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SECTION 6. PHARMACOLOGICAL AND TOXICOLOGICAL IMPLICATIONS OF SMOKE CONSTITUENTS ON CARDIOVASCULAR DISEASE

Introduction

Cardiovascular diseases are the leading causes of death in most of the technologically advanced countries of the Western Hemisphere, accounting for approximately half of all deaths annually in the United States (see Appendix A). The most common among these diseases are atherosclerosis and coronary heart disease; their ischemic complications result in increased morbidity and mortality. Coronary heart disease is the leading cause of death in the United States, accounting for two-thirds of all cardiovascular deaths (*96*).

It is generally acknowledged that coronary heart disease is a multifactorial process; that is, a variety of factors are involved in the development and clinical manifestations of this disease. Therefore, it is not a simple task to determine the etiology and time course of atherogenic development. In addition, the study of atherosclerosis is singularly difficult because no model in the experimental animal exactly replicates the human disease in physiological, morphological, and clinical detail. Investigations in human subjects are further limited by the inability to diagnose the disease in preischemic phases (*44*). Most studies of the pathology of cardiovascular diseases (CVD) have been based on autopsies by coroners or on hospital populations in which only a limited fraction of decedents have been examined. Individuals may show considerable variance in the degree of atherosclerosis identified at autopsy, limiting the value of retrospective analysis (*137*).

In 1971, the U.S. Government established the Task Force on Arteriosclerosis to assess research needs and to make recommendations on priorities for future program plans in this area. Most of the recommendations of this task force have been implemented during the past decade, and important advances have been made in basic and clinical research (*96*). Most important, major epidemiological associations of cardiovascular disease risk not only have been established, but also have been supported by examinations of the arterial wall itself, enabling an increased understanding of the basic mechanisms of disease processes.

Research in cell and molecular biology has provided new information about the interaction of blood-borne components, such as cholesterol, with the arterial wall. Basic research regarding this risk will help to increase our understanding of the effects of other circulating components, such as inhaled cigarette smoke constituents, and will elucidate the susceptibility of arterial cells to these effects.

The most firmly established modifiable risk factors for atherosclerotic CVD are hypercholesterolemia, hypertension, and cigarette smoking. In addition to these, diabetes mellitus, lack of exercise, obesity, and type A behavior have all been suggested as contributors to the multifactorial process known as atherogenesis (*82*). The

assessment of any risk factor, such as cigarette smoking, must be made within the constellation of other risks, i.e., susceptibility to disease that is predicted by multifactorial analysis (*53, 82*).

In the case of cigarette smoking, we are faced with an extremely difficult effort in determining direct cause and effect phenomena that are attributable to single factors. Over 4,000 different compounds have been identified in tobacco smoke (*45*), and the determination of the direct or indirect actions of each upon the arterial wall seems an impossible task.

We will attempt, however, to examine the major components believed to be associated with increased risk for CVD and to remember the multiple risk factors that might be associated with the development of cardiovascular dysfunctions in cigarette smokers.

The variety of possible pharmacological and toxicological implications of smoke and its constituents—and the absence of firm proof of what mechanisms are precisely involved in the unequivocal cause and effect relationship between smoking and cardiovascular disease—should not detract from our confidence in the epidemiologically and clinically irrefutable evidence of the cause and effect role of cigarette smoking in contributing importantly toward heart disease.

Tobacco Smoke: Physical Nature and Chemical Composition

Inside the burning cone of a cigarette, a variety of physical and chemical processes occur in an oxygen-deficient, hydrogen-rich environment at temperatures up to 900°C. Two major regions for the smoke formation are primarily observed—the heat-producing combustion zone and the pyrolysis-distillation zone (*11*). The mainstream smoke (MS) is formed during puff drawing; the sidestream smoke (SS) is generated largely by the smoldering of the cigarette between puffs. Throughout this review, data are discussed relating to cigarette smoke generated by smoking machines, unless otherwise noted. The standard machine smoking parameters for cigarettes were primarily developed for comparing smoke yields obtained under identical conditions. Today, these smoking parameters do not reflect the smoking behavior of many of the cigarette smokers and especially not that of smokers of low-yield cigarettes who tend to draw puffs of greater volume more frequently (*64, 68, 145*).

The mainstream smoke of tobacco products represents a very dense aerosol. In the case of a cigarette without a filter tip, it contains about 5×10^9 spherical particles per milliliter. The size of the particles varies between 0.1 and 1.0 μm, with an average diameter of 0.4 μm (*12*). Three to eight percent of the weight of the total mainstream smoke of a cigarette without a filter tip is attributable to the particulate matter. The remainder consists of vapor phase components with nitrogen (50 to 70 percent), oxygen (10

TABLE 1.—Approximate number of smoke compounds identified in some major compound classes

Compound class	Number identified
Amides, imides, lactones	237
Carboxylic acids	227
Lactones	150
Esters	474
Aldehydes	108
Ketones	521
Alcohols	379
Phenols	282
Amines	196
N-Heterocyclics	921
Hydrocarbons	755
Nitriles	106
Anhydrides	11
Carbohydrides	42
Ethers	311
Total	4,720

SOURCE: Dube and Green (*45*).

to 15 percent), carbon dioxide (10 to 15 percent), and carbon monoxide (3 to 6 percent) as major constituents (*27, 106*). Of the more than 4,000 components identified in cigarette smoke, 400 to 500 are present in the vapor phase (*27, 45*). Table 1 lists some major classes of smoke components as recently recorded by Dube and Green (*45*). The total number of 4,720 in the table exceeds by far the total number of identified compounds because of repeated listing of the compounds that contain multifunctional groups. The acute toxicity of tobacco smoke is influenced not only by the chemical composition, aerosol concentrations, and particle sizes of the smoke, but also by the smoke pH. With a pH greater than 6.2, the smoke contains increasing amounts of unprotonated nicotine, which is the most toxic form of this habituating agent (Figure 1) (*26*). The unprotonated nicotine is at least partially present in the vapor phase and thus is likely to be more rapidly absorbed by the smoker (*5*).

The U.S. cigarette is filled with a blend of tobaccos consisting of Bright, Burley, and Turkish types. Its mainstream smoke pH lies between 5.5 and 6.1. The smoke of cigarettes and cigars made up entirely of Burley or dark tobacco varieties has pH values of about 6.5 for the first puffs and up to 8.0 for the last puffs (*26*).

