Procedures for gross separation into basic, acidic, phenolic, and neutral fractions and for further processing of these fractions vary from laboratory to laboratory. The criteria upon which identification is based also vary. The most reliable identifications are based upon an ultraviolet absorption spectrum and/or a fluorescence spectrum in good agreement over the entire range with that of an authentic sample and include one or more of the following: Rf value observed in a paper chromatogram (41); order of elution from alumina; mass spectrometry.

COMPOUNDS OF THE PARTICULATE PHASE OTHER THAN HIGHER POLYCYCLICS

This brief summary is based largely on the comprehensive review by Johnstone and Plimmer of the Medical Research Council at Exeter University, England (24). It should be noted that water constitutes 27 percent of the particulate phase. The major groups of compounds included are shown in Table 1.

Aliphatic and Alicyclic Hydrocarbons

Almost all of the possible hydrocarbons, C_1 through C_4, saturated and unsaturated, straight-chain and branched-chain, have been reported to be present in tobacco smoke. Intermediate, normally liquid paraffins are present. All the C_{26} through C_{33} *n*-alkanes have been identified, as well as the C_{27} and C_{29}-C_{33} isoparaffins.

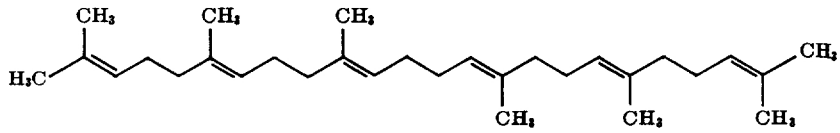
Table 1.—*Major classes of compounds in the particulate phase of cigarette smoke*

Class	Percent in particulate* phase	Number of compounds	Toxic action on lung
Acids	7.7–12.8	25	Some irritant
Glycerol, glycol, alcohols	5.3–8.3	18	Possible irritation
Aldehydes and ketones	8.5	21	Some irritant
Aliphatic hydrocarbons	4.9	64	Some irritant
Aromatic hydrocarbons	0.44	81	Some carcinogenic
Phenols	1.0–3.8	45	Irritant and possibly cocarcinogenic
	66%	254	

*Water 27%.

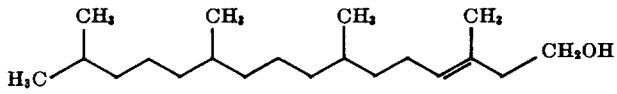
Terpenes and Isoprenoid Hydrocarbons

Isoprene, the basic unit of the terpenes and of higher terpenoids has been identified in cigarette smoke (34) as have its dimers, dipentene and 1,8-*p*-menthadiene. The triterpene squalene, consisting of six isoprene units and shown to be present in smoke (47) is of interest because of the possibility of its being cyclized to polycyclic compounds and because of its ready



Squalene

reaction with air to form hydroperoxides (which would be destroyed during attempted isolation); a hydroperoxide derived from cholesterol has been shown to be carcinogenic (cancer-causing), at least under certain conditions of administration (12). Phytadienes, products of the dehydration of the diterpene alcohol phytol, are also present in smoke and subject to air oxidation to hydroperoxides.



Phytol

Alcohols and Esters

A wide variety of mono- and dihydric alcohols, both aliphatic and aromatic, are present in tobacco smoke. Solanesol, a primary alcohol containing 9 isoprene units, has been found in both tobacco and tobacco smoke; 20 g. of pure material was isolated from 10 lbs. of flue-cured aged tobacco (0.44 percent). Grossman et al (13) found that pyrolysis of solanesol at 500° C. gives isoprene, its dimer dipentene, and other terpenoid products and concluded that the alcohol is the source of terpenoid compounds which are important factors in the flavor of tobacco smoke.

Ethylene glycol and glycerol have been found present in smoke, but it is not clear from the literature whether they are present in smoke from untreated tobacco or arise from addition of these humectant substances to tobacco to improve moistness.

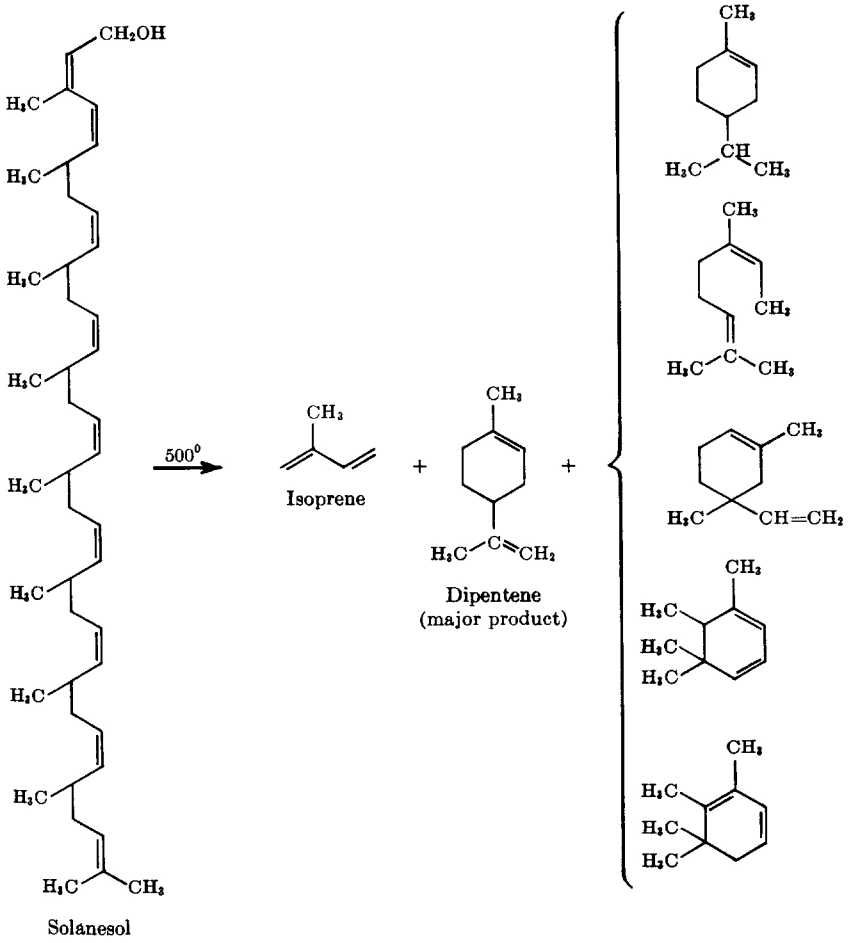
Many common esters, such as the ethyl esters of the C_2, C_3, and C_4 fatty acids, are present in smoke. Higher fatty acids are found both as free acids and as esters.

