Section 2. PHARMACOLOGY AND TOXICOLOGY

CONTENTS

LIST OF FIGURES

LIST OF TABLES

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[a]	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	13 ng
Vinyl chloride	C	1-16 ng	1.8 ng
Urethane	TI	10-35 ng	1.5 ng
Formaldehyde	CT, CoC	20-90 µg	30 µg
Hydrogen cyanide	CT, T	30-200 µg	110 µg
Acrolein	CT	25-146 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

*Cigarette may also contain such carcinogens as arsine, nicke carbonyl, and possibly volastile chlorinated olefins and nitro-olefins.

[a]C denotoes 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>95% NO; rest NO_2.

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

SOURCE: Wyder and Hoffmana *(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
Ben[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	?	?
Dibenz[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 hyrocarbons (>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	?	?
ß-Naphthylamine	BC	0-25 ng	20 ng
Other aromatic amines	BC	?	?
Unknown nitro compounds	BC	?	?
Polonium-210	C	0.03-1.8 pCi	?
Nickel compounds	C	10-600 ng	?
Cadmium compounds	C	9-70 ng	?
Arsenic	C	1-25 µg	?
Nicotine	T	0.1-20 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 1978-1976.

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).*

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 Canner 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.

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	3
Weak acids, ether soluble	5	80
Strong acids, insoluble	2	1
Strong acids, ether soluble	<1	3
Strong acids, water soluble	<2	3
Neutrals, 80% methanol soluble	2	7
Neutrals, cyclohexane soluble	≤1	13
Neutrals, nitromethane soluble	2	23

*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 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.

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 ß-naphthylamine. The presence of ß-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-

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

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

alpha$_1$-antitrypsin *activity* was reduced in smokers *(95)*. Likewise, the activity of alpha$_1$-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 viva* 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.

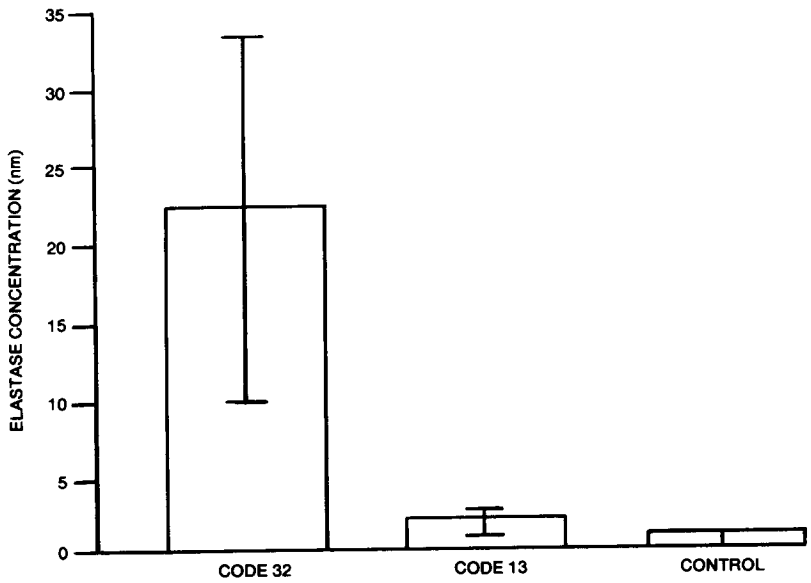

FIGURE 1.—Effect of cigarette smoke differing in selected chemical components on pancreatic elastase levels in beagle dogs after a 600-day exposure protocol of 12 cigarettes per day, 7 days per week. Bars indicate mean ±SD. Animals exposed to code 32 (high-nicotine) and code 13 (low-nicotine) cigarettes 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

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

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 corn pared. 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

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.

Agricultural Practice

The chemical composition of tobacco leaf is also affected by agricultural practice and by curing methods *(161, 163)*. High levels of nitrogen fertilizer increase nicotine and nitrate levels of the leaf. Growing plants more closely together reduces the nicotine content of the leaf. Flue-cured tobaccos are harvested, leaf by leaf, as each is ripe, but the entire plant of burley tobacco is harvested at once. Changes associated with leaf maturity depend on the harvesting practice. Enzymatic degradation of leaf constituents is halted by heat during flue curing. In contrast, burley, Maryland, and oriental tobaccos are not heated to this extent, so that more extensive enzymatic changes occur. As a consequence, there is a markedly lower sugar content in burley tobacco along with a markedly higher content of pigment polymers. Homogenized leaf curing (HLC), if commercially developed, could permit better control over these chemical changes. Furthermore, specific leaf constituents such as soluble proteins may be removed during homogenized leaf processing. Cigarettes made with HLC tobacco yielded smoke containing significantly less dimethylnitrosamine and condensate having significantly less sebaceous gland sup pression activity *(165, 169)*.

Reconstituted Sheet and Modified Tobaccos

The composition of cigarette smoke is also affected by the use of reconstituted tobacco sheet and modified tobaccos *(62, 63, 64, 65)*. Reconstituted sheet can contain substantial amounts of the tobacco "stem," which has a different composition from that of the leaf lamina. The stem is noteworthy for having a low nicotine content. In addition, the physical nature of reconstituted sheet can be controlled to change its burning characteristics and hence the composition of the smoke.

In recent years, some cigarette tobacco has been "expanded" or "puffed." Using this material, less tobacco is required to fill the cigarette. The manner in which the tobacco is shredded also affects the burning rate and therefore the composition of the smoke *(47)*. Cellulose-based substitutes have been used as a replacement for tobacco *(17, 35)*. These materials cause substantial differences in the total yield and chemical composition of the smoke.

Additives

Humectants and flavoring agents have long been used as additives in cigarette manufacture. The advent of reconstituted tobacco sheet (RTS) technology expanded the possibilities for the addition of substances to the sheet during the processing of tobacco for the manufacture of cigarettes *(174, 188)*. It is possible to add substances to the tobacco slurry or suspension for extraction of specific constituents, for dilution of the sheet, for burn rate acceleration or retardation, for

ash cohesion, and for enhancement of flavor (smoke aroma and taste) *(65, 151).* Additionally, one process for curing tobacco leaf calls for the addition of exogenous enzymes to tobacco *(169),* and as noted above, artificial tobacco substitutes are also available. In recent years, cigarette manufacturers' advertisements have focused on the flavor of new lower "tar" and nicotine cigarettes, enhanced presumably by the addition of tobacco constituents or by the addition of new flavoring materials, such as natural or synthetic chemicals. The identities and amounts of the additives actually used in the manufacture of U.S. cigarettes are not known. Systematic information has not been published or made available on the influence of these additives on the composition or biological activity of cigarette smoke.