Sidestream smoke, which is formed between puff drawings, is freely emitted into the air from the smoldering tobacco products. The peak temperature in the burning cone of a cigarette during puff drawing is about 900°C and between puffs it is about 600°C (*162*). This is an important factor for the divergence of specific toxic agents generated in mainstream and sidestream smoke. Another major

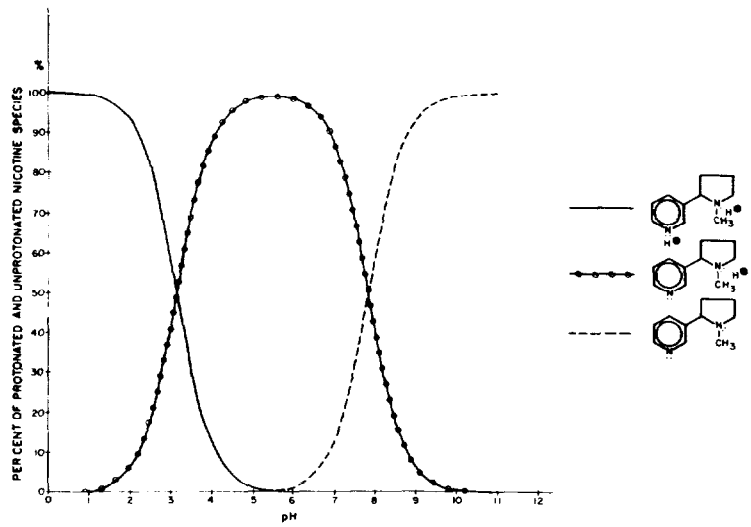

FIGURE 1.—Protonation of nicotine
SOURCE: Brunnemann and Hoffmann (*26*).

difference is that the sidestream smoke leaving the site of formation is subjected to greater air dilution and faster temperature decline than is the mainstream smoke, which travels through the tobacco column and is then inhaled as a concentrated aerosol. These conditions for sidestream smoke generation favor formation of aerosol particles of smaller size (0.01 to 0.1 μm) than those occurring in the mainstream smoke (0.1 to 1.0 μm) (*12*). The pH of sidestream smoke of a U.S. blended cigarette varies between 6.7 and 7.5, compared with pH values of less than 6.2 for the mainstream smoke of the same cigarette (*26*). This sidestream smoke thus contains free nicotine, which is essentially absent in mainstream smoke. Furthermore, during smoldering, sidestream smoke is generated in a zone that is even more oxygen deficient than the zones involved in mainstream smoke generation during puff drawing. Consequently, components that are primarily formed in a reducing atmosphere are released into the environment to a greater extent than those formed in mainstream smoke that is inhaled by the smoker. Table 2 lists the amounts of some selected toxic compounds in mainstream smoke and the ratios of undiluted sidestream smoke components to mainstream smoke components (*69*).

The air dilution of sidestream smoke emitted into the atmosphere is, of course, a determining factor for any assessment of human exposure. Nonetheless, in risk assessment, consideration must also

TABLE 2.—Distribution of selected toxic compounds in cigarette mainstream smoke (MS) and sidestream smoke (SS) of nonfilter cigarettes

Compound	MS	SS/MS
Gas phase		
Carbon monoxide	10–23 mg	2.5–4.7
Carbon dioxide	20–60 mg	8–11
Formaldehyde	70–100 μg	0.1–≃50
Acrolein	60–100 μg	8–15
Acetone	100–250 μg	2–5
Pyridine	20–40 μg	10–20
3-Vinylpyridine	15–30 μg	20–40
Hydrogen cyanide	400–500 μg	0.1–0.25
Nitrogen oxides (NO_X)	100–600 μg	4–10
Ammonia	50–130 μg	40–130
N-Nitrosodimethylamine	10–40 ng	20–100
N-Nitrosopyrrolidine	6–30 ng	6–30
Particulate phase		
Particulate matter	15–40 mg	1.3–1.9
Nicotine	1–2.3 mg	2.6–3.3
Phenol	60–120 μg	2.0–3.0
Catechol	100–280 μg	0.6–0.9
Aniline	360 ng	30
2-Toluidine	160 ng	19
2-Naphthylamine	1.7 ng	30
Benz[a]anthracene	2.0–7.0 ng	2–4
Benzo[a]pyrene	20–40 ng	2.5–3.5
Quinoline	500–2,000 ng	8–11
N'-Nitrosonornicotine	200–3,000 μg	0.5–3
N-Nitrosodiethanolamine	20–70 ng	1.2
Nickel	20–80 ng	13–30
Polonium-210	0.03–0.5 pCi	?

SOURCE: Hoffmann et al. (*70*).

be given to the fact that nitrogen oxide (NO), emitted into the environment as a sidestream smoke component, is rapidly oxidized to the more toxic nitrogen dioxide (NO_2) (*27*).

Nicotine

Chemistry

A number of observations have supported the concept that nicotine is the major habituating agent in tobacco and tobacco smoke (*90*). In addition to nicotine, tobacco contains a large variety of other alkaloids, most of which are 3-pyridyl derivatives (Figure 2). In the blended U.S. cigarette, nicotine constitutes 85 to 95 percent of the total alkaloids. Its concentration in the leaf depends primarily on the tobacco type and variety, stalk position, and cultivating practices (*140*). A study on the fate of ^{14}C-labeled nicotine, added in the form of

a salt solution to the tobacco rod of a filter cigarette, revealed that 14.9 percent of labeled nicotine emerged in the mainstream smoke and 37 percent appeared in the sidestream smoke; 18.5 percent of ^{14}C-nicotine was deposited in the butt, and the remainder ($\cong$ 30 percent) was broken down into pyrolysis products (Table 3) (*73*). The major pyrolysis products of nicotine in MS and SS of cigarettes are carbon dioxide, carbon monoxide, 3-vinylpyridine, 3-methylpyridine, pyridine, myosmine, and 2,3'-dipyridyl (*130*).

In most countries, cigarettes have shown a gradual and significant reduction over the last three decades in the sales-weighted average delivery of nicotine. In the United States the sales-weighted average nicotine yields decreased from 2.7 mg in 1955 to $\geq$1.0 mg in 1982 (*146*). These nicotine reductions have been achieved primarily by technological modifications and perhaps some agricultural changes. The technological methods encompass extraction, oxidation or transformation of nicotine into less toxic compounds (*91*), formulation, and whole leaf curing. Reduction of nicotine delivery may be achieved by lowering the transfer of the alkaloid from tobacco into the smoke. This is accomplished by use of expanded tobacco laminae, adding leaf mid-veins and stems in the form of tobacco sheets (reconstituted tobacco), and by modifications of cigarette paper and by filtration (air dilution). From an agricultural standpoint, breeding lines have been developed with low levels of nicotine; however, these are not being used in commercial varieties at present (*35*).