Sterols

Stigmasterol, β-sitosterol, and γ-sitosterol have been isolated from tobacco smoke. Indeed the sterol fraction is reported (29) to constitute approximately 0.15 percent of whole tar. The sterols are of interest as possible precursors of polycyclic aromatic hydrocarbons and because of the evidence, noted above, that sterol hydroperoxides can be carcinogenic.

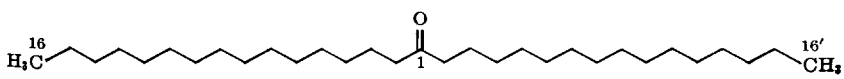
Aldehydes and Ketones

Most common aldehydes of low molecular weight (acetaldehyde, propionaldehyde, acetone, methyl ethyl ketone, etc.) have been found present



Solánesol

in tobacco smoke, as have such dicarbonyl compounds as glyoxal and diacetyl. Dipalmityl ketone exemplifies ketones of high molecular weight isolated from tobacco smoke.



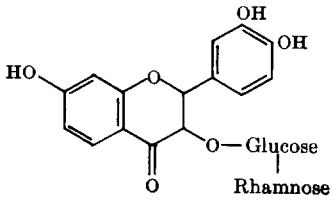
Dipalmityl ketone

Acids

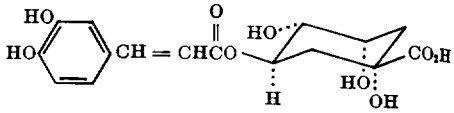
A large number of volatile and nonvolatile acids of low molecular weight are present in tobacco smoke. Fatty acids of chain length C_{13} to C_{18} are reported to constitute 1 percent of the whole tar and the bulk of these acids are present in the free form (46). Unsaturated fatty acids and keto acids (e.g., pyruvic acid) are also present.

Phenols and Polyphenols

Since the phenols and polyphenols present in tobacco leaf play an important role in the curing and smoking quality of tobacco, a great deal of investigative work has been done on the estimation, separation, and identification of complex tobacco phenols such as rutin and chlorogenic acid. The presence of simple phenols in tobacco smoke was established as early as 1871. The phenol content of smoke became of increasing importance with



Rutin

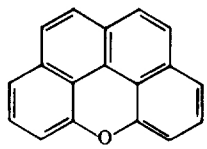


Chlorogenic acid

the demonstration that phenol and substituted phenols can function as cocarcinogens; that is, they promote the appearance of skin tumors in mice following application of a single initiating dose of a known carcinogen (4). Furthermore, the smoke from one cigarette contains as much as 1 mg. of phenols (7). In addition to simple alkylphenols, naphthols, and the polyphenols, resorcinol and hydroquinone are also present.

Alkaloids, Nitrogen Bases, and Heterocyclics

Pyridine, nicotine, nornicotine, and other substituted pyridine bases constitute some 8–15 percent of whole tar; nicotine and nornicotine constitute about 7–8 percent of the total tar. The companion bases are products of the pyrolysis of the alkaloids present in tobacco leaf. Quinoline and three polycyclic heterocyclic compounds have also been identified in smoke (45) and will be discussed later since the three polycyclic compounds are carcinogenic. A pentacyclic compound related to xanthene, namely 1,8,9-perinaphthoxanthene, has been identified in smoke (45).



1,8,9-Perinaphthoxanthene

Amino Acids

Although tobacco leaf contains a number of amino acids, relatively few have been found present in smoke; among these are glutamine and glutamic acid.

Inorganic Components

It is estimated that the main-stream smoke from one cigarette contains about 150 μg. of metallic constituents, which are mainly potassium (90 percent), sodium (5 percent), and traces of aluminum, arsenic, calcium, and copper. Arsenic is reported to be present to the extent of 0.3–1.4 μg. in the smoke of one cigarette. The inorganic compounds are most likely chlorides, but metals themselves may be present.

Apparently beryllium is present in tobacco in trace quantities, but is not volatilized in the smoking process (48). Nickel is present in cigarettes in trace amounts and may occur in main-stream smoke to a small extent, probably as the chloride (31). Spectrographic analysis has shown the presence of chromium in smoke at a level of less than 0.06 μg. per cigarette. This level appears too low to represent a hazard (48).

