- More data are also needed on cigarette flavor additives and their combustion products. Flavoring agents and additives should be studied by cigarette companies for carcinogenicity and toxicity before their commercial use is permitted, and the results of such studies should be made available.

- Research should be done on the distribution, partitioning, and penetration of lower "tar" and nicotine cigarette smoke in the lung, with consideration of potential changes in smoking patterns by those who smoke lower "tar" and nicotine cigarettes. Cigarette smoking-machines currently in use and the techniques by which animals inhale cigarette smoke in research models may not be representative of the human situation because human smokers are able to take larger, more frequent, and higher velocity puffs. To conduct meaningful assays of cigarette yields and the biological activity of cigarette smoke, it must be determined how smokers actually smoke various types of commercial cigarettes. When this information is available, it will be possible to design smoking-machines that yield more accurate estimates of human risk.

- Controlled studies are needed to determine the role of nicotine as a primary reinforcer in cigarette smoking and to determine whether there are other chemicals in addition to nicotine that may contribute to or reinforce the smoking habit. By analyzing the mechanisms whereby nicotine reinforces smoking behavior, it may be possible to design more efficacious methods of smoking cessation.

- Research should be conducted to define what effects modifications of the physical and chemical properties of leaf tobaccos have on the pharmacology of cigarette smoke. Since tobacco culturing and curing practices are continually changing, it is important to determine whether such changes as the use of new pesticides also alter the composition and biological activity of cigarette smoke.

- Standardized experimental cigarettes have frequently proved unpalatable and unacceptable for behavioral research. Prototype cigarettes should be especially designed to deliver a wide range of constituent concentrations, particularly those that approximate commercial cigarettes. This would allow researchers to predict the behavior of smokers of new types of cigarettes more accurately.

26

Section 2. PHARMACOLOGY AND TOXICOLOGY

CONTENTS

LIST OF FIGURES

LIST OF TABLES

30

Table 3.—Coefficients and standard deviations of
coefficients for Prediction Model 10

Table 4.—Comparison of mutagenic and tumor-promoting
activity of fractions of cigarette smoke condensate

Introduction

Tobacco and tobacco smoke are very complex mixtures. In 1968, Stedman (155) reported that they contained more than 1,200 clearly identified substances in addition to a number of polymer classes, such as pigments, resins, and proteins, that were not resolved into specific compounds. Since that time, many additional compounds have been isolated; at least a thousand additional constituents were found in tobacco and tobacco smoke in the following 10 years (67). Cigarette smoke components arise through distillation of volatile and semivolatile materials from the leaf and from the pyrolytic decomposition of leaf constituents. In addition, nonvolatile components of tobacco leaf can be transferred to the smoke without degradation. Thus, the components of smoke are very diverse. Many suspected or proved toxic agents have been identified in the gas phase (Table 1) or in the particulate matter (Table 2) of smoke (190). It is not surprising that chronic exposure to such a complex mixture will lead to a variety of pharmacologic and toxicologic responses.

TABLE 1.—Major toxic agents in the gas phase of cigarette smoke (unaged)*

Agent	Biologic activity*	Concentration/cigarette	
		Range reported	U.S. cigarettes[b]
Dimethylnitrosamine	C	1–200 ng	13 ng
Ethylmethylnitrosamine	C	0.1–10 ng	1.8 ng
Diethylnitrosamine	C	0–10 ng	1.5 ng
Nitrosopyrrolidine	C	2–42 ng	11 ng
Other nitrosamines (4 compounds)	C	0–20 ng	?
Hydrazine	C	24–43 ng	32 ng
Vinyl chloride	C	1–16 ng	12 ng
Urethane	TI	10–35 ng	30 ng
Formaldehyde	CT, CoC	20–90 μg	30 μg
Hydrogen cyanide	CT, T	30–200 μg	110 μg
Acrolein	CT	25–140 μg	70 μg
Acetaldehyde	CT	18–1,400 μg	800 μg
Nitrogen oxides (NO$_x$)[c]	T	10–600 μg	350 μg
Ammonia	T?[d]	10–150 μg	60 μg
Pyridine	T?[d]	9–93 μg	10 μg
Carbon monoxide	T	2–20 mg	17 mg

*Cigarettes may also contain such carcinogens as arsine, nickel carbonyl, and possibly volatile chlorinated olefins and nitro-olefins.

[a] C denotes carcinogen; TI, tumor initiator; CoC, cocarcinogen; CT, cilia toxic agent; and T, toxic agent.

[b] 85 mm cigarettes without filter tips bought on the open market 1973–1976.

[c] NO$_x$ >96% NO; rest NO$_x$.

[d] Not toxic in smoke of blended U.S. cigarettes because pH <6.5, and therefore ammonia and pyridines are present only in protonated form.

SOURCE: Wynder and Hoffmann (190).

TABLE 2.—Major toxic agents in the particulate matter of cigarette smoke (unaged)*