In 1982, about 90 percent of the U.S. cigarettes sold had filter tips made of cellulose or cellulose acetate or combinations of these with charcoal. Twenty-five percent of these filter cigarettes were perforated to allow greater air dilution of the drawn smoke puffs. More recent filter construction utilizes longitudinal air channels in addition to perforation for maximal smoke dilution by air (*70, 146*).

From the machine smoking of cigarettes, using standardized parameters of taking one puff per minute of 2 seconds' duration with a volume of 35 ml, the U.S. Federal Trade Commission reported in March 1983 that the nicotine values of 208 commercial brands ranged from $<$0.05 to 2.0 mg per cigarette (*146*). However, many people who smoke these cigarettes derive very different levels of smoke components from them, primarily because nicotine delivery in the mainstream smoke influences human smoking behavior and causes many smokers of low-yield products to draw puffs more frequently, take larger puff volumes, and inhale more deeply. This phenomenon has been observed by determining the smoking profiles of individuals or by assaying nicotine and cotinine in the sera of smokers (*64, 65, 127*). Cigarette filter construction that allows partial occlusion of the perforations or air channels of the filter tip may also lead to delivery of higher concentrations of mainstream smoke (*89*). Nicotine in mainstream and sidestream smoke of tobacco products is

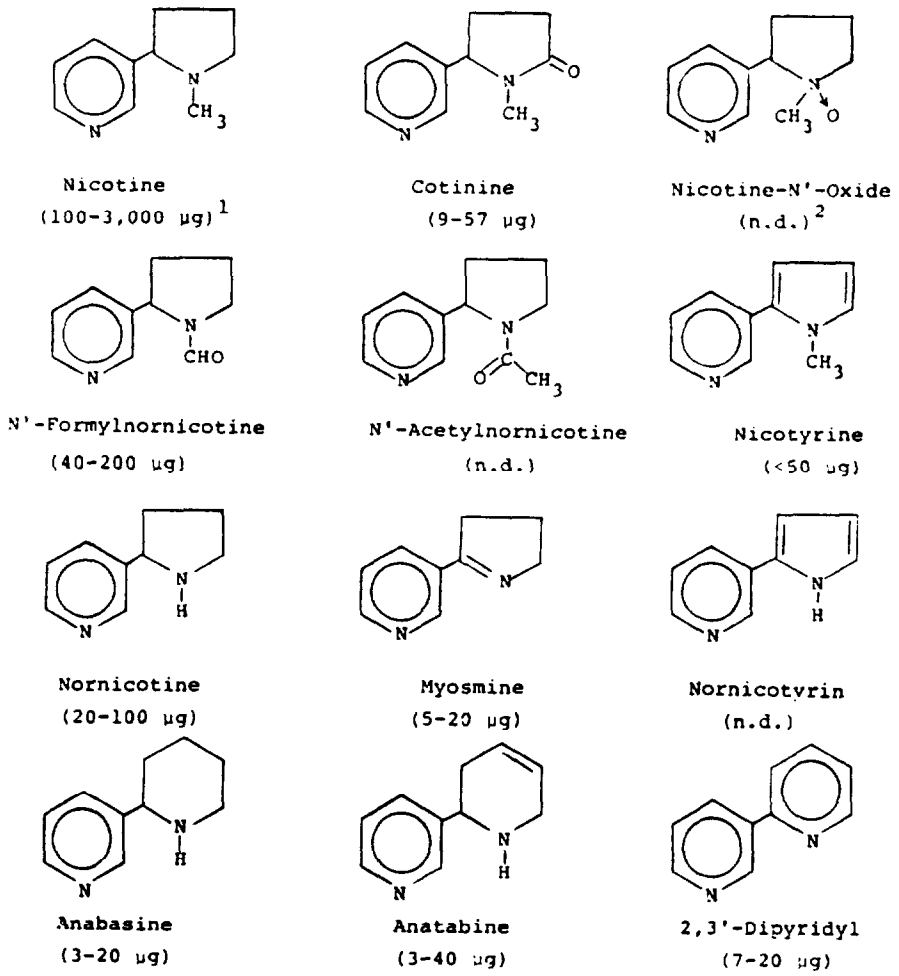

FIGURE 2.—Major alkaloids in tobacco and tobacco smoke

[1] Numbers within parentheses denote µg in the smoke of one cigarette.
[2] n.d.: Not determined.
SOURCE: Schmeltz and Hoffmann (*130*).

today primarily quantitated by gas chromatography (*151*). In physiologic fluids such as saliva, serum, or urine, smokers' exposure to nicotine is assessed by measuring the alkaloid itself or its major metabolite, either by gas chromatography with a special detector (*63*)

TABLE 3.—Distribution of nicotine and its pyrolysis products in cigarette smoke

Smoke products	Nicotine (percent)	Nicotine pyrolysis products (percent)
Mainstream particulates	14.9	0.6
Mainstream vapor phase	0.0	4.1
Sidestream particulates	37.0	4.1
Sidestream vapor phase	0.0	16.3
Butt (deposition)	18.5	1.7
Ash	0.0	0.0
Total	70.4	26.8

SOURCE: Houseman (*73*).

or, for large volumes of analyses, preferably by radioimmunoassay (RIA) (*92*).

Metabolism

The quantity of nicotine absorbed by the smoker depends on a number of factors, such as its concentration in the smoke, the individual's smoking pattern, and the smoke pH. As discussed earlier, pH values greater than 6.2 increase the amount of unprotonated nicotine in the smoke (Figure 1) (*26*). In the oral cavity, nicotine absorption varies between 4 and 45 percent (*5*). In the case of cigar smoke, the presence of free nicotine in the vapor phase, which is readily absorbed, and the harsh nature of alkaline smoke appear to be the major reasons for the cigar smoker's tendency to avoid inhalation of this smoke (*144*). That levels of cotinine in the serum of cigar smokers approximate those of cigarette smokers supports the concept that nicotine from cigar smoke is absorbed through the mucous membranes of the oral cavity. Smokers of blended cigarettes and of British types of cigarettes ($pH < 6.2$) tend to inhale the smoke. In general, between 15 and 25 percent of the nicotine in tobacco appears in cigarette mainstream smoke, from which up to 90 percent is absorbed (*162*). The extraction of nicotine from the lungs occurs quite efficiently (*143*). Nicotine enters the pulmonary capillary blood and reaches the brain via the arterial systems (*4*).

