter range and a number of metabolites are also present. Cotinine concentrations in blood are generally about tenfold greater than nicotine concentrations, and as a result, less sensitive analyti-

cal methodology may be acceptable. Methods with adequate sensitivity for determination of nicotine and cotinine in smokers' blood include gas chromatography (GC) (Curvall, Kazemi-Vala, Enzell 1982; Davis 1986; Feyerabend, Levitt, Russell 1975; Hengen and Hengen 1978; Jacob, Wilson, Benowitz 1981; Vereby, DePace, Mulé 1982), radioimmunoassay (RIA) (Langone, Gjika, Van Vunakis 1973; Castro et al. 1979; Knight et al. 1985), enzyme-linked immunosorbent assay (ELISA) (Bjercke et al. 1986), high performance liquid chromatography (HPLC) (Machacek and Jiang 1986; Chien, Diana, Crooks, in press), and combined gas chromatograph-mass spectrometry (GC-MS) (Dow and Hall 1978; Gruenke et al. 1979; Jones et al. 1982; Daenens et al. 1985). For reasons of sensitivity, specificity, and economy, GC and RIA are the most frequently used methods. GC-MS is a highly sensitive and specific technique, but the expense has discouraged its routine use. HPLC is less sensitive than GC for nicotine and cotinine determination. Although recently reported methods (Machacek and Jiang 1986; Chien, Diana, Crooks, in press) appear to have adequate sensitivity for determining concentrations in plasma, relatively large sample volumes are required. Concentrations of nicotine and cotinine in urine are tenfold to hundredfold greater than concentrations in plasma or saliva (Jarvis et al. 1984), and a variety of chromatographic and immunoassay techniques meet sensitivity requirements.

The choice of a particular method depends on the biological fluid to be assayed; the need for sensitivity, precision, and accuracy; and economic considerations. Chromatographic methods, particularly those utilizing high-resolution capillary columns and specific detectors such as nitrogen-phosphorus detectors or a mass spectrometer, provide the greatest specificity. On the other hand, immunoassay techniques are operationally simpler, generally require smaller samples, and may be less expensive than chromatographic methods. A drawback to immunoassay methods is the potential for cross-reactivity of the antibody with metabolites or endogenous substances. There is generally a good correlation between results obtained by GC and RIA for plasma cotinine concentrations ($r = 0.94$) (Gritz et al. 1981; Biber et al. 1987). In an interlaboratory comparison study (Biber et al. 1987), cotinine concentrations in smokers' urine measured by RIA were generally higher than concentrations determined by GC, whereas in nonsmokers' urine spiked with cotinine RIA and GC values were similar. These results suggest that nicotine metabolites cross-react with the antibody against cotinine, at least in some of the RIA methods.

Pharmacodynamics of Nicotine

General Considerations

This Section will focus on the relationship between nicotine levels in the body and their effects on behavior and physiological function

(pharmacodynamics). These data show how pharmacodynamic factors determine some of the consequences of cigarette smoking. Two issues are particularly relevant in understanding the pharmacodynamics of nicotine: a complex dose-response relationship and the level of tolerance that is either preexisting or is produced by administration of nicotine.

Dose-Response

The relationship between the dose of nicotine and the resulting response (dose-response relationship) is complex and varies with the specific response that is measured. In pharmacology textbooks, nicotine is commonly mentioned as an example of a drug which in low doses causes ganglionic stimulation and in high doses causes ganglionic blockade following brief stimulation (Comroe 1960). This type of effect pattern is referred to as "biphasic." Dose-response characteristics in functioning organisms (in vivo) are often biphasic as well, although the mechanisms are far more complex. For example, at very low doses, similar to those seen during cigarette smoking, cardiovascular effects appear to be mediated by the CNS, either through activation of chemoreceptor afferent pathways or by direct effects on the brain stem (Comroe 1960; Su 1982). The net result is sympathetic neural discharge with an increase in blood pressure and heart rate. At higher doses, nicotine may act directly on the peripheral nervous system, producing ganglionic stimulation and the release of adrenal catecholamines. With high doses or rapid administration, nicotine produces hypotension and slowing of heart rate, mediated either by peripheral vagal activation or by direct central depressor effects (Ingenito, Barrett, Procita 1972; Porsius and Van Zwieten 1978; Henningfield, Miyasato, Jasinski 1985).