Agent	Biologic activity[a]	Concentration/cigarette	
		Range reported	US cigarettes[b]
Benzo[a]pyrene	TI	8–50 ng	20 ng
5-Methylchrysene	TI	0.5–2 ng	0.6 ng
Benzo[j]fluoranthene	TI	5–40 ng	10 ng
Benz[a]anthracene	TI	5–80 ng	40 ng
Other polynuclear aromatic hydrocarbons (>20 compounds)	TI	?	?
Dibenz[a,j]acridine	TI	3–10 ng	8 ng
Dibenz[a,h]acridine	TI	?	?
Dibenzo[c,g]carbazole	TI	0.7 ng	0.7 ng
Pyrene	CoC	50–200 ng	150 ng
Fluoranthene	CoC	50–250 ng	170 ng
Benzo[g,h,i]perylene	CoC	10–60 ng	30 ng
Other polynuclear aromatic hydrocarbons (>10 compounds)	CoC	?	?
Naphthalenes	CoC	1–10 µg	6 µg
1-Methylindoles	CoC	0.3–0.9 µg	0.8 µg
9-Methylcarbazoles	CoC	0.005–0.2 µg	0.1 µg
Other neutral compounds	CoC	?	?
Catechol	CoC	40–460 µg	270 µg
3- & 4-Methylcatechols	CoC	30–40 µg	32 µg
Other catechols (>4 compounds)	CoC	?	?
Unknown phenols and acids	CoC	?	?
N'-Nitrosonornicotine	C	100–250 ng	250 ng
Other nonvolatile nitrosamines	C	?	?
β-Naphthylamine	BC	0–25 ng	20 ng
Other aromatic amines	BC	?	?
Unknown nitro compounds	BC	?	?
Polonium-210	C	0.03–1.3 pCi	?
Nickel compounds	C	10–600 ng	?
Cadmium compounds	C	9–70 ng	?
Arsenic	C	1–25 µg	?
Nicotine	T	0.1–2.0 mg	1.5 mg
Minor tobacco alkaloids	T	0.01–0.2 mg	0.1 mg
Phenol	CT	10–200 µg	85 µg
Cresols (3 compounds)	CT	10–150 µg	70 µg

* Incomplete list.

[a] C denotes carcinogen; BC, bladder carcinogen; TI, tumor initiator; CoC, cocarcinogen; CT, cilia toxic agent; and T, toxic agent.

[b] 85 mm cigarettes without filter tips bought on the open market 1973-1976.

SOURCE: Wynder and Hoffmann (190).

Experimental Systems for Assay of Relative Risks of Cigarette Smoking

Lung Cancer

Animal Models

The mouse skin carcinogenesis assay is thus far the most fruitful method of evaluating smoke condensates from different types of cigarettes for carcinogenic potency for the human lung (46, 51, 89, 106).

34

This model for the development of cancer dates back to 1915 (*191*). A large body of laboratory experience has provided consistent evidence for the quantitative validity of this relationship. Procedures providing good dose-response relationships are in use in many laboratories. Assays can be standardized to give relatively consistent results within a laboratory, and probably among laboratories (*62, 63, 64, 65*).

The assay depends on a number of similarities between the laboratory model and human experience. The epithelium of both the skin and lung is directly exposed to the presumptive carcinogenic agent—in this case, cigarette smoke or cigarette smoke condensate. Rabbit and mouse skin develop tumors after exposure to coal tar, a known occupational carcinogen. Mouse skin assays have predicted occupational induction of human lung cancer by bis-chloromethyl ether (*148, 177*).

It is conceivable that the mouse skin carcinogenesis assay may give a misleading measure of the relative risk of various types of cigarettes. Skin is covered with a lipid film, and the pilo-sebaceous apparatus is particularly suited for penetration of lipid materials into the skin. In contrast, the airway surface is covered by an aqueous film and might be less readily penetrated by fat-soluble materials. There is no evidence, however, that such a difference is important. Indeed, the response of mouse skin to different types of experimental cigarettes is roughly parallel to the response of hamster larynx to the same materials (*49, 50, 189*).

The hamster larynx has been used for comparative studies of different types of cigarettes (*17, 50, 52*). Invasive carcinomas of the larynx were induced in 37 percent of inbred hamsters exposed to cigarette smoke for 59 to 80 weeks. Both the cancer incidence and the incidence of other epithelial changes were dose related. Exposure of rats and mice to cigarette smoke for up to 2½ years resulted in a small incidence of respiratory tract tumors, primarily pulmonary adenomas (*44, 68, 72*). Cigarette smoke produced changes in cultured human gastric epithelial cells suggestive of malignancy (*158*).

Lung Carcinogens in Cigarette Smoke

Experience in man and with the mouse skin system indicates that two or more distinct classes of carcinogenic stimuli lead to the occurrence of tumors (*16, 26, 48*). Tumor initiators appear to alter the genetic constitution of the cell; tumor promoters accelerate and enhance the neoplastic expression of previously initiated cells. Both may play a role in the induction of tumors. Other types of cocarcinogens may also play a role in the induction of mouse skin tumors by cigarette smoke condensate (*16, 74, 89, 176*). If similar mechanisms act in man, it may not be possible to differentiate between a human carcinogen in the conventional sense and a cocarcinogen or tumor

promoter acting on a diverse population already exposed to low levels of a variety of tumor initiators.

Two prominent classes of tumor initiators are found in smoke condensates of commercial cigarettes—polycyclic aromatic hydrocarbons (PAH) and tobacco-specific nitrosamines (TSNA). Other carcinogens or tumor initiators are present in cigarette smoke as well; however, they appear to be less significant because they either are less potent or are present at lower concentrations than are PAH or TSNA.

Polycyclic Aromatic Hydrocarbons

A large variety of PAH molecules are formed by the pyrolytic process during combustion of the cigarette (*87, 105*). Of the PAHs, benzo[a]pyrene (BaP) is the most prominent and has been studied most intensively. Chemical assays for BaP in smoke condensates are well established, and it has been suggested that such assays can serve as indicators of production of all of the PAHs. This appears to be generally true. Among smoke condensates from 98 experimental cigarettes, the correlation coefficient between BaP and benz[a]anthracene content was 0.78 (*15*). Although highly significant, the value is sufficiently low to indicate that real differences do exist in the ratios of these cyclic molecules in the various cigarette smokes. Nevertheless, BaP appears to be the most important single member of this class of compounds, taking into consideration both its concentration and its relative carcinogenic potency.