Variations in Human Smoking Behavior

It does not appear possible to fully monitor smoking behavior in humans without the subjects' knowledge. Butt lengths can be measured in a variety of settings, and puff frequency can be observed without distorting smoking behavior. Measurement of puff volume and duration and of intensity of inhalation, however, requires instrumentation that may lead to alteration of usual smoking behavior. Nevertheless, despite these limitations in objectivity, recent studies provide better data than those available in the past.

Smoking measurements reported from England, Germany, and Canada differ from those used for smoking-machines in the United States *(139, 141, 150).* If the average American smoker, as well, is taking larger puffs with a greater frequency than is the machine, the absolute yields of smoke constituents are under-reported in the United States. This is not to say that the relative yield of "tar" between cigarettes is compromised; however, if smokers puff different types of cigarettes in different ways, the relative yields may be grossly distorted. For example, some smokers block the perforations in the mouthpiece of ventilated cigarettes *(102).* These smokers receive substantially more "tar," nicotine, and gas phase constituents than would be predicted from machine-smoked cigarette yields. Because this action would affect the yield only of ventilated filter cigarettes, the relative ranking of cigarettes by yields would be affected. Similarly, smokers' behavioral compensation for low nicotine delivery can affect the relative yields of filter-tipped cigarettes *(80, 142).*

Research Needs

Many gaps in our assessment of the pharmacological properties of cigarette smoke can be filled by a coordinated, welldirected research program. In comparison with the economic and medical costs of cigarette smoking, the size of the required program is modest. Besources sufficient for implementation of a meaningful program are

available. For example, except for assays of "tar," nicotine, and carbon monoxide yield, new types of cigarettes are not being monitored regularly for the delivery of potentially harmful smoke constituents. Scientists currently conducting sophisticated assays of cigarette delivery of various smoke constituents could serve as resource personnel in the design of an appropriate approach to assays of new cigarettes for suspected toxic agents. Other scientists are investigating short-term end points indicative of long-term risk from many diseases. These laboratories could assist in modifying these procedures specifically for cigarette smoke and its constituents.

Surveillance of New Cigarettes

The chief research need for the study of reduced "tar" and nicotine cigarettes is the routine and frequent surveillance of current and new cigarettes for specific chemical constituents and biological activity. The chemical constituents should include nicotine, benzo[a]pyrene, phenols, catechols, nitrosamines, carbon monoxide, nitrogen oxides, volatile aldehydes, and radionuclides. The biological assays should include sebaceous gland suppression assays, mutagenesis assays, studies of the effects of smoke on airway and ciliary function and on the increase of urinary metabolites related to the activity of elastase, and such other biological assays as may appear predictive of human disease in the future.

Inherent in this recommendation is the use of quantitative short-term end points for various conditions associated with human disease. We do not have proven animal models for quantitative evaluation of risks of chronic obstructive pulmonary disease, sudden death due to cardiovascular disease, or complications of pregnancy and infancy. Emphasis should be given to developing short- and long-term bioassays aimed particularly at these diseases.

Determination of Parameters of Human Cigarette Smoking

Smokers may smoke different types of cigarettes differently with respect to puff volume, duration, and frequency, inhalation profiles, and the manner in which the cigarette is held by the fingers and in the mouth. To conduct meaningful assays of cigarette yields and of the biological activity of cigarette smoke, it is important to know how smokers consume each type of commercial cigarette. Only when this information is available can smoking-machines be designed to yield the most accurate estimate of human dose. We must know both the average and the range of variation in smoking pattern.

The available studies compare smokers' behavior with commercial cigarettes found to deliver different amounts of "tar" or nicotine. Other changes that occur in the product are often unknown. A second type of study should use prototype cigarettes specifically designed to deliver a wide range of concentrations of a desired constituent; for

example, with high or low nicotine to "tar" ratios. Such a study would define the behavior of smokers of new types of cigarettes before or as they are marketed. These studies, however, would require a particular resource that is not accessible to most investigators. There are a large number of experimental cigarettes differing widely in several respects *(62, 63, 64, 65).* Unfortunately, they were developed without concern for smoker acceptability and cannot be used to evaluate human response to design changes. A coordinated program should be established to develop a series of clinically acceptable experimental cigarettes that resemble a "reference standard" as closely as possible, differing only in one or two well-defined characteristics. These should then be made available to appropriate investigators for the study of human smoking behavior.

Evaluation of Health Effects of Nicotine

Nicotine has pharmacological significance for man and animals *(92).* The alkaloid is suspected of playing a role in sudden death due to cardiovascular disease, to the complications of pregnancy and infancy, and possibly to chronic obstructive pulmonary disease. Nicotine in cigarettes leads to the formation of tobacco-specific nitrosamines in the smoke. These are potent carcinogens. Nicotine itself is a significant cocarcinogen in mouse skin carcinogenesis assays of smoke condensate.

It is important to determine whether nicotine acts as a cocarcinogen under the conditions of dosage achieved by cigarette smokers and whether the levels of nicotine-derived nitrosamines play a role in human malignant disease. Resources for such study are available and should be employed in a comprehensive evaluation of the potential carcinogenic effects of new types of cigarettes.

Nicotine should be tested alone, and in the presence of other noxious agents such as carbon monoxide, in animal systems designed to serve as models for nonmalignant diseases associated with cigarette smoke.

Experimental cigarettes with a range of nicotine content have been produced for studies of carcinogenesis. Many of these cigarettes are still available. Those experimental cigarettes that might be needed for pharmacological studies of nicotine should be identified and distributed to appropriate laboratories as the need develops.

The Effects of Smoking-Machine Parameters on Relative and Absolute Yields of Smoke Components From Various Types of Cigarettes

Smoking-machine assays of cigarettes fulfill two needs. The FTC ratings of "tar" and nicotine yields measure an implied risk to the smoker. Smoking-machine data guide experimenters in elucidating the mechanisms of induction of smoking-related disease. Absolute levels of smoke constituents may be very important for experiments, so the experimenter must have reliable information about the comparability

of machine and human smoking. The use of machine data to monitor risk has somewhat different requirements. If the *relative* yields of different cigarettes are not greatly affected by smoking conditions, present smoking-machine standards will be adequate to indicate relative risk of new cigarettes. We know, however, that the relative yield of many constituents is affected by butt length, puff frequency, and degree of ventilation. We need to determine how the variations in these smoking parameters affect relative yields of the several substances in smoke that are of toxicological interest.