Tolerance

A second pharmacologic issue of importance is development of tolerance; that is, after repeated doses, a given dose of a drug produces less effect or increasing doses are required to achieve a specified intensity of response. Functional or pharmacodynamic tolerance can be further defined as where a particular drug concentration at a receptor site (in humans approximated by the concentration in blood) produces less effect than it did after a prior exposure. Dispositional or pharmacokinetic tolerance refers to accelerated drug elimination as a mechanism for diminished effect after repeated doses of a drug. Behavioral tolerance refers to compensatory behaviors that reduce the impact of a drug to adversely affect performance. Such tolerance can occur following intermittent exposures to a drug such that there is minimal development of functional or dispositional tolerance.

Most studies of drug tolerance have focused on tolerance which develops as a drug is chronically administered. If the tolerance develops within one or two doses, it is referred to as acute tolerance or tachyphylaxis. If tolerance develops after more prolonged use, the tolerance is referred to as acquired or chronic tolerance. Individual differences in sensitivity to the first dose of a drug also frequently exist. Those individuals who exhibit a reduced response to a specified drug dose or require a greater dose to elicit a specified level of response are said to be tolerant to the drug. This form of tolerance is referred to as first-dose tolerance, drug sensitivity, or innate drug responsiveness. For sake of clarity, this Report will reserve the term tolerance to describe reduction in the response to nicotine during the course of or following a previous exposure and will use acute drug sensitivity to describe responsiveness to an initial dose.

Studies of tolerance to nicotine began in the late 19th century. In a series of studies of fundamental importance to the understanding of the nervous system, as well as to understanding the pharmacology of nicotine, Langley (1905) and Dixon and Lee (1912) studied the effects of repeated nicotine administration on a variety of animal species and on *in vitro* tissue preparations. Several findings emerged which have been widely verified and extended to other species and responses. These include: (1) With repeated dosing, responses diminished to nearly negligible levels; (2) After tolerance occurred, responsiveness could be restored by increasing the size of the dose; (3) After a few hours without nicotine, responsiveness was partially or fully restored.

After smoking a cigarette, people who have not smoked before ("naive smokers") usually experience a number of effects that become generally uncommon among experienced smokers. For example, retrospective reports by smokers indicate that initial exposure to tobacco smoke produced dizziness, nausea, vomiting, headaches, and dysphoria, effects that disappear with continued smoking and are rarely reported by chronic smokers (Russell 1976; Gritz 1980). Tolerance may also develop to toxic effects, such as nausea, vomiting, and pallor, during the course of nicotine poisoning, despite persistence of nicotine in the blood in extremely high concentrations (200 to 300 ng/mL) (Benowitz, Lake et al. 1987).

A systematic analysis of the various forms of tobacco smoke tolerance has not been carried out. There are a few studies comparing the effects elicited by an acute exposure to tobacco in nonsmokers and smokers. Clark and Rand (1968) studied the effect of smoking cigarettes of varying nicotine content on the knee-jerk reflex and reported that high-nicotine cigarettes suppressed this reflex to a greater degree than did low-nicotine cigarettes. This effect was more pronounced at each nicotine dose in nonsmokers and light smokers compared to heavy smokers. These findings suggested that

tolerance is due to altered sensitivity to nicotine. Tolerance to nicotine is not complete because even the heaviest smokers experience symptoms such as dizziness, nausea, and dysphoria when they suddenly increase their smoking rates (Danaher 1977). Evidence indicates that the majority of the psychological actions of tobacco smoke result from nicotine (Russell 1976; Chapter VII). Thus, most of the tolerance to effects of tobacco smoke that occurs following chronic tobacco use is due to the development of tolerance to nicotine.