The contribution of BaP or PAH in general to mouse skin carcinogenesis by cigarette smoke condensate cannot be fully measured at this time. Wynder and Hoffmann (*188*) found a correlation between BaP levels and carcinogenic activity of smoke condensates from several types of cigarettes. A much larger series of experimental cigarettes was studied in the smoking and health program of the National Cancer Institute. No significant dependence of carcinogenic potency on BaP content was observed (*62, 63, 64, 65*). The relationship between chemical composition of the experimental smoke condensates and the biological activity of this series was examined extensively by Bayne (*15*). He employed the linear terms, squared terms, and all interaction terms between any 2 of 10 independent variables. Starting with a 66-term regression equation, he searched for simpler prediction models that would provide useful estimates of carcinogenic activity. The simplest model (Table 3) that retained good predictability contained nine terms. The interaction of BaP with the nicotine term was one that appeared important.

BaP and other tumor initiators are particularly important because humans are already exposed to a number of initiators in the environment. The effect of initiators is cumulative and irreversible. Hence, any additional exposure to initiators such as the PAH might be expected to increase tumor incidence in smokers.

36

TABLE 3.—Coefficients and standard deviations of coefficients for Prediction Model 10

Terms[a]	Coefficients	Standard deviation of coefficients
1 Intercept	2.637	0.292
2 C	-3.793 E-2	0.274 E-2
3 C^2	4.688 E-4	0.408 E-4
4 pH	-4.434 E-1	0.980 E-1
5 VWA	1.242 E-1	0.555 E-1
6 N x N	2.450 E-5	0.588 E-5
7 pH x pH	3.663 E-2	0.875 E-2
8 N x pH	-7.078 E-4	1.664 E-4
9 N x BAP	-1.770 E-3	0.377 E-3

[a] C = Concentration (mg/day); VWA = very weak acids (mg/g); N = nicotine (mg/g); and BAP = benzo[a]pyrene (μg/g).

SOURCE: Bayne (15).

Tobacco-Specific N-Nitrosamines

During tobacco curing, fermentation, and burning, nornicotine gives rise to N'-nitrosonornicotine (NNN), nicotine to NNN and to 4-(N-methyl-N-nitrosamino)-1-(3-pyridil)-1-butanone (NNK), and anatabine to N'-nitrosoanatabine (NAT). NNN is a moderately active carcinogen, inducing tumors in the respiratory tract of mice, rats, and hamsters. NNK is a strong carcinogen, inducing lung carcinoma in each of the three animal species (75, 84, 86). The concentration of these carcinogens in cigarette smoke is very high in comparison with usual environmental exposures, being 1 to 85 ppm in tobacco and 1 to 9 μg in the smoke of a cigarette (57). These tobacco-specific N-nitrosamines may play a role in the development of several types of human cancer. NNN is metabolically activated by human liver microsomes (76) and, together with NNK and NAT, may be formed in vivo from the tobacco alkaloids.

Other Mutagenic or Co-mutagenic Agents

It is generally believed that tumor initiators are mutagens that can be detected by one or more short-term biological assays (2, 103). A number of fractions of cigarette smoke condensate are positive in the Ames assay system (93, 101). The agents responsible for this activity have not been fully identified, but probably include products of protein pyrolysis (119). Ames test activity, however, does not predict the activity of fractions in the mouse skin carcinogenesis assay. Fractions of smoke condensate that show activity as complete carcinogens (89) or in a promotion assay that would detect skin carcinogens as well as tumor promoters (24) are not correspondingly active in the Ames system (Table 4). It cannot be determined whether the unidentified mutagens in cigarette smoke are an important cause of lung cancer in

TABLE 4.—Comparison of mutagenic and tumor-promoting activity of fractions of cigarette smoke condensate

Sample	Mutagenic activity— as a percentage of whole condensate (Kier et al. (101))	Promoting activity*— tumor yield as a percentage of that seen with whole condensate (Bock et al. (24))
Whole condensate	100	100
Reconstituted	89	115
Bases before, insoluble	21	4
Bases after, insoluble	26	11
Bases, ether soluble	11	4
Bases, water soluble	1	2
Weak acids, insoluble	30	8
Weak acids, ether soluble	5	80
Strong acids, insoluble	2	1
Strong acids, ether soluble	<1	8
Strong acids, water soluble	<2	8
Neutrals, 80% methanol soluble	2	7
Neutrals, cyclohexane soluble	≤1	18
Neutrals, nitromethane soluble	2	28

*From tests of fractions, equivalent to 30% condensate.

humans; however, added exposure to any tumor initiators probably carries an incremental risk of cancer.

Weak Acids

Cigarette smoke contains weak organic acids that exhibit tumor-promoting or cocarcinogenic activity (24, 74, 176). The concentration of very weak acids in cigarette smoke condensates was one of the terms predictive of the skin carcinogenic activity of smoke condensates (Table 3). Of the weak acids, catechol appears to be the most important on the basis of concentration and activity (74, 176).

It is probable that the weakly acidic constituents of smoke act as tumor promoters or cocarcinogens rather than as tumor initiators. This is true for phenols and for catechol (27, 176). There is no reason to believe that tumor promoters or other types of cocarcinogens exhibit either a cumulative or an irreversible effect. Indeed, for tumor promotion in mouse skin by croton oil, clear thresholds for frequency of application and for the amount of promoter in each applied dose are apparent (26). If this is also true for man, the risk of very small doses of weak acids might be negligible. Phenol (126, 188), but not catechol (29), can be selectively removed by filters. The extent to which the cocarcinogenic weak acids are reduced by selective filtration cannot be determined at this time.