The metabolism of nicotine occurs principally in the liver. Its main metabolite, cotinine, appears in the blood within a few minutes after inhalation, and significant amounts of other metabolites appear in the tissues after about 5 minutes. The kidneys and lungs may, to a minor extent, also be involved in the metabolism of nicotine (*24, 143*).

Figure 3 illustrates the pathways of the metabolism of nicotine (*54*). Cotinine is the major metabolite of nicotine. It appears within a few minutes in the blood of smokers and has a half-life time of between 20 and 30 hours (*92*). Cotinine has been detected in

concentrations up to 10 μg per milliliter in the urine of smokers and up to 40 ng in the urine of nonsmokers who remained in a heavily polluted indoor environment for a minimum of 1 hour (*65, 69*). Nicotine-N′-oxide has been detected in the urine of smokers (*55*). In the oral cavity of man, nicotine-N′-oxide is reduced to nicotine (*78*). It has also been found that ingested nicotine-N′-oxide is reduced to nicotine by the gut flora or by intestinal enzymes (*54*). Nornicotine has been observed to be formed by N-demethylation of nicotine (*54*). γ-(3-Pyridyl)-γ-methylamino butyric acid has been identified in human urine as 3-pyridylacetic acid, the major end product of nicotine metabolism (*54*).

Association With Cardiovascular Diseases

The pharmacological effects of nicotine absorbed by inhalers might be considered small and transient, but they are repeated many times each day and act directly on the sympathetic and parasympathetic cells of the central nervous system (CNS). Nicotine exerts a direct effect on ganglion cells, producing transient excitation followed by depression or transmission blockade. At the level of the central nervous system, nicotine causes CNS stimulation followed by CNS depression (*133*).

Nicotine, like acetylcholine, discharges adrenaline from the adrenal glands and other chromaffin tissue, and releases noradrenaline from the hypothalamus. It also releases antidiuretic hormone from the pituitary (*33*), and by excitation of chemoreceptors in the carotid body, augments various reflexes (*36*).

The cardiovascular responses to nicotine, in general, parallel those that follow stimulation of the sympathicoadrenal system. Because nicotine has both stimulant and depressant effects, the responses of the cardiovascular system represent the sum of several different modes of action of this compound.

Recently, progress has been made in identifying nicotine receptor sites in the brains of animals (*81*). When microinjected into the third cerebral ventricle, nicotine increased cardiac and respiratory rates, but it did not alter these parameters when microinjected into the periaqueductal gray.

Most studies have shown that in people with known coronary heart disease, cigarette smoking increases the incidence of angina pectoris, although angina directly precipitated by smoking is rare. With additive risk factors such as hypertension, the threshold for attacks of angina can be significantly lowered by daily cigarette smoking. A long-term followup study of part of the Framingham cohort demonstrated a lack of association between smoking at diagnosis and subsequent cardiovascular events, however (*74*). This appeared to be related to changes in habits following diagnosis, implying that improved prognosis could be achieved by withdrawal

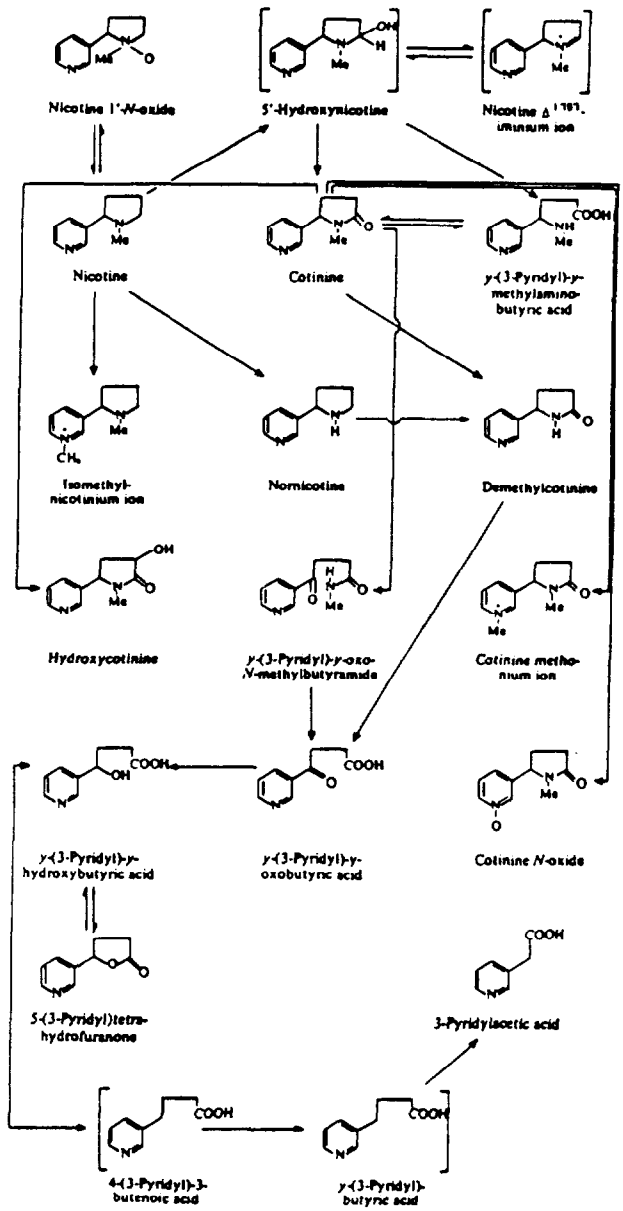

FIGURE 3.—Pathways of nicotine metabolism
SOURCE: U.S. Department of Health, Education, and Welfare (*144*).

TABLE 4.—Effects of nicotine on the cardiovascular system

Increases in blood pressure
Increases in heart rate
Electorcardiographic changes
Nonspecific ST and T-wave changes
Increased conduction velocity, propensity toward arrhythmias
Exacerbation of angina in coronary patients
Diminished left ventricular performance in coronary patients

SOURCE: Stimmel (*136*).

of daily cigarette smoking. The relationship here cannot be attributed solely to removal of nicotine; it can also be related to improvements in cardiovascular status such as increased oxygen saturation.

The exact mechanism whereby nicotine could trigger a cardiovascular event is unknown. However, a common initial feature of several suggested mechanisms is a sympathetic discharge (Table 4) (*14*). This stimulation of sympathetic nerves, with consequent release of the neurotransmitter norepinephrine within the myocardium, has been shown to lower the ventricular fibrillation threshold in animals (*38*). Responses to sympathetic activity such as hemodynamic parameters of heart rate and systolic blood pressure generally reflect plasma nicotine concentrations (*88, 112, 135*). Increase in pulse rate and systolic pressure, decrease of pressure pulse transit time, and digital blood flow are well correlated with nicotine levels of the cigarettes smoked (*88*); the same effects can be noted with intravenous injection of varying doses of L-nicotine (*150*).