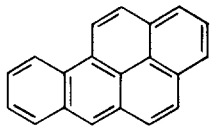
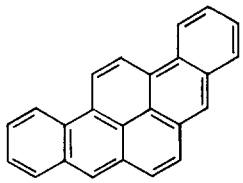
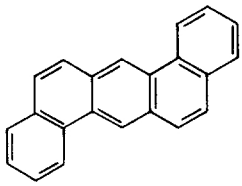
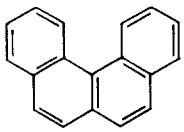
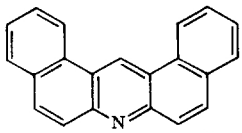
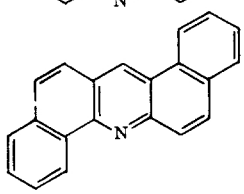
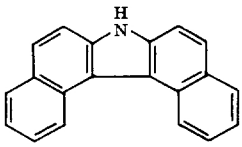
Noncarcinogenic Aromatic Hydrocarbons

The aromatic hydrocarbons present in tobacco smoke have received an enormous amount of attention since some of them are carcinogenic. Noncarcinogenic hydrocarbons of smoke containing one to three rings include benzene, toluene and other alkylbenzenes, acenaphthene, acenaphthylene, fluorene, anthracene, and phenanthrene. Hydrocarbons of established carcinogenicity to mice all contain from four to six condensed rings. However, no less than 27 hydrocarbons containing four or more condensed rings which have been tested for carcinogenicity with negative results have been isolated from tobacco smoke tar. As methods of separation and identification improve, it is almost certain that additional hydrocarbons will be found present in smoke, because almost every conceivable ring system has been demonstrated to be present and the number of possible alkylated polycyclics is very large indeed.

CARCINOGENIC HYDROCARBONS AND HETEROCYCLICS IN TOBACCO SMOKE

In 1925–30 Kennaway et al. in seeking to identify the active substance in high-boiling fractions of coal tar distillates of established carcinogenicity to mice, discovered that dibenzo(a,h)anthracene (for formula, see Table 2) prepared by synthesis evokes skin cancer when applied to the skin of mice (11). The hydrocarbon was recognized as different from the carcinogen of coal tar because its fluorescent spectrum did not match the characteristic three-banded spectrum of the tars. In 1933 Cook and co-workers (11) isolated the coal tar constituent responsible for the characteristic fluorescence and identified it as benzo(a)pyrene. It is one of the most potent of all the carcinogens now known.

TABLE 2.—*Carcinogenic Polycyclic Compounds Isolated From Cigarette Smoke*

Compound	Structure	Carcinogenicity	Amount reported, μg/1000 cigarettes
1. Benzo(a)pyrene		++++	16 (ave. of 10 reports)
2. Dibenzo(a,i)pyrene		++++	0.02-10 (2 reports)
3. Dibenzo(a,h)anthracene		++	4 (1 report)
4. Benzo(c)phenanthrene		+	not stated
5. Dibenz(a,j)acridine		+	2.7 (1 report)
6. Dibenz(a,h)acridine		+	0.1 (1 report)
7. 7H-Dibenzo(c,g)carbazole		+	0.7 (1 report)

Since the discovery of carcinogenic hydrocarbons, a large number of polycyclic hydrocarbons and heterocyclic analogs have been tested for carcinogenicity to mice and to rats in many laboratories, both by application to the skin and by subcutaneous injection. Bioassays in different laboratories, often on independently prepared samples, are remarkably consistent and place a series of hydrocarbons in the same relative order of potency. A compilation (and its supplement) prepared by J. L. Hartwell (16) of the National Cancer Institute lists 2108 compounds of which 481 were reported to cause malignant tumors in animals. All but one of the polycyclic hydrocarbons listed in Table 2 as having been identified in tobacco smoke have already been documented in the Hartwell report and can be assigned a rating as very potent (++++), potent (+++), moderately carcinogenic (++), or weakly carcinogenic (+) (31). Many other such compounds studied are reported in the Hartwell survey and in another by Arthur D. Little, Inc. (31). The rating assigned to dibenzo(a,i) pyrene is based on experiments with over 10,000 inbred mice in which one subcutaneous injection in the groin of 0.5 mg. of hydrocarbon in tricaprylin produced 50 percent sarcomas at the injection site in 14 weeks and 98 percent tumors in 24 weeks (20). Benzo(a)pyrene is one of the two most potent of the seven carcinogens detected in tobacco smoke and it is present in much larger quantity than any of the other carcinogens listed. Two polycyclic hydrocarbons isolated from tobacco smoke but not yet adequately tested for carcinogenicity are: benzo(j)fluoranthene and dibenzo(a,l)pyrene.

Identification of benzo(a)pyrene is reported in 19 separate investigations; the amount given in the table per 1000 cigarettes (70 mm. long, weighing about 1.0 g. each) is the average of 10 values selected on the basis of the quality of criteria used for identification (31). Compounds 1, 2, 3, 4, and benzo(j)fluoranthene were identified in one laboratory over a period of years and are listed together in a review by Van Duuren (44). Isolation of the three heterocyclic carcinogens (5,6,7) is reported by Van Duuren (45).

Because of losses in the process of fractionation and purification, the amount of carcinogens reported in a given investigation may be less than the amount actually present. Wynder and Hoffman (50) investigated this point by adding a known amount of radioactive C^{14}-labelled benzo(a)pyrene to a smoke condensate and applied the usual procedure for isolation of benzo(a)pyrene, which involved, in the last stages, chromatographing twice on silica gel and four times on paper. The activity of the benzo(a)pyrene finally isolated indicated a loss of 35–40 percent of carcinogen during processing. The amount of benzo(a)pyrene given in Table 2 thus should be multiplied by a factor of 1.5 to give the estimated true amount. Probably the amounts of the other carcinogens in smoke are also at least 1.5 times the reported amounts.

Relatively little work has been done on the components of smoke produced with cigars and pipes. Table 3 summarizing a comparative study made in one laboratory (5) indicates that the amount of benzo(a)pyrene, the only carcinogen in the group studied, increases sharply from cigarettes to cigars to pipes.