38

Nicotine

Nicotine exhibits neither complete carcinogenic activity nor tumor-promoting activity. The nicotine content of cigarette smoke condensate did not affect its carcinogenic activity when suspended in beeswax-tricaprylin pellets implanted in rat lungs (43); however, in mouse skin bioassays, this alkaloid is an important cocarcinogen (20). Not only is nicotine active in models with other compounds such as BaP and 12-O-tetradecanoylphorbol-13-acetate (TPA), but also the measured carcinogenic potency of cigarette smoke condensates appears to depend on the nicotine content of the "tar." Of all of the individual compounds of smoke condensates assayed in the smoking and health program of the National Cancer Institute, nicotine was most closely related to carcinogenic activity (62, 63, 64, 65). In the simplest predictive model developed by Bayne, every term but one involved nicotine concentration, pH, or the concentration of crude condensate (Table 3). The availability of nicotine to the tissues depends on the pH and concentration of condensate. Hence, available nicotine was a factor of all but one term of the prediction model.

Nicotine may also play a role in the development of oral cancer in tobacco chewers. Aqueous extracts or unburned tobacco exhibit tumor-promoting activity when tested on mouse skin. This activity depends on the presence of nicotine acting together with a fraction having a molecular weight greater than 13,000 daltons (21). In addition, nicotine gives rise to carcinogenic N-nitrosamines during tobacco chewing (84).

Data of Morosco and Goeringer (122) suggest that nicotine reduced serum alpha$_1$-antitrypsin activity and elevated pancreatic elastase levels in dogs exposed to cigarette smoke. These workers believe that interference with the protease–protease inhibitor balance may be a factor in carcinogenesis (123).

It must be pointed out that the relationship between carcinogenic activity of smoke condensates and their nicotine contents may be caused in part by the conversion of nicotine to tobacco-specific nitrosamines or to the co-occurrence of nicotine and some other unidentified carcinogen. For example, the nicotine level of tobacco is dependent on the amount of nitrate fertilizer used in tobacco culture (166). High levels of tobacco-specific nitrosamines were found in the unburned tobaccos usually raised with high levels of nitrogen fertilizer (77). The level of volatile nitrosamines in cigarette smoke also depends on nitrate fertilizer (170). One may postulate that the nicotine level of cigarette smoke condensates is an indicator of such nitrogenous carcinogens that were not measured directly. At present, however, there is no direct evidence that this is the case. In any event, the carcinogenic activity of mixtures of pure BaP and TPA are enhanced by the concomitant application of nicotine under conditions such that nitrosamine formation would not be expected (20).

Whether the cocarcinogenic effects of nicotine are important for man is a matter of speculation. Tumor-promoting activity of croton oil exhibits a threshold both for frequency of application and for the quantity of agent present with any given treatment (26). The animal studies in which nicotine acts as a cocarcinogen employ nearly lethal levels of nicotine administered once or twice a day. In contrast, smokers are exposed to a large number of low doses of nicotine daily. If a threshold amount of nicotine per dose is required for cocarcinogenic activity, human smokers may not be affected in a manner similar to that of the mouse skin system.

Polonium 210

There have been repeated suggestions that ^{210}Po might contribute to the carcinogenic activity of cigarette smoke in man (137). Polonium levels in tobacco result primarily from the use of phosphate fertilizers that are contaminated with radium decay products, particularly ^{210}Pb, a precursor of ^{210}Po (162, 168). Very little ^{210}Po is found in tobacco leaf, but some is transferred to the smoke. Yields of 10 to 15 fCi of alpha emitters were recently reported for experimental cigarettes and 490 fCi/gm for commercial cigarette smoke condensate (36). Most of the radioactivity was due to insoluble forms of ^{210}Po. Cancer may arise from a single affected cell. It has been suggested that small amounts of insoluble ^{210}Po concentrated in small areas might deliver an effective carcinogenic dose to a target cell (112). Harley et al. (71), however, found very few "hot spots" in the lungs of deceased smokers. Based on human experience with radon daughters, they assumed a lifetime risk of lung cancer of 1×10^{-2} for a dose of one rad/year. At most, the radioactivity they detected was estimated to explain only 10 percent of the lung cancers suffered by cigarette smokers. They consider polonium 210 a questionable risk factor in human carcinogenesis.

Polonium 210 contamination of tobacco can be effectively reduced by selection of plant types and sources of phosphate fertilizer, and by removal using chelating agents (71, 171).

Volatile N-Nitrosamines

Tobacco smoke contains a number of secondary and tertiary amines. These amines, together with nitrogen oxides, may give rise to the *in vivo* formation of nitrosamines. Although the formation of most nitrosamines is favored at low pH (110), a small amount of volatile nitrosamines is found in cigarette smoke and may be formed in the lungs under normal conditions (30, 84, 170). The volatile N-nitrosamines are organ-specific carcinogens, which in mice give rise to tumors of the liver and kidney. At present, there is no reason to assume that volatile nitrosamines cause lung cancer in smokers. Nevertheless, it is prudent to limit the presence of any carcinogen in cigarette smoke.

Volatile nitrosamines in smoke can be reduced by selective filtration and by limiting the nitrate content of tobaccos (*30, 121*).

Bladder Cancer

The induction of bladder cancer in animals has been studied intensively over the past several decades. The bladder appears to be a particularly sensitive target for agents that are metabolized in the liver and excreted in the urine. Among the compounds known to produce bladder cancer in both man and animals is β-naphthylamine. The presence of β-naphthylamine in cigarette smoke has been demonstrated (*85*), along with other carcinogenic aromatic amines (*129*). The yield was so low, however, that they did not believe these agents contributed significantly to the risk of bladder cancer in smokers.