In a recent study on the cardiovascular effects of infused nicotine, Benowitz and colleagues (*18*) showed that nicotine infusions could achieve plasma concentrations and cardiovascular effects similar to those induced by cigarette smoking. Heart rate increased after low concentrations of nicotine were administered and reached a plateau, beyond which increasing blood concentrations of nicotine had no effect. In these studies, as in those of Cryer et al. (*38*), elevations in pulse rate and blood pressure occurred promptly after the start of nicotine exposure and preceded the investigators' measurement of increased increments of plasma epinephrine and norepinephrine concentrations. These hemodynamic effects of nicotine are probably not mediated by circulating catecholamine levels, but are due to local release of the sympathetic neurotransmitter norepinephrine from adrenergic axon terminals.

The increase in circulating catecholamines has been demonstrated to be nicotine dose dependent by Hill and Wynder (*66*), who found an increase in epinephrine levels in plasma to be proportional to the nicotine content of the cigarette smoked. Although elevated with smoking, norepinephrine levels were not correlated with plasma

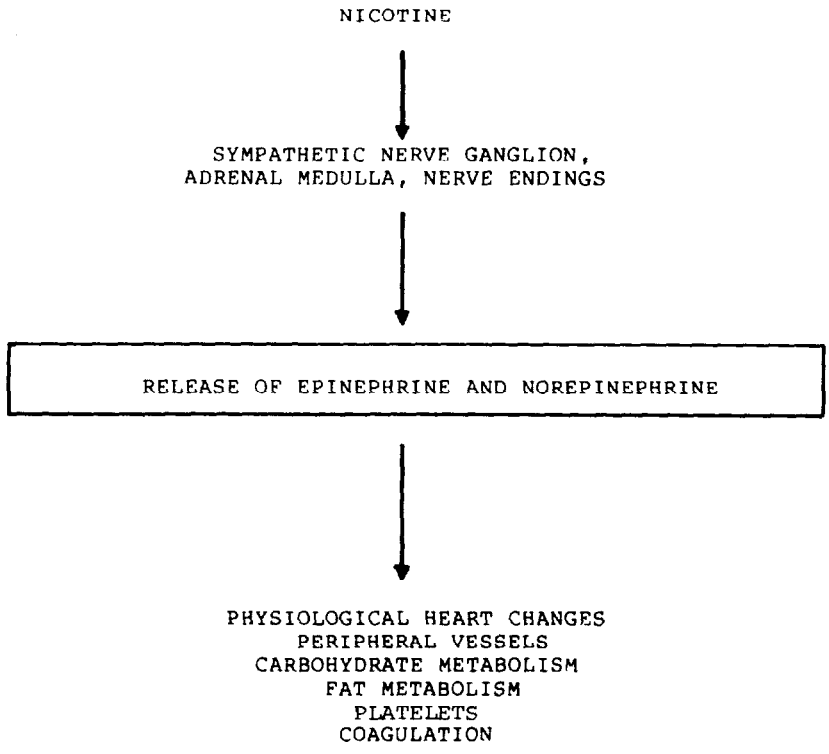

FIGURE 4.—Net results of catecholamine elevations as mediated through nicotine stimulation

SOURCE: Schievelbein and Eberhardt (*129*).

nicotine. The release of epinephrine is probably mediated through the adrenal medulla and chromaffin tissue. A more recent study showed that when the pH of the smoke is altered to favor more effective absorption of the alkaloid, smokers compensate to adjust nicotine intake, resulting in modulation of the epinephrine response (*59*).

The net results of catecholamine elevations as mediated through nicotine stimulation are summarized in Figure 4. In general, the cardiovascular responses of increased heart rate and augmented contractility create a variable oxygen need; nicotine also dilates the coronary arteries, which exerts a measurable effect only in healthy individuals without coronary heart disease (*129*).

Available evidence suggests that the release of adrenergic transmitters by nicotine and other cholinergic agonists is mediated by nicotine receptors (*159*). Norepinephrine outflow from adrenergically innervated tissues is evoked by nicotine and the nicotinic agonist dimethylphenylpiperazinium, but not by the muscarinic agonists methacholine and oxytremorine (*20*). Studies using a variety of inhibitors of nicotinic receptors have resulted in a number of possible and related mechanisms of action of nicotine on the central nervous system and the cardiovascular system: (a) prevention of inactivation of the adrenergic transmitter after its release, (b) facilitation of adrenergic transmitter release, (c) involvement of histamine (*31*), (d) mediation of nicotine by neuronal uptake, (e) induction of nerve action potentials, (f) depolarization of nerve terminals, and (g) exocytotic release of adrenergic transmitter. These mechanisms are not necessarily mutually exclusive.

Through these mechanisms, nicotine might exert powerful effects on the sympathicoadrenal control of cardiovascular function. Many issues remain to be elucidated and understood, however, such as the effects of drug interactions with nicotine (*42*), the interindividual variability in metabolism of this alkaloid (*18*), and tolerance (*132*), as well as the habituating mechanisms of the cigarette smoking habit itself (*77*).

In addition to hemodynamic perturbations, nicotine intake has been implicated in atherosclerotic changes in the arterial wall itself. Nicotine has been associated with acceleration of atherosclerotic disease, the initiation of which is becoming more clearly understood. Experimental evidence is emerging that links atherogenesis to tobacco smoke inhalation, possibly through mechanically induced intimal damage as well as through hypoxia. The pathogenesis of vascular injury might occur via two mechanisms. The first mechanism is accelerated by high levels of blood fat, particularly cholesterol in the low density lipoprotein fraction, and it is the abnormal accumulation of this lipid in the lining of large arteries that leads to changes in cells of the arterial wall, causing a thickened intima rich in fat and smooth muscle cells.

The other mechanism of atherogenesis resides in vessel injury and thrombosis, with the thrombus persisting and becoming organized, leading to vessel wall thickening (*103*). Plaque development may therefore involve damage to the intima and a complex interaction between intima and the circulating blood, resulting in (a) platelet release of mitogenic factors, (b) increased uptake of serum lipoproteins, and (c) intracellular deposition of lipid (*123*). It has been shown that platelets and cells, such as macrophages, can make available at sites of injury a protein that will cause smooth muscle cells to proliferate and another that will make them migrate into the arterial intima (*122*).