Influence of Raw Product Modification on the Pharmacology of Cigarette Smoke

The composition of smoke is determined by the physical and chemical properties of leaf tobacco. Modification of the raw product therefore changes the pharmacology of cigarette smoke. The diversity of available tobacco germplasm along with known genetic techniques permits reduction of hazards in cigarettes through plant breeding and selection. Cultural and curing practices are constantly changing in response to market demands and the needs of farmers. Pesticides currently registered for use on tobacco have been tested as contributors to the carcinogenic activity of cigarette smoke condensates. When used as directed, these materials caused no significant change in biological activity *(65, 166)*. However, the pesticides used in tobacco farming change from time to time in response to the occurrence of new plant pests; for example, the recent spread of blue mold in tobacco growing regions has led to the use of a new pesticide. It is not known whether the use of such materials may result in changes in the hazards of cigarette smoke.

Present tobacco curing processes may vary somewhat from farm to farm. Furthermore, marked differences in agricultural practices such as close spacing of tobacco plants, bulk curing, and homogenized leaf curing might be introduced in the future. We need to determine the consequences of changes (genetic, cultural, and curing methodologies) on both the chemical composition and the biological effect of cigarette smoke.

Physical and Chemical Properties of Smoke From Cigarettes Delivering Less Than 10 mg of "Tar"

In the past few years, cigarettes delivering less than 10 mg of "tar" by FTC test have been placed on the market. These cigarettes apparently employ efficient filters together with various degrees of smoke dilution. The extreme reduction of "tar" and nicotine delivery by these cigarettes suggests significant differences in combustion processes. Substantial differences in the chemical nature of both mainstream and sidestream smoke might result from such changes.

Some or all of the new lower "tar" and nicotine cigarettes are manufactured by processes that involve the use of chemicals or flavor additives to improve consumer acceptability. The nature of these additives, and their combustion products, that are currently used in marketed cigarettes is not available to the public or to the Government. Likewise, there are no published data on the biologic effects of these additives or their combustion products.

Very low yield cigarettes may add to present concerns with respect to sidestream smoke (5, *157, 184)*. While these cigarettes may deliver such low levels of "tar," nicotine, and gas phase constituents that smokers cannot compensate completely, the delivery of sidestream smoke may not be reduced. Indeed, the sidestream smoke might contain more of some substances (e.g., pyrolytic products of flavor additives) than does the sidestream smoke of higher yield cigarettes. For very low yield cigarettes, the risk of the sidestream smoke may equal that of the mainstream smoke. The chemical and physical nature of sidestream smoke should be determined on new cigarettes.

Development and Validation of Analytical Methods

Methods for determining "tar" and nicotine yield were developed before very low yield cigarettes were an important segment of the market. It is questionable whether existing procedures can measure accurately the "tar" delivery of the cigarettes yielding 0.1 mg of "tar." Other techniques giving acceptable results must be developed. Procedures for determining "tar" yields of low magnitude through measurement of fluorescence have been recommended *(159)*. These methods must be validated by determining intra- and inter-laboratory reproducibility. Furthermore, fluorescence measurements may be compromised by additives that interfere with fluorescence, either directly or through the behavior of their pyrolytic products. Fluorescence measurements may not be satisfactory for use with new commercial cigarettes.

Analytical procedures must also be validated for a number of chemical constituents in smoke such as aldehydes, nitrogen oxides, phenols and catechols, aromatic hydrocarbons, and nitrosamines. Several laboratories are conducting such assays with favorable results. However, coordinated comparisons among laboratories to measure the degree of intra- and inter-laboratory variability have not been reported.

Other Research Needs

A number of other research needs of lesser priority should be addressed:

1. It is necessary to study the interaction of smoking with occupational and environmental exposure to other noxious materials. The incidence of lung cancer is greatly increased in asbestos workers or uranium miners who smoke cigarettes *(9, 70, 117)*. The

risk of using contraceptive hormones is also greater in cigarette smokers *(132, 174)*. Laboratory models of cocarcinogenesis should be used to measure the potential effect of combined smoking and exposure to other environmental toxins. Animal models should be developed to investigate the possible synergism of smoking and the environment in causing other diseases.

2. It is necessary to determine the threshold, if any, for carbon monoxide with respect to cardiovascular effects, pregnancy, and psychological performance. Carbon monoxide delivery of cigarettes can be controlled by ventilation *(66, 126)*. To determine the carbon monoxide risk of lower "tar" and nicotine cigarettes, we need to know whether thresholds for carbon monoxide activity exist and whether these thresholds vary for individuals of different ages, medical histories, or genetic backgrounds. Evaluation of risk due to carbon monoxide must take environmental exposure into consideration *(152)*.
3. It is necessary to define the extent of smoker compensation for differences in nicotine delivery of cigarettes. To the extent that smokers compensate for lower nicotine delivery, they will probably obtain more of other constituents from lower nicotine than from higher nicotine cigarettes. For example, the smoker might take more puffs to obtain the same dose of nicotine, and thus receive a greater dose of carbon monoxide *(80, 145)*. It should be determined at what point smokers can no longer compensate for lower nicotine levels and whether compensation is a permanent behavior change of smokers who switch to lower "tar" and nicotine cigarettes. To carry out such studies, standardized noninvasive procedures to indicate smoke uptake from cigarettes yielding various amounts of "tar," nicotine, and carbon monoxide should be validated. Analyses of blood, urine, and expired air have been used for these purposes *(25, 179, 181)*. Analysis of saliva for nicotine might also be useful. With any procedure, inter-laboratory comparisons using standardized methods are needed.
4. Many gas phase components of cigarette smoke are ciliatoxic in the experimental setting. They may overcome physiologic defense barriers against pulmonary toxins. To some extent, the ciliatoxic agents are absorbed in the mouth and upper airways and do not reach the deeper portions of the lung. Experimental systems may not be capable of duplicating the anatomic and behavioral factors that may affect human response to ciliatoxic agents. Nevertheless, short-term sequellae of smoking can be measured in human smokers of different types of cigarettes. Further evaluation of these effects in man should be undertaken.
5. Attention to chemical habituation evoked by cigarette smoking is centered on nicotine, which is the most active acute pharmacolog-

ic agent in cigarette smoke. It is necessary to determine whether there may be other chemicals present in cigarette smoke that contribute to cigarette smoking reinforcement.