The urine of 10 smokers and 21 nonsmokers was examined by Yamasaki and Ames (*192*) for mutagens or for substances that were converted to mutagens by rat liver microsomes. Increased levels of mutagens were found in the urine of seven smokers, but in none of the nonsmokers. If promutagens in urine are responsible for the bladder cancers occurring in cigarette smokers, it is possible that certain individuals are particularly sensitive to bladder carcinogenesis by cigarette smoke. If true, this sensitivity may be exploited for disease prevention. Large quantities of mutagen-containing urine can be collected from sensitive individuals. Isolation and identification of the promutagens might permit removal of the precursors from cigarette smoke.

Laryngeal Cancer

Hamsters develop laryngeal cancer after long-term inhalation of diluted cigarette smoke (*17, 50, 52*). The effect is dose related and has been used to compare different cigarettes. Tobacco-specific nitrosamines induce cancer in the trachea and lungs of hamsters and may be of particular importance in the induction of human cancer of the larynx (*84*). Other carcinogens and cocarcinogens of cigarette smoke that are active in the mouse skin bioassay system may also contribute to induction of laryngeal cancer. Both organ systems involve epithelial tissue directly exposed to the carcinogenic mixture.

Other Cancers

Cigarette smoking is also associated with cancer of the kidney, pancreas, oral cavity, and esophagus (*173*). No animal model of these cancers has been developed to the point where it could be used for quantitative comparisons of different types of cigarettes. Oral cavity and esophageal tumors may be induced by direct exposure to smoke carcinogens. NNN, when given in the drinking water of rats, induces cancer of the esophagus (*84*). This finding suggests that tobacco-

41

specific nitrosamines may be active as "contact" carcinogens. Alternatively, the carcinogens might be produced through metabolism at distant sites, such as the liver, and then transported to the target site, where they can be further activated. Pancreatic cancer was induced in hamsters with diisopropylnitrosamine (134). This observation suggests the possibility of a similar action of smoke nitrosamines. Any carcinogen in cigarette smoke might contribute to induction of cancer distant from the exposure site. To this extent, elimination of the carcinogens causing lung cancer or bladder cancer would reduce the induction of cancer in other organs as well.

Alcohol usage and cigarette smoking show synergistic effects in the induction of cancer in the upper digestive tract (113, 172). The effect of alcohol in this circumstance may result from the induction of microsomal enzymes, which are believed to metabolize carcinogens to their active forms (113).

Early End Points Suggestive of Carcinogenic Potential

It is generally considered that the induction of cancer requires a specific genotoxic event that may be preceded or followed by ill-defined and less specific epigenetic changes that enhance the manifestation of the genetic event (182). In the two-stage carcinogenesis system of mouse skin, the first step—initiation—appears to be genotoxic, and the second step—promotion—appears to be epigenetic. Several other forms of cocarcinogenesis have been described (16). Tobacco smoke owes its carcinogenic activity to several carcinogens and cocarcinogens (24, 87, 176, 188).

Agents capable of producing genetic change can often be detected by mutagenesis assay systems (2). Most carcinogens are mutagens. Conversely, agents capable of inducing mutations are suspect as possible carcinogens. Cigarette smoke condensates and some of their fractions are mutagenic in the Ames salmonella assay systems (93, 119). These fractions are clearly of interest because they possess the capability of inducing genetic changes that might lead to tumor formation. Mutagenesis assays may provide a basis for the quantitative comparisons of new cigarettes when the relative importance of the genetic and epigenetic factors in smoke-induced cancer is understood. The Ames test gives poor results for fractions of smoke condensate that appear to be most active in systems designed to detect tumor-promoting activity (Table 4). Furthermore, mutagenesis assays of a series of experimental cigarettes have not provided consistent results (167). The complexity of carcinogenesis by tobacco smoke condensates renders mutagenesis assays of uncertain value for quantitative comparisons of relative carcinogenicity.

Several in vitro systems measure the transformation of normal cells into malignant cells after exposure to carcinogens. These systems are sensitive to both genetic and epigenetic processes (90, 186). Such assays

42

may prove to be useful short-term indicators of the relative potency of different types of cigarette smoke. The toxicity of most experimental smoke condensates may interfere with the conduct of such studies, however. Experimental cigarettes that yield smoke condensates with a wide range of carcinogenic activity are now available. It should be possible to determine the usefulness of *in vitro* systems with this material. For organ-specific carcinogens, the DNA repair test is a good predictor of relative carcinogenic activity (*186*).

Most chemicals that are carcinogenic to mouse skin selectively destroy the sebaceous glands of the treated skin (*23*). The sebaceous gland suppression assay is a good predictor of the activity of experimental smoke condensates as carcinogens in mouse skin (*22*).

Chronic Obstructive Lung Disease

No animal models for chronic obstructive lung disease are available to measure the potency of smoke from various types of cigarettes. Long-term inhalation studies with hamsters, dogs, and primates have not given rise to disease states comparable to emphysema observed in humans (*17, 50, 52, 114*). In two experiments, Sprague-Dawley and CD rats exposed to cigarette smoke for 6 to 26 months developed emphysematous changes (*104, 124*). Similar results were not reported in other long-term studies with rats (*44, 68*).

A number of pulmonary function tests have been evaluated as measures of early lung disease in man (*31, 61, 73, 100, 135, 154*). Thus far, similar tests have not proved useful as animal assays. They might, however, be useful in comparing the effects of different types of cigarettes on human smokers. Exposure of CD rats to whole tobacco smoke for 6 months led to a loss of lung parenchymal tissue distal to the terminal airways (*124*). This was indicated by a 21 percent decrease in parenchymal tissue and 12 percent decrease in alveolar surface area.