TABLE 3.—*Polycyclic hydrocarbons isolated from tobacco smoke*

[μg. per 1000 g. of tobacco consumed]

Hydrocarbon	Cigarettes	Cigars	Pipes
Benzo(a)pyrene	9	34	85
Acenaphthylene	50	16	291
Anthracene	109	119	1,100
Pyrene	125	176	755

COCARCINOGENS

Assays of tobacco smoke tars for carcinogenicity are done by applying a dilute solution of tar in an organic solvent with a camel's hair brush to the backs of mice beginning when the animals are about six weeks old. Application is repeated three times a week for a period of a year or more. The results of a number of such assays present a puzzling anomaly: the total tar from cigarettes has about 40 times the carcinogenic potency of the benzo(a)pyrene present in the tar. The other carcinogens known to be present in tobacco smoke are, with the exception of dibenzo(a,i)pyrene, much less potent than benzo(a)pyrene and they are present in smaller amounts. Apparently, therefore, the whole is greater than the sum of the known parts (27, 33, 49).

One possible or partial explanation of the discrepancy is that the tar contains compounds which, although not themselves carcinogenic, can enhance the cancer-producing properties of the carcinogens. Berenblum and Shubik (3), reporting on cocarcinogenesis, described the potentiating effect of croton oil, which itself is noncarcinogenic except in certain strains of mice (4a), on the action of hydrocarbon carcinogens. Phenol is reported to have a similar potentiating effect (4, 50) and, as noted above, cigarette smoke contains considerable phenolic material. Long-chain fatty acid esters (39) and free fatty acids (19) have been shown to function as cocarcinogens, and substances of both types occur abundantly in tobacco smoke. It is possible that the potentiating action of croton oil is due to the presence of fatty acids and their esters. A further observation of possible importance is that some polycyclic hydrocarbons, though very weak or inactive as carcinogens, are capable of initiating malignant growth under the influence of a promoter. Thus benz(a)anthracene, identified in cigarette smoke, is very weak or inactive in initiating malignant growth by itself, but initiates carcinogenesis under the influence of croton oil as promoter (15).

If more were known about the possible cocarcinogenicity of the many inactive components of tobacco smoke, some of the apparent discrepancy between isolation and bioassay data might disappear. It is possible that some of the carcinogenicity of smoke is due to hydroperoxides formed from unsaturated smoke components and destroyed in the isolation procedures. Furthermore both sets of data are far from precise; for example, one estimate of the amount of the highly potent dibenzo(a,i)pyrene per 1000 cigarettes (Table 2) is 0.02μg. and another is 10μg.

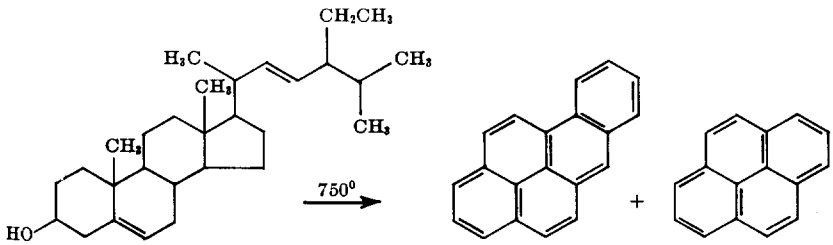
However, it is not necessary to wait for an exact balance of the two sets of data to draw a conclusion from each. The isolation experiments, taken

alone, indicate that cigarette smoke contains a number of identified chemicals which are carcinogenic to mice. The bioassays suggest that cigarette smoke probably contains components which, acting in a manner as yet undescribed, are involved in the induction of tumors in mice.

Assessment of all conceivable synergistic effects presents a gigantic problem for exploration. Tobacco smoke contains considerable amounts of phenols and fatty acids, both of which, as previously mentioned, enhance the activity of known carcinogens. Cellulose acetate filters now in use remove 70–80 percent of acidic constituents of tobacco smoke.

MECHANISM OF THE FORMATION OF CARCINOGENS

Most of the carcinogenic compounds identified in cigarette smoke tar are not present in the native tobacco leaf but are formed by pyrolysis at the high burning temperature of cigarettes. Van Duuren (44) reports formation of benzo(a)pyrene and pyrene on pyrolysis of stigmasterol, a smoke com-



Stigmasterol Benzo(a)pyrene Pyrene

ponent. Similar pyrolysis of pyridine or of nicotine gives dibenzo(a,j) acridine and dibenzo(a,h)acridine, both of which are carcinogenic (Table 2). Pyrolysis of nontobacco cigarettes made from vegetable fibers and spinach resulted in formation of benzo(a)pyrene (50).

Hurd and co-workers (22) by careful experimentation have elaborated plausible mechanisms for the formation of polycyclic aromatics by pyrolysis of materials of low molecular weight at temperatures in the range 800–900° C. Postulated radical intermediates are:

(a) $CH_2{=}C{=}\dot{C}H \longleftrightarrow \dot{C}H_2{-}C{\equiv}CH$

(b) $\ddot{C}H{-}CH{=}\dot{C}H \longleftrightarrow \dot{C}H{=}CH{-}\ddot{C}H$

(c) $\dot{C}H{=}CH{-}CH{=}\dot{C}H$

These radicals can arise from propylene, toluene, picoline, or pyridine. A variety of polycyclic hydrocarbons can be generated by reaction of these radicals with themselves or with other small radicals present in the heating zone. For example, dimerization of (b) should give benzene.

It thus appears that the pyrolysis of many organic materials can lead to the formation of components carcinogenic to mice. Cigarette paper consists essentially of cellulose. Pyrolysis of cellulose has been shown to produce benzo(a)pyrene. The observation (2) that treatment of tobacco with copper nitrate decreases the benzo(a)pyrene content of the cigarette smoke suggests a possibility for improvement by the use of additives or catalysts. The fact that side-stream smoke contains three times more benzo(a)pyrene than main-stream smoke has been cited (50) as evidence that more efficient oxidation could conceivably lower the content of carcinogenic hydrocarbons.