6. A variety of short-term animal models with quantitative end points predictive of the development of tobacco-associated diseases should be developed. Except for cancer, long-term animal models suitable for quantitative comparisons of disease risk are not adequate. Even if successful long-term animal models are developed, the costs in time and resources may prevent the timely evaluation of new cigarettes.
7. It is necessary to develop methods for dissemination of information regarding the delivery of various noxious agents by cigarettes. The smoke content of "tar," nicotine, carbon monoxide, phenolic constituents, volatile aldehydes, nitrogen oxides, aromatic hydrocarbons, and nitrosamines may all contribute to the risks incurred by smokers. The Federal Trade Commission releases its findings of "tar" and nicotine yields of cigarettes and has announced its intention to assay carbon monoxide delivery. As additional monitoring assays are conducted, it will be necessary to present the new information to the public and to health professionals in a meaningful way.
8. It is necessary to evaluate the health hazard posed by passive inhalation by nonsmokers of the sidestream smoke from new types of cigarettes. Lower "tar" and nicotine cigarettes are designed to reduce the mainstream smoke received by the smoker. There is no evidence that the amount of sidestream smoke or its quality is improved by these design changes. Indeed, if additives are used to insure acceptability of the cigarettes by the smoker, their pyrolytic products may occur in the sidestream smoke. New types of cigarettes should be monitored for the qualitative and quantitative risks they might impose on the nonsmoker.
9. It is necessary to evaluate cigarettes with lower "tar" to nicotine ratios than are currently found in the market place. Compensation by smokers of lower "tar" and nicotine cigarettes appears to be based on nicotine delivery. The "tar" to nicotine ratio may limit the delivery of smoke constituents to the smoker. A low ratio might be a desirable strategy for lower risk cigarettes. It should be determined whether smoke from cigarettes with unusually low "tar" to nicotine ratios has unusual pharmacologic or toxicologic properties.
10. It is necessary to develop a low "tar" and nicotine reference cigarette. Several laboratories will need these reference cigarettes as a standard for comparisons of lower "tar" and nicotine commercial cigarettes. Commercial products cannot serve as a reference because design changes are made without announce-

ment and because the identity of additives is not disclosed. Without a stable reference, intra-laboratory comparisons conducted at different periods of time and many inter-laboratory studies will be compromised. Reference cigarettes are available for a limited range of "tar" and nicotine deliveries. A reference cigarette delivering very low levels of "tar," nicotine, and gas phase constituents is needed. To produce a reference of sufficient quality, large numbers of cigarettes must be made. Because an effort of this magnitude cannot be undertaken by individual researchers, a centralized facility to provide reference cigarettes to appropriate scientists is desirable.

Summary

1. Several thousand constituents have been identified in tobacco and tobacco smoke. Of these, nicotine appears to be the most important acute-acting pharmacologic agent. Nicotine's physiologic effects include increased heart rate and blood pressure. Nicotine also can permit the formation of tobacco-specific nitrosamines, which are potent carcinogens, and nicotine itself may be a significant cocarcinogen. The carcinogenic potency of cigarette smoke condensates appears to depend on the nicotine content of the "tar." This relationship may be due in part to the conversion of nicotine to tobacco-specific nitrosamines or to the coexistence of nicotine and some other unidentified carcinogen. Whether the carcinogenic effects of nicotine as determined in animal studies are directly applicable to humans is not known at present.
2. In an important study to predict the carcinogenic activity of cigarette smoke condensate, the amount of available nicotine delivered to the mice was found to be a factor in every term but one of the predictive model.
3. Polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines are two prominent classes of tumor initiators found in the smoke condensates of commercial cigarettes. Of the polycyclic aromatic hydrocarbons formed during combustion, benzo[a]pyrene (BaP) may be the most important and has been studied the most extensively. A correlation has been found between benzo[a]pyrene levels and the carcinogenic activity of smoke condensates from several types of cigarettes, but other studies have failed to show that carcinogenic potential is significantly dependent on benzo[a]pyrene content. However, the interaction of BaP with nicotine does appear important in carcinogenesis.
4. The tobacco-specific nitrosamines (TSNA) are formed during curing and fermentation of tobacco leaves and combustion of

cigarettes. TSNAs induce cancer in the lungs and trachea of hamsters and may be of particular importance in the induction of human laryngeal cancer. They may be active as contact carcinogens, or their metabolism at distant sites may produce carcino gens that are then transported to a target site.

5. It is not known whether the unidentified mutagens in cigarette smoke are an important cause of lung cancer in humans, but added exposure to any tumor initiators probably carries an increased risk of cancer.
6. Cigarette smoke contains oxidants that have been shown to reduce the activity of $alpha_1$-antitrypsin in animals and man. This inhibitory function is distinct from the effect whole smoke has on increasing levels of elastolytic enzymes released by neutrophils and macrophages.
7. The great variety of tobacco types makes it possible to manipulate the plant genetically to change the content of the constituents of the leaf. The chemical content of the leaf is also affected by agricultural practices and curing methods. The nicotine content of tobacco, for example, is related to the amount of nitrate fertilizer used in cultivation. Modification of tobacco as reconstituted sheet incorporates substantial amounts of tobacco stems that contain less nicotine than the leaf. The physical nature of reconstituted sheets can be controlled to change their burning characteristics and smoke composition.
8. Vapor-phase constituents of cigarette smoke inhibit ciliary motility and mucous flow in experimental animals.
9. Cigarette smokers metabolize several compounds more rapidly than do nonsmokers. This effect is believed to be caused by the induction of microsomal oxidases, which include aryl hydrocarbon hydroxylase (AHH). Induction of AHH activity appears to be caused by systemic exposure to the smoke compounds themselves or to the metabolites of those compounds. The AHH system may be involved in the metabolic formation of ultimate carcinogens from procarcinogen precursors.
10. In recent years, a number of flavoring additives or cellulose-based tobacco substitutes may have been included in manufactured cigarettes. The nature and amounts of such additives as actually used are not known, nor is it known what influence these additives may have on the chemical composition or subsequent biological activity of cigarette smoke.
11. Cigarette design has a major effect on smoke composition. The filter is the design characteristic that has the most impact on "tar" yield; it can also selectively remove nitrosamines and semivolatile phenols from smoke. The porosity of cigarette paper and the presence of holes in the mouthpiece influence smoke

composition because ventilation reduces the quantity of “tar” and dilutes the gas phase of smoke.

12. Because of the complexity of cigarette smoke, the total impact of any cigarette modification on smoke composition will probably never be fully known.
13. Many laboratory studies of the effects of smoke constituents have been carried out using smoking machines that control puff volume, frequency and duration, butt length, and other factors according to standardized parameters. However, the most widely used parameters were established in 1967, and the type of cigarettes generally smoked today are substantially different with respect to length, paper porosity, “tar” and nicotine content, and concentration of gas phase constituents. Evaluation of the toxicological and pharmacological properties of smoke from new types of cigarettes requires detailed knowledge of the manner in which those cigarettes are smoked, as well as of how smoking patterns affect smoke composition.

References

(1) AKIN, F.J., BENNER, J.F. Induction of aryl hydrocarbon hydroxylase in rodent lung by cigarette smoke: A potential short-term bioassay. *Toxicology and Applied Pharmacology* 36(2): 331-337, May 1976.