Recent evidence suggests that emphysema results from a shift in the balance of elastase production and elastase inhibition in the lung (*97*). A few individuals with genetically determined very low levels of alpha$_1$-antitrypsin, an elastase inhibitor, are particularly prone to develop the disease (*53*). When purified elastase is instilled into the lungs of dogs, emphysematous changes appear in as little as 90 minutes (*96, 98*).

Cigarette smoke can act on this system in two ways. *In vitro* tests with cigarette smoke condensate show that this material suppressed the antiprotease activity of human serum, pulmonary lavage fluid, and purified human alpha$_1$-antitrypsin (*94*). The suppression of protease inhibitors by cigarette smoke is blocked by the presence of phenolic antioxidants, suggesting that oxidants or free radicals of the smoke were responsible for the effect (*107*). In one study, the serum levels of alpha$_1$-antitrypsin in smokers were higher than in nonsmokers (*76*). Another study found, however, that immediately after smoking, serum

alpha₁-antitrypsin *activity* was reduced in smokers (*95*). Likewise, the activity of alpha₁-antitrypsin in lung lavage fluid from Sprague-Dawley rats was reduced by 30 to 40 percent after 3 to 6 puffs of cigarette smoke. Similar reductions were observed in lavage fluid from the lower respiratory tract of asymptomatic smokers (*58*). Even greater differences were seen between smokers and nonsmokers with idiopathic pulmonary fibrosis. Cigarette smoke also stimulates the release of elastase from macrophages *in vitro* and *in vivo* and from polymorphonuclear leukocytes *in vitro* (*19, 143, 185*). Thus, smoke may increase the elaboration of elastase in the lung and at the same time suppress its inactivation. The techniques used in these studies could be applied to smoke from various types of cigarettes; they might then serve as short-term end points to evaluate relative cigarette risk.

Dogs exposed to cigarette smoke through tracheostomies for 600 days had significantly higher levels of pancreatic elastase than sham-smoked controls (*122*). The greatest effects were seen in animals exposed to higher nicotine cigarettes, although the blood carboxyhemoglobin levels were the same for both higher and lower nicotine smokers (Figure 1). The lower nicotine cigarettes in this study were produced by removal of the alkaloid by a commercial process (*65*). It cannot be stated with confidence that other constituents were not removed as well.

Sudden Death Due to Cardiovascular Disease

Animal Models

No animal model permitting the quantitative comparison of death rates due to cardiovascular disease induced by different types of cigarettes is presently available. Long-term inhalation studies using smoke-exposed rats, hamsters, dogs, and primates have been conducted (*17, 44, 50, 52, 68, 104, 114*). None has provided an end point comparable to sudden death observed in human smokers. There are, however, several avenues of investigation whose intermediate experimental observations might indicate a mechanism for mortality caused by cardiovascular effects. Much attention has been given to changes induced by nicotine-induced catecholamine release (*138, 156, 160*). Methods to follow these effects in animals are well established. Other short-term end points being studied include lipoprotein levels (*79*), alteration of arterial morphology (*9, 10, 32, 111*), and changes in arachidonic acid metabolism (*12, 82*). These procedures might be adapted for estimation of the relative potency of various types of cigarettes, but there is no direct evidence that any of these changes are either necessary or sufficient indicators of the risk of sudden death due to heart disease.
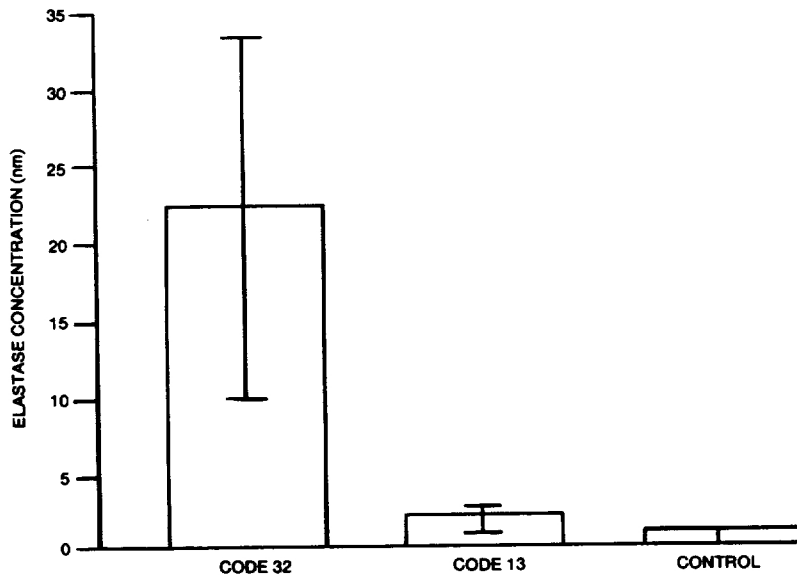
44

FIGURE 1.—Effect of cigarette smoke differing in selected chemical components on pancreatic elastase levels in beagle dogs after a 600-day exposure protocol of 12 cigarettes per day, 7 days per week. Bars indicate mean ±SD. Animals exposed to code 32 (high-nicotine) and code 13 (low-nicotine) cigarettes differed significantly (p<0.05) in pancreatic elastase levels from corresponding sham-exposed controls. Significant differences were also observed (p<0.05) between code 32 and code 13 cigarette smokers (Student t-test).

SOURCE: Morosco and Goeringer (122).

Nicotine

It has long been known that nicotine elevates blood pressure and heart rate and may increase the onset of angina pectoris attacks. These effects were summarized in the 1976 report, *The Health Consequences of Smoking* (175). Nicotine readily passes through biological membranes. The level in the breast fluid of smoking women is similar to that found in the plasma (81). The heart rate of fetuses of smoking women is elevated, apparently caused by transplacental passage of nicotine (127, 136). Thus, nicotine causes widespread effects in the smoker.