Unfortunately, we have relatively little evidence about how the endothelium can be injured. Blood flow, particularly at a high shear rate, can damage the endothelium, and high serum lipid levels can be associated with vessel injury (*125*). Immune reactions have been shown to damage vessels, and products from cigarette smoke components are suspected of causing injury to the cellular lining of vessels (*87*).

Nicotine can be implicated in the processes of atherogenesis through several of its known actions on the cardiovascular system (*47*). Via catecholamine release and the subsequent increase in free fatty acids, nicotine affects different steps in blood coagulation pathways, including platelet aggregation (*48, 129*) and increased fibrinolytic activity, with possible injurious effects to the cells lining the arterial wall. Blood pressure and heart rate can influence the blood shear rate, and transitory increases in blood sugar can influence basic metabolic rates (*42*).

A desquamating effect of nicotine on the vascular endothelium has been demonstrated in rabbits when nicotine was given in relatively low doses, as compared with those received by cigarette smoking (*23*). In a clinical assay, smoking two cigarettes increased the number of circulating endothelial cells by 50 percent. The interaction of nicotine and other cigarette smoke constituents could influence this increase in endothelial cells released into the bloodstream (*137*).

The possible role of prostaglandins in the promotion of atherogenesis is tied in with the nicotine induction of altered prostaglandin activity (*155, 157*). Evidence suggests that smoking causes a thromboxane (TxA_2):prostacyclin ($PG1_2$) imbalance. Nicotine increases platelet activity in vitro, and cigarette smoking in general has been shown to increase this activity in humans (*95*) as well as to potentiate platelet aggregability in the presence of hyperlipidemia (*67*). Nicotine inhibits the ability of coronary arteries to synthesize prostacyclin-like substances in vitro, an effect that is more pronounced in vascular cultures derived from patients who smoke than from nonsmoking control subjects (*156*). This phenomenon and alterations in endothelial barrier functions have also been investigated in umbilical arteries derived from smoking and nonsmoking mothers (*7*). Pronounced changes were seen in the endothelium of the vessels derived from smoking mothers. These consisted of swelling of the cells with numerous blebs on the luminal membrane. In addition, extensive edema of the subendothelial spaces was a regular feature, as was an increase in basement membrane thickness. These changes are consistent with an increase in endothelial permeability (*7*).

Cigarette smoking and nicotine in particular may therefore alter platelet function and prostaglandin production in potentially deleterious ways, suggesting that smoking may interfere with vascular

defense against platelet deposition, a factor in atherogenesis (*124*). Specifically, nicotine might alter the occupancy of endothelial receptors for β-adrenergic catecholamines, increasing the intracellular concentration of cyclic AMP and inhibiting the release of arachidonic acid from endothelial cell phospholipids, and thereby reducing prostacyclin synthesis (*1*).

Nicotine has been implicated at other levels of arterial cell function. This alkaloid has been found to produce an exposure-time- and concentration-dependent effect on the lysosomes of endothelial cells through increases in lysosome fragility and formation of large acid phosphatase positive vacuoles, presumably lysosomes, in rat endothelial cells (*158*). These findings suggested that the lysosomal vacuolar system should be examined as a possible target for cellular dysfunction following chronic exposure to nicotine.

Alterations in the levels of lysosomal enzymes in atherosclerotic tissue have been demonstrated in the presence of single risk factors including hypercholesterolemia (*60*), hypertension, and diabetes mellitus (*160*) in animals, as well as in diseased areas of human vessels (*49*). Inhalation experiments have shown that aortic tissue from animals chronically exposed to cigarette smoke exhibited increased levels of several lysosomal enzymes as well as cholesterol and cholesteryl esters (*57*). Studies are currently being carried out to determine the specific cigarette smoke constituents responsible for these changes.

Cigarette smoking has been implicated in elevation of serum lipids and changes in lipoprotein distribution (*117*). Reports in the literature run from no alteration (*118*) to significant changes in the direction of those levels promoting atherogenesis (*25, 71*). By stimulating sympathetic nervous activity and catecholamine release, nicotine can cause an elevation in plasma free fatty acids and increased secretion of very low density lipoprotein triglycerides (*21*). Kirchbaum and colleagues (*84*) found that the rise of free fatty acids in plasma produced by smoking was twice as high in patients who had suffered a myocardial infarction as in controls. This rise in free fatty acids is probably a result of catecholamine-mediated lipolysis and may be an important mediator in the production of endothelial cell injury (*161*). In addition, cigarette smoking in general is associated with a reduction in high density lipoprotein apoprotein components (*19*) and may attenuate this lipoprotein's antiatherogenic properties by altering surface phospholipid constituents (*62*).

Carbon Monoxide

Chemistry

The formation of carbon monoxide (CO) occurs in and close to the burning cone of a cigarette by thermal decomposition, by reaction of

tobacco with atmospheric oxygen, and by secondary reactions of tobacco with carbon dioxide, water, and other primary pyrolysis products (*13, 69*). Studies with labeled precursors have shown that CO is formed at temperatures above 460°C and that about 30 percent of it derives from thermal decomposition of tobacco, 36 percent from combustion of tobacco, and 23 percent from reduction of carbon dioxide (*13*). The yield of CO in the mainstream smoke of a cigarette depends on the amount of tobacco and the type of paper burned during puff drawing, the concentration of pyrolytic precursors, the temperature profile of the tobacco during puff drawing, and the permeability of the wrapper for the outward diffusion of CO (*111*). The CO concentration in the inhaled smoke is also a function of the permeability of the cigarette paper and of the physical and chemical properties of the filter (*86, 153*).

The importance of the air velocity created during puff drawing is demonstrated by the significant increase in the smoke with increasing puff volume (Figure 5) (*69*). The somewhat higher CO yields in the smoke of cigarettes with conventional filter tips compared with those from nonfilter cigarettes have been attributed to the loss of air dilution and to the increased smoke velocity that occurs when the last 15 to 25 mm of the tobacco column are replaced by a filter tip with a nonporous wrapper (*69, 153*). This concept is also reflected in the higher CO yield for a filter cigarette smoked with puffs of different volumes (Figure 5).

In recent years, cigarettes with perforated filter tips have gained in market share. The air entering through the perforations in these filter tips dilutes the mainstream smoke and thus reduces the concentration of CO in the smoke (Figure 5). In addition, carbon monoxide is selectively reduced, most likely because of the reduced velocity of air entering the burning cone (*86*). Finally, the newly introduced filter cigarettes with longitudinal air channels reduce CO in the smoke even further (*68*). However, as discussed before, because of the compensatory changes in smoking behavior that smokers make in order to satisfy their need for a certain amount of nicotine and because of the possible obstruction of perforated filter tips or their air channels during puff drawing, the smokers may not fully benefit from the intended air dilution of the smoke (*64, 65, 68, 89, 127*).