(2) AMES, B.N. Identifying environmental chemicals causing mutations and cancer. *Science* 204(4393): 587-593, May l1, 1979.

(3) ARCHER, V.E., WAGONER, J.K., LUNDIN, F.E., JR Uranium mining and cigarette smoking effects on man. *Journal of Occupational Medicine* 15(3): 204-211, March 1973.

(4) ARMITAGE, A.K., DAVIES, RF., TURNER, D.M. The effects of carbon monoxide on the development of atherosclerosis in the White Carneau pigeon. *Atherosclerosis* 23(2): 333, March-April 1976.

(5) ARONOW, W.S. Effect of passive smoking on angina pectoris. *New England Jorunal **of** Medicine* 299(1): 21-24, July 6, 1978.

(6) ARONOW, W.S. Effect of non-nicotine cigarettes and carbon monoxide on angina. *Circulation* 61(2): 263-265, February 1980.

(7) ARONOW, W.S., ISBELL, M.W. Carbon monoxide effect on exercise-induced angina pectoris. *Annals of Internal Medicine* 79(3): 392-395, September 1973.

(8) ARONOW, W.S., SWANSON, A.J. The effect of low-nicotine cigarettes on angina pectoris. *Annals of Internal Medicine* 71(3): 599-601, September 1969.

(9) ASMUSSEN, I. Ultrastructure of the human placenta at term. Observations on placentas from newborn children of smoking and nonsmoking mothers. *Acta Obstetricia et Gynecologica Scandinavica* 56(2): l19-126, 1977.

(10) ASMUSSEN, I. UItrastructure of human umbilical veins. Observations on veins from newborn children of smoking and nonsmoking mothers. *Acta Obstetricia et Gynecologica Scandinavica* 57(3): 253-255, 1978.

(11) ASTRUP, P. *Carbon* monoxide as a contributor to the health hazards of cigarette smoking. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 75-80.

(12) BAKHLE, Y.S., HARTIALA, J., TOIVONEN, H., UOTILA, P. Effects of cigarette smoke on the metabolism of vasoactive hormones in rat isolated lungs. *British Journal of Pharmacology* 65(3): 495-499, March 1979.

(13) BATTISTA, S.P. Cilia toxic components of cigarette smoke. In: Wynder, E.L., Hoffmann, D., Gori, G.B. (Editors). Proceedings of the Third World Conference on Smoking and Health, New York, June 2-5, 1975. Volume I. *Modifying the Risk for the Smoker*. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, DHEW Publication No. (NIH) 76-1221, 1976, pp. 517-534.

(14) BATTISTA, S.P. Inhalation studies of toxicity of tobacco smoke. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 51-62.

(15) BAYNE, C.K. *A Probability Prediction Model of Mouse Skin Tumors Based on Chemical Components of Cigarette Smoke Condensates*. Oak Ridge, Tennessee, U.S. Department of Energy, Oak Ridge National Laboratory, Publication No. ORNL/TM-7037, October 1979, 33 pp.

(16) BERENBLUM, I. A reevaluation of the concept of cocarcinogenesis. *Progress in Experimental Tumor Research* 11: 21-30, 1969.

(17) BERNFELD, P., HOMBURGER, F., SOTO, E., PAI, K.J. Cigarette smoke inhalation studies in inbred Syrian golden hamsters. *Journal of the National Cancer Institute* 63(3): 675-689, September 1979.

(18) BILIMORIA, M.H., JOHNSON, J., HOGG, J.C., WITSCHI, H.P. Pulmonary aryl hydrocarbon hydroxylase: Tobacco smoke-exposed guinea pigs. *Toxicology and Applied Pharmacology* 41(2): 433-440, August 1977.

(19) BLUE, M.-L, JANOFF, A. Possible mechanisms of emphysema in cigarette smokers. Release of elastase from human polymorphonuclear leukocytes by cigarette smoke condensate in vitro. *American Review of Respiratory Disease* 117(2): 317-325, February 1978.

(20) BOCK, F.G. Cocarcinogenic properties of nicotine. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 129-139.

(21) BOCK, F.G., CLAUSEN, D.F. Further fractionation and co-promoting activity of the large molecular weight components of aqueous tobacco extracts. *Carcinogenesis* 1: 317-321, April 1980.

(22) BOCK, F.G., GORI, G.B. *Sebaceour Gland Suppression Tests as an Indicator of the Carcinogenic Activity of Experimental Cigarette Smoke condensates (SCS).* Proceedings of the Sixty-Ninth Annual Meeting of the American Association for Cancer Research 19: 43, March 1978.

(23) BOCK, F.G., MUND, R. A survey of compounds for activity in the suppression of mouse sebaceous glands. Cancer Research 18(8): 887-892, September 1958.

(24) BOCK, F.G., SWAIN, A.P., STEDMAN, R.L. Bioassay of major fractions of cigarette smoke condensate by an accelerated technic. *Cancer Research* 29(3): 584-587, March 1969.

(25) BORGERS, D., JUNGE, B. Thiocyanate as an indicator of tobacco smoking. *Preventive Medicine* 8(3): 351-357, May 1979.

(26) BOUTWELL, R.K. Some biological aspects of skin carcinogenesis. *Progress in Experimental Tumor Research* 4: 207-250, 1964.

(27) BOUTWELL, R.K., BOSCH, D.K. The tumor-promoting action of phenol and related compounds for mouse skin. *Cancer Research* 19: 413-424, May 1959.

(28) BRUNNEMANN, K.D., HOFFMANN, D., WYNDER, E.L., GORI, G.B. Chemical studies on tobacco smoke. XXXVII. Determination of tar, nicotine, and carbon monoxide in cigarette smoke. A comparison of international smoking conditions. In: Wynder, E.L., Hoffmann, D., Gori, G.B. (Editors). Proceedings of the Third World Conference on Smoking and Health, New York, June 2-5, 1975. Volume I. *Modifying the Risk for the Smoker.* U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, DHEW Publication No. (NIH) 76-1221, 1976, pp. 441-449.

(29) BRUNNEMANN, K.D., LEE, H.-C., HOFFMANN, D. Chemical studies on tobacco smoke. XLVII. On the quantitative analysis of catechols and their reduction. *Analytical Letters* 9(10): 939-955,1976.

(30) BRUNNEMANN, K.D., YU, L., HOFFMANN, D. Assessment of carcinogenic volatile N-nitrosamines in tobacco and in mainstream and sidestream smoke from cigarettes. *Cancer Research* 37(9): 3218-3222, September 1977.