An estimate of the relative potency of various cigarettes with respect to the acute cardiovascular effects of nicotine can be determined by direct chemical assay of relative levels of nicotine in the smoke. By measurement of urinary excretion of nicotine and its major

metabolite, cotinine, it is possible to estimate the individual smoker's actual exposure to nicotine.

Nicotine appears to have measurable effects on performance by smokers (*149, 183*). This may account for the apparent role of nicotine in the reported tendency of some individuals to compensate when switched from higher to lower "tar" and nicotine cigarettes (*60, 139, 142, 146, 147*).

Carbon Monoxide

The effects of carbon monoxide in reducing the oxygen-carrying capacity of the blood are well known. More recently a body of evidence has linked carbon monoxide directly to disease states and to early end points that might be predictive of disease (*11, 109*). Aronow has shown that carbon monoxide, along with nicotine, decreased the duration of exercise achieved before angina (*6, 7, 8*). In his studies, a non-nicotine cigarette made of Indian herbal leaves was employed. Smoke from these cigarettes was more active than expected on the basis of its carbon monoxide content. Aronow (*6*) attributed this effect to a "tobacco component" other than nicotine or carbon monoxide. The effect, however, could well have been caused by a specific herb constituent. Models using pigeons, rabbits, pigs, and primates have been employed to study early end points for carbon monoxide effects (*4, 11, 114*). To the extent that carbon monoxide is responsible for cardiovascular disease, determination of the relative potency of various cigarettes in affecting cardiovascular disease can be made by chemical assay of cigarette carbon monoxide yield.

Other Agents

It has been suggested that agents of tobacco smoke other than nicotine and carbon monoxide contribute to its cardiovascular effects (*4, 116*). Until these agents are identified or an alternative explanation for tobacco effects is established, animal models predictive of cardiovascular death in smokers will be important.

Complications of Pregnancy and Early Childhood

A full understanding of the potential effects of smoking on pregnancy and early infancy is still being developed. Most of the current information available was reviewed in the 1980 report, *The Health Consequences of Smoking for Women* (*174*). Maternal smoking causes changes in the vascular structure of the placenta and increased fetal heart rate (*9, 10, 127, 136*). Maternal carboxyhemoglobin (HbCO) is elevated in smokers, leading to an elevated fetal HbCO and thus to a reduced oxygen content of the fetal blood (*108*).

Some, if not all, of the smoking-related complications of pregnancy are attributed to nicotine and carbon monoxide (*108*). The relative

46

hazards of lower "tar" and nicotine cigarettes with respect to these agents can be determined by chemical assays of carbon monoxide and nicotine. Actual disease risk, however, will be affected by the delivered dose of these constituents, which in turn depends upon the individual's style of smoking. Other constituents of smoke might also contribute to complications of pregnancy. Comparisons of various types of cigarettes should be possible through epidemiological study, coupled perhaps with evaluation of the vasculature of human placenta (9, 10).

Recent reports indicate that cigarette smoke might contain active transplacental carcinogens (54, 125, 140). The importance of this in human cancer will probably not be determined soon. No animal assays have yet been applied to assess the relative health hazard of varying cigarettes in transplacental carcinogenesis.

Nonspecific End Points of Toxicologic Significance

Cigarette smoke and its components cause several conditions that may relate to human disease in nonspecific ways. Using assays with these end points may provide useful measures of potential risks due to smoking.

Reduction of Lung Defense Mechanisms

Vapor-phase constituents of cigarette smoke inhibit ciliary motility and mucous flow in experimental animals (13, 14). With ciliary paralysis, removal of other toxic materials from the lung will be inhibited. Animal models suffer some limitations in attempts to duplicate the human situation. For example, many of the ciliastatic agents in the gas phase of smoke are absorbed in the upper airways of man and may not reach areas in the lung where they could affect bronchial cilia (45). Furthermore, the concentration of ciliatoxic agents in cigarette smoke will depend on the amount of dilution of smoke by air that occurs during inhalation. Accordingly, the interpretation of animal studies requires care. Similar effects occur in humans, however. Clearance of Fe_3O_4 dust from the lungs of smokers is dramatically slower than from the lungs of nonsmokers (37).

Induction of Microsomal Oxidase

Cigarette smokers metabolize several compounds more rapidly than nonsmokers (38, 39, 99, 187). This effect is believed caused by the induction of microsomal oxidases, which include aryl hydrocarbon hydroxylase (AHH). The level of AHH itself is much higher in placentas from smoking women than from nonsmokers (130, 131, 178). Activation of these enzymes has also been observed in the lungs of rats, hamsters, and mice exposed to cigarette smoke (1, 59). Guinea pigs, in contrast, showed a reduction in pulmonary AHH after smoke exposure (18). Induction of AHH activity appears to result from

47

systemic exposure to the smoke compounds themselves or to the metabolites of those compounds. Some carcinogens, including PAH, induce AHH (*38*). More important, the AHH system is involved in the metabolic formation of ultimate carcinogens from procarcinogen precursors (*118*). Cigarette smoke may play an indirect role in carcinogenesis among smokers through this mechanism. Assay of the inducibility of AHH as a measure of individual sensitivity to cigarette smoke has not proved useful (*115, 128*); however, screening of enzyme activity in tissues of human or animal smokers of different types of cigarettes might prove useful for indicating the relative potency of the different cigarettes.

Changes in Genetic Status

To the extent that an early step of carcinogenesis involves genetic change, one would expect that exposure to cigarette smoke might cause detectable changes in genetic material. It is reported that heavy smokers have higher incidences of chromosomal aberrations and higher rates of sister chromatid exchange than do nonsmokers (*91*). Animal models with such end points are feasible, but have not been applied to assays of the toxicity of various cigarettes.