Acute Sensitivity

Human Studies

Studies which have indicated that individuals differ in response to tobacco smoke or nicotine have used smokers as the experimental subjects. Consequently, whether individual differences are due to differences in acute sensitivity to nicotine that have persisted during chronic tobacco use or are due to differences in the development of tolerance is unknown.

Nesbitt (1973) and Jones (1986) noted that individual smokers differ with respect to the effects of smoking a standard cigarette on heart rate, but it is not clear from these studies whether these differences in responsiveness are due to differences in sensitivity to nicotine or to differences in the dose and kinetics of nicotine. Benowitz and colleagues (1982) observed individual differences in the effects of i.v. injections of nicotine on heart rate, blood pressure, and fingertip skin temperature. Differences were not explained by differences in blood levels, indicating differential sensitivity to nicotine.

Animal Studies

Studies using laboratory animals indicate that differences in acute sensitivity to nicotine exist. Inbred rat and mouse strains differ in sensitivity to the effects of nicotine on locomotor activity (Garg 1969; Bättig et al. 1976; Schlatter and Bättig 1979; Hatchell and Collins 1980; Marks, Burch, Collins 1983b). Mouse strains also differ in the direction of the effect (increased or decreased activity). The mouse strains that differ in sensitivity to the effects of injected nicotine on locomotor activity also differ in the magnitude of response to a standard dose of tobacco smoke (Baer, McClearn, Wilson 1980). Inbred mouse strains also differ in sensitivity to the effects of nicotine on body temperature, heart rate, and acoustic startle response (Marks, Burch, Collins 1983a; Marks et al. 1985, 1986), as well as in sensitivity to nicotine-induced seizures (Tepper, Wilson, Schlesinger 1979; Miner, Marks, Collins 1984, 1986). These findings indicate that genetic factors may influence the sensitivity of rats and

mice to the first dose of nicotine. The importance of genetically determined differences in human sensitivity to the effects of nicotine administered in tobacco smoke remains to be determined.

Mechanisms of Differences in Acute Sensitivity

Differences between inbred mouse and rat strains in sensitivity to the effects elicited by a single injected dose of nicotine do not appear to result from differences in rate of nicotine metabolism (Petersen, Norris, Thompson 1984) or from differences in brain nicotine concentration following intraperitoneal injection (Hatchell and Collins 1980; Rosecrans 1972; Rosecrans and Schechter 1972). Thus, rat and mouse strains differ in tissue sensitivity to the effects of nicotine. Differences among mouse strains in sensitivity to nicotine do not appear to be due to differences in the number or affinity of brain nicotine receptors that are measured via the binding of ³H-nicotine (Marks, Burch, Collins, 1983b). Mouse stocks that are more sensitive to nicotine-induced seizures do have greater numbers of hippocampal nicotine receptors that bind ¹²⁵I-bungarotoxin (BTX) (Miner, Marks, Collins 1984, 1986). Some of the differences in sensitivity to nicotine between genetically defined stocks of animals may be related to differences in the number of nicotine receptors in specific regions of the brain.

Tachyphylaxis (Acute Tolerance)

Human Studies

Systematic studies of tachyphylaxis or acute tolerance to effects of tobacco in nonsmokers have not been reported. There is evidence that tachyphylaxis does develop to effects of tobacco and nicotine in humans. Smokers frequently report that the first cigarette of the day is the best and that subsequent cigarettes are "tasteless" (Russell 1976; Henningfield 1984). Smoking a single standard cigarette after 24 hr of abstinence increases heart rate, whereas smoking an identical cigarette during the course of a normal day fails to change heart rate (West and Russell 1987). Fewer standard puffs were required to produce nausea at the beginning of the day (following 8 to 10 hr of tobacco abstinence) or from high-nicotine cigarettes than at the end of the day or from low-nicotine cigarettes (Henningfield 1984). Complete tolerance to nausea and vomiting developed over 8 hr in a woman in the course of an accidental nicotine poisoning, despite persistently toxic blood levels of nicotine (Benowitz, Lake et al. 1987). These findings suggest that tolerance which is lost and regained during short periods of abstinence from tobacco is tolerance to nicotine.