(31) BUIST, A.S., GHEZZO, H., ANTHONISEN, N.R., CHERNIACK, R.M., DUCIC, S., MACKLEM, P.T., MANFREDA, J., MARTIN, RR., MCCARTHY, D., ROSS, B.B. Relationship between the single-breath N_2 test and age, sex, and smoking habit in three North American cities. *American Review of Respiratory Disease* 120(2): 305-318, August 1979.

(32) BYLOCK, A., BONDJERS, G., JANSSON, I., HANSSON, H.-A. Surface ultrastructure of human arteries with special reference to the effects of smoking. *Acta Pathologica et Microbiologica Scondinavica (Section A: Pathology)* 87(3): 201-209, May 1979.

(33) CARP, H., JANOFF, A. Possible mechanisms of emphysema in smokers. In vitro suppression of serum elastase-inhibitory capacity by fresh cigarette smoke and its prevention by antioxidanta. *American Review of Respiratory Disease* 118(3): 617-621, September 1978.

(34) CHAPLIN, J.F. Genetic influence on chemical constituents of tobacco leaf and smoke. *Beitrage zur Tabakforschung* 8(4):233-240, December 1975.

(35) CLAPP, M.J.L., CONNING, D.M., WILSON, J. Studies on the local and systemic carcinogenicity of topically applied smoke condensate from a substitute smoking material. *British Journal of* Cancer 35(3): 329-341, March 1977.

(36) COHEN, B.S., EISENBUD, M., HARLEY, N.H. Alpha radioactivity in cigarette smoke. *Radiation Research* 83(1): 190-196, July 1980.

(37) COHEN, D., ARAI, SF., BRAIN, J.D. Smoking impairs long-term dust clearance from the lung. Science 204(4392): 514-517, May 4, 1979.

(38) CONNNEY, A.H., LEVIN, W. Carcinogen metabolism in experimental animals and man. In: Montesano, R., Tomatis, L. (Editors). *chemical carcinogenesis Essays*. International Agency for Research on Cancer Scientific Publications, No. 10, 1-24, 1974.

(39) COOKSLEY, W.G.E., FARRELL, G.C., CASH, G.A., POWELL, L.W. The interaction of cigarette smoking and chronic drug ingestion on human drug metabolism. *Clinical and Experimental Pharmacology and Physiology* 6(5): 527-533, September-October 1979.

(40) CREIGHTON, D.E., LEWIS, P.H. The effect of different cigarettes on human smoking patterns. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences.* New York, Churchill Livingstone, 1978, pp. 289-300.

(41) CREIGHTON, D.E., LEWIS, P.H. The effect of smoking pattern on smoke deliveries. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences*. New York, Churchill Livingstone, 1978, pp. 301-314.

(42) CREIGHTON, D.E., NOBLE, M.J., WHEWELL, R.T. Instruments to measure, record and duplicate human smoking patterns. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences*. New York, Churchill Livingstone, 1978, pp. 277-287.

(43) DAGLE, G.E., MCDONALD, K.E., SMITH, L.G., STEVENS, D.L., JR. Pulmo nary carcinogenesis in rate given implants of cigarette smoke condensate in beewax pellets. *Journal of the National Cancer Institute* 61(3): 905-910, September 1978.

(44) DALBEY, W.E., NETTESHEIM, P., GRIESEMER, R., CATON, J.E., GUERIN, M.R. Chronic inhalation of cigarette smoke by F344 rats. *Journal of the National Cancer Institute* 64(2): 383-333, February 1980.

(45) DALHAMN, T., EDFORS, M.L., RYLANDER, R. Mouth absorption of various compounds in cigarette smoke. *Archives of Environmental Health* 16(6): 831-835, June 1968.

(46) DAY, T.D. Carcinogenic action of cigarette smoke condensate on mouse skin. An attempt at a quantitative study. *British Journal of Cancer* 21(l): 56-81, March 1967.

(47) DEBARDELEBEN, M.Z., CLAFLIN, W.E., GANNON, W.F. Role of cigarette physical characteristics on smoke composition. *Recent Advances in Tobacco Science*. Valhalla, New York, Naylor Dana Institute for Disease Prevention, American Health Foundation, Series 4, 1978, pp. 85-111.

(48) DOLL, R., PETO, R. Cigarette smoking and bronchial carcinoma: Doss and time relationships among regular smokers and lifelong non-smokers. *Journal of Epidemiology and Community Health* 32(4): 393-313, December 1978.

(49) DONTENWILL, W. Biological evaluation of carcinogens in tobacco and tobacco smoke. In: Wynder, E.L., Hoffmann, D., Gori, G.B. (Editors). Proceedings of the Third World Conference on Smoking and Health, New York, June 2-5, 1975. Volume I. *Modifying the Risk for the Smoker*. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, DHEW Publication No. (NIH) 76-1221, 1976, pp. 209-220.

(50) DONTENWILL, W., CHEVALIER, H.-J., HARKE, H.-P., KLIMISCH, H.-J., KUHNIGK, C., RECKZEH, G., SCHNEIDER, B. Untersuchungen uber den Effekt der chronischen Zigarettenrauchinhalation beim syrischen Goldhamster and uber die Bedetung des Vitamin A auf die bei Berauchung gefunden Organveranderungen. [Studies on the effect of chronic cigarette smoke inhalation in Syrian golden hamsters and the importance of Vitamin A on morphological alterations after smoke exposure.] *Zeitschrift fur Krebsforschung und Klinische Onkologie* 89(2): 153-180, 1977.

(51) DONTENWILL, W., CHEVALIER, H.-J., HARKE, H.-P., KLIMISCH, H.-J., RECKZEH, G.. FLEISCHMANN, B., KELLER, W. Experimentelle Untersuchungen uber die tumorerzeugende Wirkung von Zigarettenrauch-Kondensaten an der Mausehaut. VII. Mitteilung: Einzelvergleiche von Kondensaten verschiedener modifizierter Zigaretten. [Experimental investigations on the tumorigenic activity of cigarette smoke condensate on mouse skin. VII. Comparative studies of condensates from different modified cigarettes.] *Zeitschrift fur Krebsforschung* und *Klinische Onlcologie* 89(2): 145-151, 1977.

(52) DONTENWILL, W., CHEVALIER, H.-J., HARKE, H.-P., LAFRENZ, U., RECKZEH, G., SCHNEIDER, B. Investigations on the effects of chronic cigarette-smoke inhalation in Syrian golden hamsters. *Journal of the National Cancer Institute* 51(6): 1781-1832, December 1973.

(53) ERIKSSON, S. Studies on a_1-antitrypsin deficiency. *Acta Medica Scandinavica* 177 (Supplement 432): 5-85, 1965.

(54) EVERSON, R.B. Hypothesis: Individuals transplacentally exposed to maternal smoking may be at increased cancer risk in adult life. *Lancet* 2(8186): 123-126, July 19, 1980.