Changes in Immune Status

Recent reports suggest that smoking causes changes in immune function (*56, 69, 144*), but the contribution of these effects to major disease states is unclear. Men with malignant melanoma who smoke are more likely to develop metastases than are nonsmokers, perhaps as a consequence of impaired immune systems (*153*).

Composition of Smokes From Various Types of Cigarettes

Smoking-Machine Design

Laboratory smoking-machine parameters historically have been standardized to permit interlaboratory comparisons and to provide reproducible baselines with which modified cigarettes can be compared. Somewhat different parameters are used in different countries (*28*). In the United States, the most widely used standards are those employed by the Federal Trade Commission (*133*). The machines deliver a 35 ml puff from the cigarette over a 2-second period with a bell-shaped puff profile. The cigarettes are puffed once each minute to the defined butt length of 23 mm (nonfiltered cigarettes), or to a butt length 3 mm longer than the filter overwrap (filter-tipped cigarettes). The butt length is different from cigarette to cigarette, according to the length of the overwrap.

These parameters were established in 1967 when the great majority of cigarettes consumed in the United States were nonfiltered and 70 or

48

85 mm in length. They were based, in part, on observed smoking patterns in a limited number of human smokers. The types of cigarettes smoked today are substantially different with respect to length, paper porosity, pressure drop, "tar" and nicotine yield, and the concentration of gas phase constituents.

Cigarette smoking-machines can be designed, however, to control puff volume, frequency of puffing, duration of puff, the profile of puff pressure over time, butt length, position of cigarette during and between puffs (e.g., horizontal or vertical), and "restricted" or "free" smoking between puffs (i.e., whether the butt end is closed or open). The puff volume can be measured in terms of the air entering the cigarette or the air plus combustion gases leaving the cigarette. Smoking-machines could be designed to change the puff frequency and the nature of the puffs during the course of smoking a single cigarette (41, 42).

Human smoking patterns are diverse and span a wide range from one individual to another (40, 78, 139). Some individuals compensate for lower yield cigarettes by changing their style of smoking (80, 139, 142, 146, 180). These changes can include increasing puff volume, duration, or frequency, or changing the puff pressure profile. In summary, human smoking behavior may be quite different from standard smoking-machine behavior. Furthermore, the average smoker may have a different smoking pattern for each different type of cigarette.

The chemical composition of smoke is affected by smoking-machine parameters. "Tar" yield per puff depends on puff volume, puff frequency, butt length, and the frequency of puffing at different stages of cigarette consumption (188, 193, 194). The concentrations of several specific chemical constituents of "tar" are controlled by the puff frequency, volume, and duration (Chortyk, O.T., and Schlotzhauer, W.S.S., personal communication). If the human smoking pattern varies systematically with the type of cigarette, the relative yield of various chemical constituents delivered to the smoker may vary substantially from that measured by machine. Accordingly, evaluation of the toxicological and pharmacologic potential of the smokes from new types of cigarettes will require knowledge of the manner in which those cigarettes are smoked by the consumer and of the effect of smoking patterns on the composition of smoke.

Dependence of Smoke Composition on Cigarette Design

The composition of smokes from different types of cigarettes can be described by absolute yields per cigarette or per puff, or by the concentration of constituents per unit weight of "tar" or per unit volume of smoke. Modifications of cigarette design can affect yield (quantitative change) or composition of the smoke (qualitative change). Information with respect to individual constituents is available for many modifications. However, modifications affecting the

concentration of one substance will also affect the levels of other substances as well.

Because of the complexity of cigarette smoke, the full impact of any cigarette modification on the composition of the smoke in either absolute or relative terms can never be ascertained. For this reason, bioassays with appropriate end points are essential to determine the relative toxicities of new types of cigarettes. Several modifications of cigarettes reduce the mouse skin carcinogenic activity of the smoke condensate. These include choice of leaf variety, use of reconstituted sheet, and use of tobacco substitutes.

Filters

The design characteristic of commercial cigarettes that most affects the cigarette yield is the filter. In 1980, the "tar" yield of cigarettes, as reported by the Federal Trade Commission or by advertisements, ranged from 30 mg for unfiltered, king-size cigarettes to as low as 0.1 mg for some filter-tipped brands (55). Filters selectively remove nitrosamines and semivolatile phenols from the smoke (88, 120, 126, 188). Thus, not only the absolute delivery of these constituents but also their relative concentration in cigarette "tar" depend on the filter.

Ventilation

A second major influence on the composition of cigarette smoke is ventilation of the cigarette by the use of paper with a high degree of porosity or by the presence of holes in the mouthpiece. When more air is drawn through the paper or through the mouthpiece, the amount of air drawn through the burning coal of the cigarette is reduced. This effect will reduce the quantity of "tar." By altering the burn temperature, it will also change the combustion process and thus the composition of the smoke. Ventilation also dilutes the gas phase of the smoke with air, causing a marked reduction in the concentration of gas phase constituents in the smoke (66, 83, 126).

Tobacco Variety

A substantial collection of tobacco lines is available to plant geneticists. These include 63 species related to tobacco and about 1,000 different tobacco varieties (164). The wealth of this material permits genetic manipulation of the leaf, which could be used selectively to enhance or to reduce the content of specific constituents. Among flue-cured tobacco lines available at present, the nicotine concentration varies from 0.2 to 4.75 percent (34). Among various burley lines the concentration varies from 0.3 to 4.58 percent. The ranges could be extended by agronomists, should that be desired. Changes in yield of many other smoke constituents might be achieved by genetic modification.