Association With Cardiovascular Diseases

The health effects of exposure to carbon monoxide are not fully known. However, research findings in selected population groups indicate that carbon monoxide acts as an added stress factor to precipitate cardiac symptomatology or ischemic episodes in individuals already compromised by coronary disease (*3*). Additionally, excessive levels of carbon monoxide in the blood have been found by

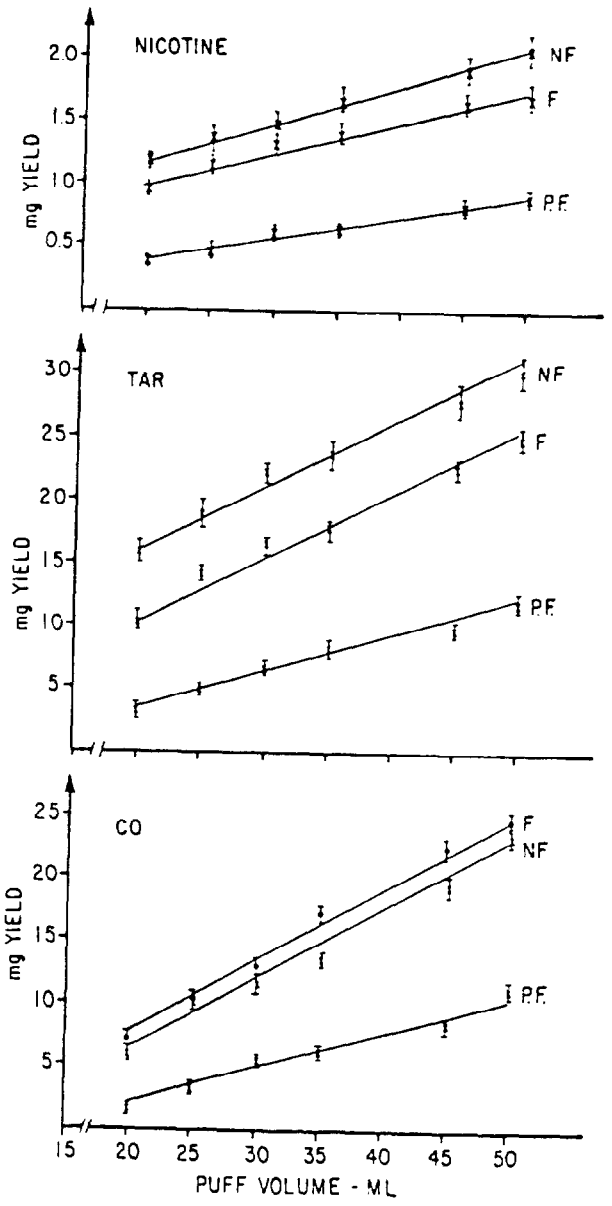

FIGURE 5.—Carbon monoxide, tar, and nicotine in the smoke of cigarettes

NOTE: NF = Nonfilter; F = Filter; PF = Perforated Filter.
SOURCE: Hoffmann et al. (69).

some investigators to impair certain perceptual and motor functions (*104*).

Carbon monoxide is a common industrial pollutant generated by any burning process. It is odorless and tasteless and gives no warning of its presence in most circumstances, thus allowing for chronic exposure over extended periods of time. The early symptoms of carbon monoxide poisoning often resemble those of a variety of diseases; thus, tissue hypoxia might occur in healthy people without forewarning (*163*).

Carbon monoxide combines with hemoglobin in large quantities. Its chemical affinity for hemoglobin is over 200 times greater than that of oxygen. The ability of carbon monoxide to cause tissue hypoxia stems from two effects on circulating processes: (a) a reduction in the total amount of oxygen carried by red cells in blood, which reduces delivery of oxygen to tissues, and (b) a shift to the left of the oxyhemoglobin dissociation curve of the blood that contains both oxyhemoglobin and carboxyhemoglobin. The leftward shift of the oxyhemoglobin dissociation curve decreases the tension at which oxygen molecules are dissociated from hemoglobin, and therefore decreases the driving pressure for diffusion of oxygen into tissues and cells (*142*). The concentration of carboxyhemoglobin reached in blood will depend upon the concentration of carbon monoxide in the inhaled gas mixture as well as on the alveolar concentration, and on arterial oxygen tension, duration of exposure, and ventilation volume (*142*).

It is noteworthy that carbon monoxide binds to muscle myoglobin as well as to hemoglobin and may exert tissue hypoxia by interfering with oxygen transport to muscle mitochondria. It can also have an effect on other hemoproteins such as those present in the cytochrome oxidase system (*35*). Cytochrome P-450, a mixed-function oxidase, probably does not bind sufficient carbon monoxide to cause inhibition of drug hydroxylation, even at a carboxyhemoglobin saturation of 15 to 20 percent (*126*). However, smokers and nonsmokers differ in their clinical reactivity to drugs such as phenacetin and diazapam through acceleration of biotransformation processes. This increased drug metabolism is believed to result from an induction of microsomal drug-metabolizing enzymes after chronic exposure to tobacco smoke (*148*). Although smoking in general results in accelerated enzyme induction, carbon monoxide has been implicated in decreased hepatic protein synthesis, a phenomenon that can be duplicated by chronic cigarette smoke exposure (*52*). This effect could be due to a deleterious reaction of hepatic tissue because of binding of carbon monoxide to intracellular hemoproteins.

It is possible that carbon monoxide may bind to hemoproteins other than cytochrome oxidase, hemoglobin, or myoglobin in sufficient amounts to inhibit their function. Tryptophan dioxygenase and

catalase have high affinities for carbon monoxide, which in the first case, could result in increased serotonin levels in several tissues, and through the latter enzyme, could cause the cellular accumulation of hydrogen peroxide, a toxic oxidant (*126*).

Cigarette smoking causes increased carboxyhemoglobin levels, thereby reducing the oxygen-carrying capacity of the blood and subsequent tissue oxygenation and, possibly, cellular metabolism. Interpretation of the effects of carbon monoxide absorption by healthy people is a subject of much controversy because effective threshold levels have not been established. Environmental exposure to carbon monoxide may be without chronic consequences in healthy people who appear able to compensate for any acute effects at levels of industrial exposure. However, in patients with angina pectoris, exposure to carbon monoxide has reduced exercise time until the onset of chest pain (*3*).