(55) FEDERAL TRADE COMMISSION. *Report of "Tar" and* Nicotine *Content of the Smoke of 176 Varieties of Cigarettes.* December 1979.

(56) FERSON, M., EDWARDS, A., LIND, A., MILTON, G.W., HERSEY, P. Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. *International Journal of Cancer* 23: 603-609, 1979.

(57) FINE, D.H. An assessment of human exposure to N-nitroso compounds. In: Walker, E.A., Griciute, L., Castegnaro, M., Lyle, R.E., Davis, W. (Editors). Proceedings of a conference at Durham, New Hampshire, August 22-24, 1977. Environmental *Aspects of N-Nitroso* Compounds. Lyon, France, International Agency for Research on Cancer, IARC Scientific Publication No. 19, 1978, pp. 267-278.

(58) GADEK, J.E., FELLS, G.A., CRYSTAL, R.G. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science* 206(4424): 1315-1316, December 1979.

(59) GIELEN, J.E., GOUJON, F., SELE, J., VAN CANTFORT, J. Organ specificity of induction of activating and inactivating enzymes by cigarette smoke and cigarette smoke condensate. *Archives of Toxicology* (Supplement 2): 239-251, 1979.

(60) GOLDFARB, T., GRITZ, E.R., JARVIK, M.E., STOLERMAN, I.P. Reactions to cigarettes as a function of nicotine and "tar." *Clinical Pharmacology and Therapeutics* 19(6): 767-772, June 1976.

(61) GORI, G.B. (Editor). Proceedings of the Tobacco Smoke Inhalation Workshop on Experimental Methods in Smoking and Health Research, Bethesda, Maryland, June 19-21, 1974. Proceedings *of the Tobacco Smoke Inhalation Workshop on Experimental Methods in Smoking and Health Research.* U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Smoking and Health Program, DHEW Publication No. (NIH) 75-906, 1975, 83 pp.

(62) GORI, G.B. (Editor). *Toward Less Hazardous Cigarettes. The First Set of Experimental Cigarettes.* U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Smoking and Health Program, Report No. 1, DHEW Publication No. (NIH) 76-905, 1976, 148 pp.

(63) GORI, G.B. (Editor). *Toward Less Hazardous Cigarettes. The Second Set of Experimental Cigarettes.* U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutea of Health, National Cancer Institute, Smoking and Health Program, Report No. 2, DHEW Publication No. (NIH) 76-1111, 1976, 153 pp.

(64) GORI, G.B. (Editor). *Toward Less Hazardous Cigarettes. The Third Set of Experimental Cigarettes.* U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Smoking and Health Program, Report No. 3, DHEW Publication No. (NIH) 77-l280, 1977, 152 pp.

(65) GORI, G.B. (Editor). *Toward Less Hazardous Cigarettes. The Fourth Set of Experimental Cigarettes.* U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, Smoking and Health Program, Report No. 4, March 1980.

(66) GORI, G.B., ELLIS, R.L. Reduction of carbon monoxide in cigarette smoke. *Preventive* Medicine 8(3): 358-368, 1979.

(67) GREEN, C.R. Neutral oxygenated compounds in cigarette smoke and their possible precursors. *Recent Advances in Tobacco Science.* Valhalla, New York, Naylor Dana Institute for Disease Prevention, American Health Foundation, Series 3: 94-l20, 1977.

(68) GUERIN, M. Tumeurs pulmonaires et cancer buccal chez le rat soumis a l'inhalation de fumee de cigarette. *Bulletin de l'Association francaise pour l'Elude du Cancer* 46(2): 295-309, April-June 1959.

(69) GULSVIK, A., FAGERHOL, M.K. Smoking and immunoglobulin levels. *Lancet* 1(8133): 449, February 24, 1979. (Letter)

(70) HAMMOND, E.C., SELIKOFF, I.J., SEIDMAN, H. Asbestos exposure, cigarette smoking and death rates. In: Selikoff, I.J., Hammond, E.C. (Editors). Health Hazards of Asbestos Exposure. *Annals of the New York Academy of Sciences* 330: 473-490, 1979.

(71) HARLEY, N.H., COHEN, B.S., TSO, T.C. Polonium-210: A questionable risk factor in smoking-related carcinogenesis. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 93-104.

(72) HARRIS, R.J.C., NEGRONI, G., LUDGATE, S., PICK, C.R., CHESTERMAN, F.C., MAIDMENT, B.J. The incidence of lung tumours in C57BL mice exposed to cigarette smoke:air mixtures for prolonged periods. *International Journal of Cancer* 14(l): 130-136, July 15, 1974.

(73) HARRISON, G.N., MOOR, G.R., MOHLER, J.L., SPEIR, W.A., JR Small airway disease: Comparison of tests in young smokers versus nonsmokers. *Southern Medical Journal* 71(9): 1079-1081, September 1978.

(74) HECHT, S.S., CARMELLA, S., MORI, H., HOFFMANN, D. A study of tobacco carcinogenesis. XX. The role of catechols as a major cocarcinogen in the weakly acidic fraction. *Journal of the National Cancer Institute,* in press.

(75) HECHT, S.S., CHEN, C.H.B., HOFFMANN, D. Tobacco-specific nitrosamines: Occurrence, formation, carcinogenicity, and metabolism. *Accounts of Chemical Research* 12(3): 92-98, March 1979.

(76) HECHT, S.S., CHEN, C.-H.B., MCCOY, G.D., HOFFMANN, D., DOMELLOF, L. a-Hydroxylation of N-nitrosopyrrolidine and N'-nitrosonornicotine by human liver microsomes. *Cancer Letters* 8(1): 35-41, November 1979.

(77) HECHT, S.S., ORNAF, R.M., HOFFMANN, D. Chemical studies on tobacco smoke. XXXIII. N'-Nitrosonornicotine in tobacco: Analysis of possible contributing factors and biologic implications. *Journal of the National Cancer Institute* 54(5): 1237-1243, May 1975.

(78) HENNINGFIELD, J.E., GRIFFITHS RR. A preparation for the experimental analysis of human cigarette smoking behavior. *Behavior Research Methods and Instrumentation* 111(6): 538-544, December 1979.

(79) HEYDEN, S., HEISS, G., MANEGOLD, C., TYROLER, H.A., HAMES, C.G., BARTEL, A.G., COOPER, G. The combined effect of smoking and coffee drinking on LDL and HDL cholesterol. circulation 60(1): 22-25, July 1979.

(80) HILL, P., MARQUARDT, H. Plasma and urine changes after smoking different brands of cigarettes. *Clinical Pharmacology and Therapeutics* 27(5): 652-658, May 1980.