THE GAS PHASE

The gas phase accounts for 60 percent of total cigarette smoke. Hobbs et al. (34, 35) found that 98.9 mole percent of the gas phase is made up of the following seven components:

Nitrogen	73 mole percent
Oxygen	10
Carbon-dioxide	9.5
Carbon-monoxide	4.2
Hydrogen	1.
Argon	0.6
Methane	0.6
	98.9

The approximately one percent of the gas phase not accounted for by the seven major constituents contains numerous compounds, no less than 43 of which have been identified as present in trace amounts. Some of these are listed in Table 4 (1).

TABLE 4.—*Some gases found in cigarette smoke*

Compound	Concentration	Safe level for industrial exposure*	Toxic action on lung
	(*ppm*)	(*ppm*)	
Carbon Monoxide	42,000	100	Unknown
Carbon Dioxide	92,000		None
Methane, ethane, propane, butane, etc.	87,000	500	None
Acetylene, ethylene, propylene, etc.	31,000	5,000	None
Formaldehyde	30	5	Irritant
Acetaldehyde	3,200	200	Irritant
Acrolein	150	0.5	Irritant
Methanol	700		Irritant
Acetone	1,100	200	Irritant
Methyl ethyl ketone	500	250	Irritant
Ammonia	300	150	Irritant
Nitrogen Dioxide	250	5	Irritant
Methyl Nitrite	200		Unknown
Hydrogen Sulfide	40	20	Irritant
Hydrogen Cyanide	1,600	10	Respiratory enzyme poison
Methyl Chloride	1,200	100	Unknown

*The values listed refer to time-weighted average concentrations for a normal work day.

EFFECTS ON CILIARY ACTIVITY*

An important line of investigation was opened up by the report by Hilding (18) that cigarette smoke is capable of inhibiting the transport activity of ciliated cells such as found in the respiratory tract. It has been suggested (10, 17) that failure of ciliary function to provide a constantly moving stream of mucus enables environmental carcinogens to reach the epithelial cells. Kensler and Battista (28) describe development of a method of bioassay for inhibition of ciliary transport activity involving exposure of the trachea of a rabbit to the test material. The smoke from a regular cigarette was found to inhibit transport activity by 50 percent after exposure to two or three puffs. Several commercial filter cigarettes gave essentially the same result. The fact that these filters lower the phenol content by 70 to 80 percent and trap about 40 percent of the particulate phase suggested that neither phenolic nor particulate materials are responsible for the inhibition noted. The next trial was with an absolute filter, that is, one which removes the entire particulate phase and gives nonvisible gas. The observation that such treatment did not significantly alter the inhibitory effect of the puff established that components of the gas phase are responsible for inhibition of ciliary transport activity. Assays of known components of the gas phase showed the following compounds to possess such activity: hydrogen cyanide, formaldehyde, acetaldehyde, acrolein, and ammonia, although no one of these occurs at levels high enough to produce the effect noted for smoke.

Activated carbons differ markedly in their adsorption characteristics. Carbon filters previously employed in cigarettes do not have the specific power to scrub the gas phase. It has been reported that a filter containing special carbon granules removes gaseous constituents which depress ciliary activity (28).

PESTICIDES AND ADDITIVES

Before 1930 practically the only insecticides used in the growing of tobacco were lead arsenate and paris green (the mixed acetate-arsenite salt of copper). Analysis of 6 brands of American cigarettes purchased in 1933 showed a range of 7.5–26.4 parts of As_2O_3 per million, with an average value of 13.9 ppm. (6). Cogbill and Hobbs (8) found that main-stream smoke of cigarettes containing 7.1 μg. of arsenic per cigarette contains 0.031 μg. per puff. This amount would be equivalent to 0.25 μg. of arsenic per cigarette (8 puffs), and hence a smoker consuming 2.5 packs of such cigarettes per day might inhale 12.5 μg. of arsenic per day. By comparison, analysis of the atmosphere of New York City over a 12-year period indicated an average content of 100–400 μg. of arsenic per 10 cubic meters, which is an approximate daily intake per person (38).

Extensive Federal efforts to discourage the use of arsenicals for the control of tobacco hornworms on the growing tobacco crop resulted in a sharp de-

*This topic is discussed more fully in Chapter 10.

cline in the arsenic content of cigarettes after 1950. Thus, the average arsenic content of 17 brands of cigarettes analyzed in 1958 was 6.2 ppm. of As_2O_3 (14).

It seems unlikely that the amount of arsenic derived even from unfiltered cigarettes is sufficient to present a health hazard.

Chemicals recommended by the Department of Agriculture for the control of tobacco insects are: malathion, parathion, Endosulfan, DDT, TDE, endrin, dieldrin, Guthion, aldrin, heptachlor, Diazinon, Dylox, Sevin, and chlordane (42a). Trace amounts of TDE and endrin have been detected in commercial cigarettes and cigarette smoke. Guthion and Sevin residues were detected in main-stream cigarette smoke at levels approximating 0.3 percent and 1 percent of that added to cigarettes prior to smoking. Tobacco treated with Guthion and Sevin at the recommended levels showed no measurable contamination of main-stream cigarette smoke (4b). (For discussion of carcinogenicity of tobacco pesticides, see Chapter 9.)