Tolerance develops very rapidly to several effects of nicotine. Rosenberg and colleagues (1980) studied the effects of i.v. nicotine

injections on arousal level, heart rate, and blood pressure. In these experiments, six healthy smokers, 21 to 35 years of age, received six series of nicotine injections spaced 30 min apart. Each series of injections consisted of 10 2- μ g/kg injections spaced 1 min apart. Subjects reported a pleasant sensation after the first series of injections, but this response was not observed thereafter. Heart rate and blood pressure values remained above baseline, but there was little increment with successive injections, despite nicotine blood level increases which were similar to those observed after the first series of injections. In contrast, skin temperature fell progressively during the period of nicotine dosing, gradually returning to baseline at the end of the study. These data indicated rapid development of tolerance to subjective effects and heart rate and blood pressure responses, but tolerance was not complete because heart rate and blood pressure remained above baseline. Henningfield (1984) also assessed subjective responses of human subjects after i.v. injections with nicotine at 10-min intervals. The subjective response of "liking" the effects of nicotine was lost after five or six injections. Benowitz and coworkers (1982) studied the effect of a 30-min infusion of nicotine at a rate of 1 to 2 μ g/kg/min. Shortly after initiation of infusion, heart rate and blood pressure increased, but the increase did not continue even though plasma nicotine concentrations continued to rise during the continuous infusion. Maximal cardiovascular changes were seen within 5 to 10 min, whereas maximal plasma nicotine levels were not reached until 30 min. These findings indicate that tachyphylaxis to the effects of nicotine may develop in humans within 5 to 10 min, the time required to smoke one cigarette. In contrast to heart rate, skin temperature (reflecting cutaneous vascular tone) declined and rose in association with changes in blood nicotine concentrations, showing no evidence of tolerance.

The above studies indicate rapid development of tolerance to some (but not all) actions of nicotine in people. These studies were performed with cigarette smokers who had abstained from smoking the night before the study. Since significant quantities of nicotine persist in the body even after overnight abstinence, there is probably some persistence of tolerance. Experimental data supporting this conclusion were obtained in a study of cardiovascular responses to infused nicotine in smokers following either an overnight or 7-day tobacco abstinence (Lee, Benowitz, Jacob 1987). Heart rate and blood pressure responses were significantly greater after more prolonged abstinence. However, within 60 to 90 min, the blood concentration-effect relationship in subjects after brief abstinence approximated that observed after prolonged abstinence. Thus, a significant level of tolerance persists throughout the daily smoking cycle, but is lost with prolonged abstinence. Tolerance, at least after abstinence for one week, is rapidly reestablished with subsequent exposure.

Animal Studies

Many studies demonstrate that acute tolerance or tachyphylaxis develops very quickly to actions of nicotine. Barrass and coworkers (1969) demonstrated that pretreatment of mice with a single i.v. dose (0.8 mg/kg) of nicotine resulted in an increase in the LD₅₀ (dose which is lethal to 50 percent of animals) for nicotine. Maximal protection was seen 5 min after the injection, but this protection diminished steadily over the next hour. Tachyphylaxis develops to the effects of nicotine on locomotor activity. Stolerman, Bunker, and Jarvik (1974) noted that pretreating rats with a 0.75-mg/kg dose of nicotine 2 hr before challenge doses of nicotine (0.25 to 4.0 mg/kg) resulted in a shift of the nicotine dose-response curves, indicating reduced sensitivity. The ED₅₀ values (doses that are effective in producing the measured response in 50 percent of animals) for nicotine-induced decreases in locomotor activity were nearly 2.4-fold greater in nicotine-pretreated rats than in saline-pretreated animals. Nicotine pretreatment also results in tachyphylaxis to the effects of nicotine on body temperature (hypothermia) in cats (Hall 1972), water-reinforced operant responding in rats (Stitzer, Morrison, Domino 1970), discharge of lateral geniculate neurons of cats (Roppolo, Kawamura, Domino 1970), repolarization of sartorius muscle in frogs (Hancock and Henderson 1972), blood pressure elevation in rats (Wenzel, Azmeh, Clark 1971), contraction of aortic strips in rabbits (Shibata, Hattori, Sanders 1971), respiratory stimulation in cats (McCarthy and Borison 1972), and gastrointestinal contraction in squid (Wood 1969) and guinea pigs (Hobbiger, Mitchelson, Rand 1969). More recent studies have demonstrated that pretreatment with as little as one dose of nicotine will attenuate nicotine-induced elevations of plasma corticosterone (Balfour 1980) and adrenocorticotropic hormone (ACTH) (Sharp and Beyer 1986) levels in rats (see also Chapter III).