Atherosclerosis is a multifactorial disorder in which cigarette smoking and carboxyhemoglobin levels may exert varying effects, depending upon the other risk factors present. Carbon monoxide is believed to be a contributing factor to the acceleration of the disease process. Wald and associates (*152, 153*) have suggested that carboxyhemoglobin levels in tobacco smokers correlate better than a smoking history with the development of myocardial infarction, angina pectoris, and intermittent claudication. However, it should be pointed out that in such studies, elevation in carboxyhemoglobin also reflects the absorption of other constituents of tobacco smoke.

In his earlier studies, Astrup (*8*) found that carbon monoxide or decreased oxygen tension enhanced coronary atherosclerosis in cholesterol-fed rabbits and suggested that high carboxyhemoglobin levels resulting from tobacco smoking were associated with the development of occlusive arterial vascular disease. More recent experiments, conducted in a blind fashion, confirmed the presence of myocardial lesions, but failed to produce aortic atherosclerosis (*9*), although other researchers had noted morphological alterations in coronary arteries similar to those seen in the earlier experiments (*8, 40*).

Animal experiments on accelerated atherogenesis with carbon monoxide must be considered to be unsatisfactory, and although clinical trials have linked elevated carboxyhemoglobin levels with cardiovascular symptomatology, a cause and effect phenomenon for carbon monoxide and disease of the arterial wall has not been elucidated.

Carbon monoxide exposure has been related to increases in circulating blood lipids. This was first demonstrated in rabbits in which carboxyhemoglobin levels were 15 to 25 percent, resulting in elevations of serum cholesterol for a transient 2 to 3 weeks following exposure (*10*). In an epidemiological study by Van Houte and

Kesteloot (*147*) involving 42,000 subjects, significantly higher serum cholesterol levels were demonstrated in male smokers than in nonsmokers, particularly in the 30- to 39-year age group. The early Framingham results also reported slightly higher serum cholesterol levels among smokers (*41*), whereas Blackburn et al. (*22*) report nonsignificant differences between smokers and nonsmokers. Many other epidemiological investigations have analyzed the correlation between smoking status, serum cholesterol levels, and lipoprotein distributions, but the majority of results suggest a trend rather than significance in support of a connection between smoking and elevated cholesterol (*100*). This relationship does not offer an explanation for an effect of cigarette smoking on serum lipid levels, but experimental evidence suggests that carbon monoxide might exert an effect on the metabolism of chylomicron remnants (*51*). Ascorbic acid levels could limit the conversion of cholesterol to bile acids (*85*), or carbon monoxide could diminish hepatic degradation of lipoprotein constituents (*40*).

Carbon monoxide has also been implicated in the noted decrease in patency following vascular surgery and arterial reconstruction among continuing cigarette smokers (*138*). Cigarette smoking as measured by extrapolation from carboxyhemoglobin values had a definite adverse effect on the healing process and success rate of vascular surgery (*20, 56*).

Additional Contributors

Hydrogen Cyanide

Nitrates in tobacco serve as precursors for hydrogen cyanide (HCN) in the smoke (*61, 79*). This concept has been supported by studies in which ^{15}N-labeled nitrates of potassium, sodium, or calcium, respectively, had been added to cigarette tobacco and in which the isolated HCN from the smoke was found to contain ^{15}N-HCN (*79*). However, tobacco proteins appear to be the major group of precursors for HCN in the smoke (*27, 80*). This was demonstrated in pyrolysis studies with tobacco protein and amino acids and by recovery in the smoke of ^{15}N-HCN from cigarettes spiked with ^{15}N-glycine (*27, 80*). It has been shown that HCN is formed via N-heterocyclic intermediates and from pyrolidine, the decarboxylation product of proline (*80*). From these intermediates, hydrogen cyanide is split off in the pyrolysis-distillation zone of the burning cigarette under the opening of the N-containing ring (*27, 80*).

Several methods have been explored for the selective reduction of hydrogen cyanide in cigarette smoke. Charcoal-containing filter tips can remove 70 to 80 percent of the HCN from cigarette mainstream smoke (*139*). With increasing smoke dilution through filter perforation, HCN can be selectively reduced up to 80 percent, with a 70

percent ventilation rate (*105*). The extraction of protein fractions from cigarette tobacco leads also to a selective reduction of HCN (*141*). The smoke of one cigarette contains from 20 to 480 μg of HCN, the lowest values being measured in the smoke of cigarettes with charcoal-containing filter tips (*120*).

Although only nicotine and carbon monoxide have been incriminated as contributors to the increased risk of cigarette smokers for cardiovascular disease, other gas phase constituents might also play roles in the pathogenic processes. Hydrogen cyanide is an inhibitor of several respiratory enzymes and as such can influence cellular metabolism in the myocardium and arterial wall. It is also a powerful ciliatoxic agent allowing for decreased efficiency in removal of tar constitutents from the respiratory system (*16*).

The arterial effects of hydrogen cyanide, nitric oxide, and carbonyl sulfide were investigated by Hugod and Astrup (*76*) in experiments in which rabbits were exposed to these compounds alone or in combination with carbon monoxide. The duration of exposure was from 5 days to 12 weeks, and aortas were assessed morphologically for intimal damage suggestive of early atherosclerotic changes. No histotoxic effect on intimal or subintimal morphology was noted, and a parallel experiment demonstrated no effect on the coronary arteries (*75*). The duration of exposure to these compounds must be considered short, however, with the possibility that prolonged exposure might result in morphological or enzymatic alterations.

Nitrogen Oxides

A number of studies have shown that nitrate is a major precursor for nitric oxide (NO) and traces of nitrous oxide (N_2O) and nitrogen dioxide (NO_2) found in cigarette mainstream and sidestream smoke (*79, 134*). The mainstream smoke of a cigarette contains from 6 to 600 μg of NO per cigarette, depending primarily on the nitrate content of the tobacco blend and the nature of the filter tip (*105*). The correlation of nitrate content of tobacco with nitric oxide yield in the mainstream smoke appears to be linear (*27*). The smoke of cigarettes made with Burley tobacco and particularly those made with Burley stems, which are rich in nitrate, is especially high in nitric oxide (*29, 107*). Tobacco proteins appear also to contribute to the NO yield in cigarette smoke (*79*).

The concentration of nitrogen dioxide and that of methyl nitrite in freshly generated cigarette smoke is very minute (<5 μg/cigarette); however, these agents increase rapidly as a function of the aging of mainstream and sidestream smoke. Within about 3 minutes, 50 percent of NO is oxidized to NO_2 (*149*). The best approaches toward reducing NO in cigarette smoke are by reduction of nitrate in tobacco and by dilution of smoke by air entering through the holes of perforated filter tips (*27, 105*).