Cigarette manufacture in the United States includes use of additives such as sugars, humectants, synthetic flavors, licorice, menthol, vanillin, and rum. Glycerol and methylglycerol are looked on with disfavor as humectants because on pyrolysis they yield the irritants acrolein and methylyglyoxal. Additives have not been used in the manufacture of domestic British cigarettes since the Customs and Excise Act of 1952, Clause 176, and probably longer, inasmuch as Section 5 of the Tobacco Act of 1842 imposed a widespread prohibition on the use of additives in tobacco manufacture.

SUMMARY

Of the several hundred compounds isolated from the tobacco leaf, two groups are specific to tobacco. One of these groups includes the alkaloid nicotine and related substances. The other includes compounds described as isoprenoids. Cigarette smoke is an heterogeneous mixture of gases, uncondensed vapors, and particulate matter. In investigating chemical composition and biological properties, it is necessary to deal separately with the particulate phase and gas phase of smoke.

Components of the particulate phase other than the higher polycyclics include aliphatic and alicyclic hydrocarbons, terpenes and isoprenoid hydrocarbons, alcohols and esters, sterols, aldehydes and ketones, acids, phenols and polyphenols, alkaloids, nitrogen bases, heterocyclics, amino acids, and inorganic chemicals such as arsenic, potassium, and some metals. Seven polycyclic compounds isolated from cigarette smoke have been established to be carcinogenic. They are shown in Table 2. The over-all carcinogenic potency of tobacco tar is many times the effect which can be attributed to substances isolated from it. The difference may be associated in part with the presence in tobacco smoke of cocarcinogens, several of which have been identified as smoke components.

Components of the gas phase of cigarette smoke have been shown to produce various undesirable effects on test animals or organs, one of which is suppression of ciliary transport activity in trachea and bronchi.

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Chapter 7

Pharmacology and Toxicology of Nicotine

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Figure

Chapter 7

GENERAL PHARMACOLOGIC ACTION OF NICOTINE ON NERVE CELLS

The pharmacology and chronic toxicity of nicotine, in dosage comparable to the amounts that man may absorb from smoking or other use of tobacco, are pertinent to an evaluation of health hazard.

The most notable action of nicotine involves a direct effect on sympathetic and parasympathetic ganglion cells (18). This usually occurs as a transient excitation, followed by depression, or even paralysis with effective doses. The ganglia are rendered more sensitive to acetylcholine initially and thus make preganglionic impulses more effective. Paralysis is associated with diminished sensitivity of ganglia to acetylcholine and concomitant reduction in the intensity of postganglionic discharges. Similar effects occur at the neuromuscular junction, resulting in a curariform action in skeletal muscle with adequate doses (16). In the central nervous system, as in ganglia, primary stimulation is succeeded by depression. Furthermore, nicotine like acetylcholine discharges epinephrine from the adrenal glands and other chromaffin tissue (20); it also releases antidiuretic hormone from the posterior pituitary by stimulating the supraopticohypophyseal system (3). Nicotine also augments various reflexes by excitation of chemoreceptors in the carotid body (10).

The pharmacological response of the whole organism at any one time therefore, representing as it does the algebraic sum of stimulant and depressant effects resulting from many direct, reflex, and chemical mediator influences on autonomic nervous transmission and excitability of virtually all organ systems, defies accurate description. The wide variation in smoking habits leads to every conceivable pattern of fluctuating blood levels of nicotine during the day. This suggests strongly that nicotine-sensitive cells may be shifting continuously from excitation to depression. Such activity probably accounts for the unpredictable effects observed in different individuals and in the same individual at different times. Using the classic pharmacological approach, it is therefore virtually impossible to make reliable statements regarding the effect of smoking on the many organ systems. In order to characterize the biological effects of nicotine in man, it thus becomes necessary to place heavy reliance on symptoms and signs derived from clinical and epidemiological studies.

EFFECTS ON THE CENTRAL NERVOUS SYSTEM

The action of nicotine on central nervous system functions has recently been reviewed (20). Very little of the reported work involves human

experimentation, and most of it is with doses much larger than are associated with the act of smoking. It suffices to note here that moderate doses of nicotine elicit marked increases in respiratory, vasomotor, and emetic activity, and still larger doses lead to tremors and convulsions, both in animals and man. The amounts absorbed even in heavy smoking may produce transient hyperpnea through carotid and aortic arch reflexes (5). The increase in blood pressure which is commonly observed is partly central in origin. Nausea and emesis are more pronounced in the novice smoker but may occur even in heavy smokers with excessive use of tobacco. Electroencephalographic (EEG) studies in the intact rabbit (21) indicate that nicotine, in doses of 0.5 to 3.0 milligrams per kilogram, produced an "arousal reaction" involving the hippocampus. In a later stage of the same reaction there appeared a discharge pattern similar to that noted in convulsions. Lesions in the septum abolished the "arousal reaction," chlorpromazine and evipan abolished the discharge pattern. None of the congeners of nicotine, including lobeline, produced similar patterns.

Knapp and Domino (12) found that concentrations of nicotine (10 to 20 μg/kg), a level commonly reached in man by smoking, produced EEG arousal patterns in four species of animals, the rabbit, cat, dog, and monkey, after neopontine transection. These effects did not appear to be related to fluctuations in blood pressure or to catecholamine or serotonin levels.

In a study of electrical activity (as measured by electroencephalogram) in 25 human subjects before and after smoking one cigarette, Lambiase and Serra (15) noted an 80 percent depression in voltage and an acceleration in frequency of the alpha rhythm which remained unchanged in form during the recordings. These alterations were more consistent in subjects over 35 years of age and were attributed to carbon monoxide and nicotine resulting in cerebral anoxia and/or release of epinephrine. Hauser et al. (9), who studied the EEG changes on cigarette smoking in healthy young adults, obtained highly variable responses usually toward an increase in the dominant alpha frequency of 1 or 2 cycles per second. Some subjects showed similar changes when puffing a glass cigarette stuffed with cotton and others when puffing specially prepared nicotine-free cigarettes. They concluded that the effects noted were more likely to represent a psycho-physiologic response to the act of smoking than to any substances present in cigarette smoking. Bickford (1) arrived at a similar conclusion. Wide gaps of information exist in this area and it is not meaningful to attempt inferences concerning correlations of electrical events in the central nervous system and subjective effects of smoking from the type of evidence currently available.