The interval between the pretreatment and challenge doses of nicotine is a critical factor that determines whether tachyphylaxis is observed. Aceto and coworkers (1986) examined the effect of i.v. nicotine infusion on heart rate and blood pressure in the rat. Tolerance did not develop when the interval between pretreatment and challenge doses was 30 min; marked tolerance was detected when the interval was reduced to 1 min. However, Stolerman, Fink, and Jarvik (1973) observed that after a single intraperitoneal dose of nicotine to rats, acute tolerance to a second dose did not become maximal until 2 hr after the initial injection.

Mechanisms of Tachyphylaxis

Although tachyphylaxis has been described for a wide variety of nicotine's effects, very little is known about mechanisms. A nicotine

metabolite may play a role in the development of tachyphylaxis. Barrass and colleagues (1969) argued that nicotine metabolites may block nicotine receptors and thereby antagonize nicotine's lethal effects. This argument was made because pretreatment with nicotine-N'-oxide protected mice from the lethal effects of large doses of nicotine. LD₅₀ values were increased approximately ninefold by pretreatment with nicotine-N'-oxide. These authors hypothesized that this protection may involve conversion of nicotine-N'-oxide to hydroxynicotine. Their results indicated that injection of a reduction product of cotinine, believed to be hydroxynicotine, gave immediate protection, whereas maximum protection was not seen until 40 min after injection of nicotine-N'-oxide. Thus it appears that metabolism, possibly to hydroxynicotine, is required for the protective action of nicotine-N'-oxide.

Another hypothesis is that tachyphylaxis is the result of desensitization of nicotine receptors. Desensitization of the receptor involves a conformational change that results in increased affinity of the nicotinic receptor for agonists coupled with decreased ability of the receptor to transport ions (Weiland et al. 1977; Sakmann, Patlak, Neher 1980; Boyd and Cohen 1984). Desensitization of nicotinic receptors at the motor end-plate was first described by Katz and Thesleff (1957) and has since been studied by a large number of investigators, using either skeletal muscle or the electric organs of the eel, *Torpedo californica*. Although tachyphylaxis has been commonly suggested as being due to desensitization of brain nicotinic receptors, the role of desensitization in tachyphylaxis to specific behavioral effects of nicotine has not been studied. This is because concentrations of nicotinic receptors in specific areas of the brain corresponding to the behavioral effects being measured are not high enough to use available methods.

Chronic Tolerance

Human Studies

Chronic tolerance to tobacco and nicotine has not been studied systematically in human subjects, but it is clear, as noted previously, that some tolerance does develop. Tolerance is not complete; symptoms of nicotine toxicity such as nausea appear when smokers increase their normal tobacco consumption by as little as 50 percent (Danaher 1977).

These findings are consistent with the observations that smokers increase their tobacco consumption and intake of nicotine with experience. Such escalating dose patterns may be observed for several years after initiation of either cigarette smoking or smokeless tobacco use. Cigarette smokers may achieve such increases by augmenting the number of cigarettes smoked and by increasing the amount of nicotine extracted from each cigarette. For users of