CARDIOVASCULAR EFFECTS

The cardiovascular effects of nicotine are described in Chapter 11, Cardiovascular Diseases.

GASTROINTESTINAL EFFECTS

Most but not all experimental and clinical evidence supports the popular view that smoking reduces appetite (6, 17 p. 271). This reduction has been attributed both to direct effects on gastric secretions and motility and to reflexes arising from local effects on the taste buds and mucous membranes in the mouth. The unpredictable and temporary elevation of blood sugar is probably too small to contribute significantly (17, p. 326). Nicotine effects on the hypothalamus, comparable to the appetite reduction produced by other stimulants like amphetamine, and psychological mechanisms may play significant roles (23). Hunger contractions are inhibited but gastric movements of digestion do not appear to be influenced significantly by moderate smoking (4).

Nausea, often associated with vomiting, is by far the most common symptom related to the gastrointestinal tract. This effect probably originates centrally in the medullary emetic chemoreceptor trigger zone (14). It is now generally agreed that nicotine stimulates peristalsis but the mechanism is a complex one, probably involving local, central and reflex actions. Schnedorf and Ivy (21) found wide individual variation in gastrointestinal passage time in medical student smokers and non-smokers but gained the impression that smoking tends to augment motility of the colon. These effects are probably related to actions on the parasympathetic ganglia in the bowel. The summative effects of all of these pharmacological actions on the whole intestinal tract do not produce a consistent pattern. Excessive smoking may be associated with diarrhea, constipation, or alternating patterns between the two extremes. The only consistency is that symptoms attributable to nicotine effects on the gastrointestinal tract are very common.

DISTRIBUTION AND FATE

Nicotine is actively and rapidly metabolized by man and other mammals, the metabolites being in large measure excreted in the urine. If any tissue storage occurs, it is in such small quantity as to elude current analytical technics. Nicotine is a rather unstable molecule which in neutral or alkaline conditions undergoes a variety of changes. A review of the current concepts of the known and suggested pathways for the metabolism of nicotine is shown in Figure 1 (18). The main intermediate appears to be (−)-cotenine which yields γ-(3-pyridyl)-γ-methylamino butyric acid. Cotenine has low toxicity and lacks the potent pressor activity of nicotine.

Dogs receiving 150 mg/kg/day orally for 108 days exhibited no weight loss or other objective signs (2). Man has ingested 500 mg orally at 8-hour intervals for 6 days without untoward effects. No evidence has been presented that the other known metabolites of nicotine carry any significant systemic toxicity.

SUMMARY DIAGRAM OF ROUTES FOR THE METABOLISM OF NICOTINE IN MAMMALS

(Some hypothetical intermediates are shown in brackets.)

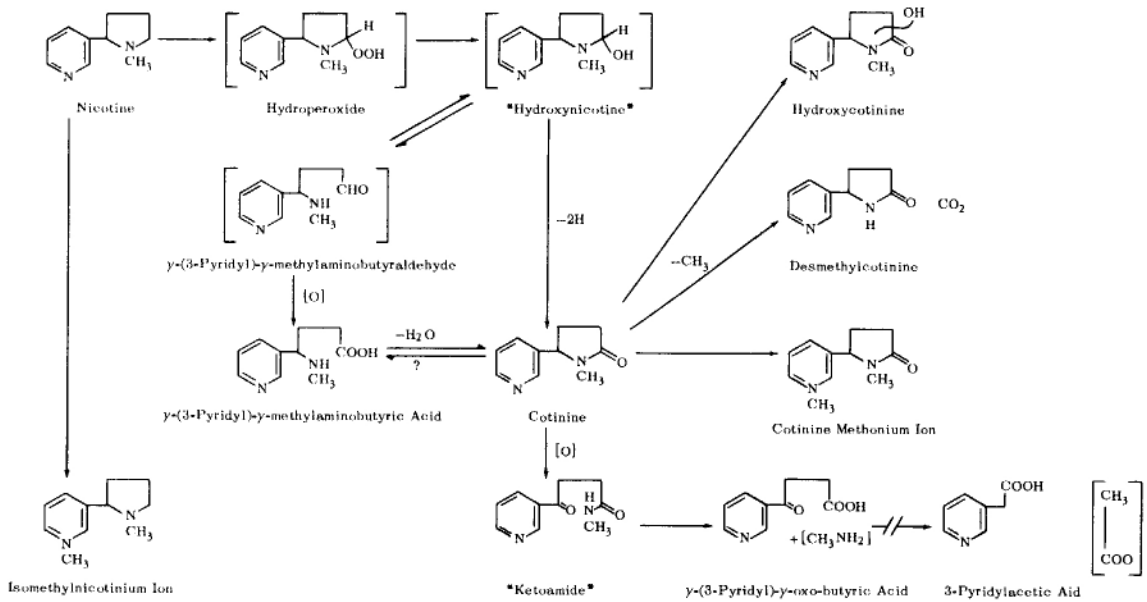

FIGURE 1.

Source: McKennis, Herbert H., Jr. (18)

CHRONIC TOXICITY

Evaluation of the chronic toxicity of tobacco smoke may be considered in several categories: (a) the systemic toxicity of nicotine or its congeners, (b) the systemic toxicity of other constituents of smoke or tobacco, carbon monoxide and other compounds, (c) specific organ toxicity in certain susceptible individuals, such as those with Buerger's disease and allergic responses, (d) local effect of irritants on mucous and pulmonary membranes by tars, phenols, the oxides of nitrogen, and others. The latter three types of potential toxicity are discussed in Chapter 9, Cancer, and Chapter 10, Non-Neoplastic Respiratory Diseases.

It might appear that the least difficult problem in this group of variables would be to assess the chronic toxicity of nicotine since we are dealing with a comparatively simple organic compound of known composition and reaction. Whereas there is a voluminous literature of studies involving chronic exposure to nicotine or tobacco smoke in many animal species (17, pp. 501–504), most of these are poorly designed and controlled and are of little value for extrapolation to man. For example, in the best nicotine experiments involving life span studies, the daily dose of nicotine was near the maximal tolerated dose (just subconvulsive), which is greatly in excess of any human smoking exposure. Even though some authors (11) observed weight loss and degenerative vascular changes in rats under these severe conditions, others (22) noted some weight loss but no histologic change. In life span experiments in rats, with tobacco smoke in amounts approximating human smoking exposure, very little systemic toxicity was noted (8, 13). Even though animal experimentation is inadequate, especially in long-term effects of nicotine on large animal species, existing data permits a tentative conclusion that the chronic systemic toxicity of nicotine is quite low in small to moderate dosage.

The clinical literature is devoid of human data concerning chronic exposure to nicotine alone, and the general statements regarding the chronic toxicity of nicotine for man represent inferences drawn from chronic exposure to tobacco in various forms, including industrial poisoning. Repeated exposure to tobacco in excessive amounts is reported to induce amblyopia, arrhythmias, digestive disturbances, cachexia and a wide variety of other signs and symptoms. But the effects of excessive dose are of little concern here. The question is whether prolonged *exposure to nicotine*, in the quantities absorbed systemically from smoking or other tobacco use, produces toxic effects which result in unpleasant symptoms, dangerous signs, specific degenerative disease, or shortening of the life span. Unfortunately even a tentative answer to this question must be obtained indirectly and by making certain assumptions. Inasmuch as nicotine is systemically absorbed from all routes of administration, smoking, chewing, snuffing, or "snuff dipping,"* it appears logical to assume that if the amounts of nicotine absorbed in the various methods of use are of the same order of magnitude, any toxic effects observed should also be in this order of magnitude. There appears to be general agreement that this is so. Calculations indicate that the nicotine

*A small amount of snuff is placed in the groove between the teeth and the lower lip or beneath the tongue and held there from 30 minutes to several hours.

absorbed (40–60 mg) from 6 cigars uninhaled equals that from 30 cigarettes inhaled (19). Chewing tobacco may yield 8 to 87 mg in 6 to 8 hours (24); in chewing snuff, 20–60 mg of nicotine (7).

The following variables play a role in the amount of nicotine absorbed (17, p. 8):

> To sum up, the rate and amount of absorption of nicotine by the smoker depend to a greater or less extent upon the following factors:
>
> 1. Length of time the smoke remains in contact with the mucous membranes;
> 2. pH of the body fluids with which the smoke comes in contact;
> 3. Degree and depth of inhalation;
> 4. Degree of habituation of the smoker (?);
> 5. Nicotine content of the tobacco smoked;
> 6. Moisture content of the tobacco smoked;
> 7. Form in which tobacco is smoked (cut [cigarettes] or uncut [cigars]) (?);
> 8. Length of butt;
> 9. Use of holder or filter;
> 10. Alkalinity or acidity of the tobacco smoke (?);
> 11. Agglomeration of smoke particles (more important in cigarette-smoking).

There is no acceptable evidence that prolonged exposure to nicotine creates either *dangerous* functional change of an objective nature or degenerative disease. The minor evidences of toxicity, nausea, digestive disturbances and the like, are similar in kind and degree with all forms of use.

The fact that the over-all death rates of pipe and cigar smokers show little if any increase over non-smokers is very difficult to reconcile with a concept of high nicotine toxicity. In view of the mortality ratios of pipe and cigar smokers, it follows logically that the apparent increase in morbidity and mortality among cigarette smokers relates to exposure to substances in smoke other than nicotine. Unfortunately, there are no useful mortality statistics in those who chew, snuff, or "dip" tobacco, and the literature regarding industrial exposure is so confusing that little help is available here. The type of projection made above, however unsatisfactory, is not inconsistent with the animal toxicity data as well as the fact that nicotine undergoes very rapid metabolism to substances of low toxicity. The evidence therefore supports a conclusion that the chronic toxicity of nicotine in amounts ordinarily obtained in common forms of tobacco use is very low indeed.

SUMMARY

The pharmacological effects of nicotine at dosage levels absorbed from smoking (1–2 mg per inhaled cigarette) are comparatively small; the response in any point in time represents the algebraic sum of stimulant and depressant actions from direct, reflex, and chemical mediator influences on the several organ systems. The predominant actions are central stimulation and/or tranquilization which vary with the individual, transient hyperpnea,

peripheral vasoconstriction usually associated with a rise in systolic pressure, suppression of appetitite, stimulation of peristalsis and, with larger doses, nausea of central origin which may be associated with vomiting.

Nicotine is rapidly metabolized by man and certain other mammals. The primary pathway through (−)-cotenine to γ-(3-pyridyl)-γ-methylaminobutyric acid is described in detail. The known metabolites have very low toxicity.

The rapidity of degradation to non-toxic metabolites, the results from chronic studies on animals, and the low mortality ratios of pipe and cigar smokers when compared with non-smokers indicate that the chronic toxicity of nicotine in quantities absorbed from smoking and other methods of tobacco use is very low and probably does not represent a significant health problem.

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