(81) HILL, P., WYNDER, E.L. Nicotine and cotinine in breast fluid. *Cancer Letters* 6: 251-254, 1979.

(82) HINMAN, L.M., STEVENS, C., MATTHAY, R.A., GEE, J.B.L. Angiotensin convertase activities in human alveolar macrophages: Effects of cigarette smoking and sarcoidosis. Science 205(4402): 202-203, July 13, 1979.

(83) HOFFMANN, D., ADAMS, J.D., WYNDER, E.L Formation and analysis of carbon monoxide in cigarette mainstream and sidestream smoke. *Preventive Medicine* 8(3): 344-350, May 1979.

(84) HOFFMANN, D., CHEN, C.-H.B., HECHT, S.S. The role of volatile and nonvolatile N-nitrosamines in tobacco carcinogenesis. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 113-l27.

(85) HOFFMANN, D., MASUDA, Y., WYNDER, E.L. a-Naphthylamine and ß-naphthylamine in cigarette smoke. Nature 221(5177): -254-256, January 18, 1969.

(86) HOFFMANN, D., RAINERI, R, HECHT, S.S., MARONPOT, EL, WYNDER, E.L. A study of tobacco carcinogenesis. XIV. Effect of N'-nitrosonornicotine and N'-nitrosoanabaaine in rats. *Journal of the National Cancer Institute* 55(4): 977-979, October 1975.

(87) HOFFMANN, D., SCHMELTZ, I., HECHT, S.S., WYNDER, E.L. Tobacco carcinogenesis. In: Gelboin, H.V., Tso, P.O. (Editors). *Polycyclic Hydrocarbons and Cancer.* Volume 1. New York, Academic Press, 1978, pp. 85-117.

(88) HOFFMANN, D., TSO, T.C., GORI, G.B. The less harmful cigarette. *Preventive Medicine* 9(2): 287-296, March 1980.

(89) HOFFMANN, D., WYNDER, E.L. A study of tobacco carcinogenesis. XI. Tumor initiators, tumor accelerators, and tumor promoting activity of condensate fractions. *Cancer* 27(4): 848-864, April 1971.

(90) HOLLSTEIN, M., MCCANN, J., ANGELGSANTO, F.A., NICHOLS, W.W. Short-term tests for carcinogens and mutagens. *Mutation Research* 65(3):133-226, September 1979.

(91) HOPKIN, J.M., EVANS, H.J. Cigarette smoke-induced DNA damage and lung cancer risks. *Nature* 283(5745): 388-390, January 24, 1980.

(92) HUDSON, RD. Central nervous system responses to cigarette smoke inhalation in the cat. *Archives Internationales de Pharmacodynamie et de Therapie* 237(2): 191-212, February 1979.

(93) HUTTON, J-J., HACKNEY, C. Metabolism of cigarette smoke condensates by human and rat homogenates to form mutagens detectable by salmonella typhimurium TA 1538. *Cancer Research* 35(9): 2461-2468, September 1975.

(94) JANOFF, A., CARP, H. Possible mechanisms of emphysema in smokers. Cigarette smoke condensate suppresses protease inhibition in vitro. *American Review of Respiratory* Disease 116(1): 65-72, July 1977.

(95) JANOFF, A., CARP, H., LEE, D.K., DREW, R.T. Cigarette smoke inhalation decreases a_1-antitrypsin activity in rat lung. *Science* 206(4424): 1313-1314, December 14, 1979.

(96) JANOFF, A., SLOAN, B., WEINBAUM, G., DAMIANO, V., SANDHAUS, R.A., ELIAS, J., KIMBEL, P. Experimental emphysema induced with purified human neutrophil elastase: Tissue localization of the instilled protease. *American Review of Respiratory Disease* 115(3): 461-477, March 1977.

(97) JANOFF, A., WHITE, R., CARP, H., HAREL, S., DEARING, R., LEE, D. Lung injury induced by leukocytic proteases. *American Journal of Pathology* 97(1): 112-129, October 1979.

(98) JOHANSON, W.G., JR., PIERCE, A.K. Effects of elastase, collagenase, and papain on structure and function of rat lungs in vitro. *The Journal of Clinical Investigation* 51(2): 233-293, February 1972.

(99) JUSKO, W.J. Influence of cigarette smoking on drug metabolism in man. *Drug Metabolism Reviews* 9(2): 221-236, 1979.

(100) KANNER, R.E., RENZETTI, A.D., JR, KLAUBER, MR., SMITH, C.B., GOLDEN, C.A. Variables associated with changes in spirometry in patients with obstructive lung diseases. *American Journal of Medicine* 67: 44-50, July 1979.

(101) KIER, L.D., YAMASAKI, E., AMES, B.N. Detection of mutagenic activity in cigarette smoke condensates. *Proceedings of the National Academy of Sciences of the United States of America* 71(10): 4159-4163, October 1974.

(102) KOZLOWSKI, L.T., FRECKER, R.C., KHOUW, V., POPE, M.A. The misuse of "less-hazardous" cigarettes and its detection: Hole-blocking of ventilated filters. *American Journal of Public Health* 79(11): 1202-1203, November 1980.

(103) LAVOIE, E., BEDENKO, V., HIROTA, N., HECHT, S.S., HOFFMANN, D. A comparison of the mutagenicity, tumor-initiating activity and complete carcinogenicity of polynuclear aromatic hydrocarbons. In: Jones, P.W., Leber, P. (Editors). *Polynuclear Aromatic Hydrocarbons.* Ann Arbor, Ann Arbor Science, 1979, pp. 705-721.

(104) LE BOUFFANT, L., MARTIN, J.C., DANIEL, H., HENIN, J.P. NORMAND, C. Action of intensive cigarette smoke inhalations on the rat lung. Role of particulate and gaseous cofactors. *Journal of the National Cancer Institute* 64(2): 273-281, February 1980.

(105) LEE, M.L., NOVOTNY, M., BARTLE, K.D. Gas chromatography/mass spectrometric and nuclear magnetic resonance spectrometric studies of carcinogenic polynuclear aromatic hyrocarbons in tobacco and marijuana smoke condensates. *Analytical Chemistry* 48(2): 405-416, February 1976.

(106) LEE, P.N., ROTHWELL, K., WHITEHEAD, J.K. Fractionation of mouse skin carcinogens in cigarette smoke condensate. *British Journal of Cancer* 35(6): 739-742, June 1977.

(107) LELLOUCH, J., CLAUDE, JR., THEVENIN, M. Alpha$_1$antitrypsine et tabac, une etude de 1296 hommes sains [Smoking and serum alpha(1)-antitrypsin in 1296 healthy men.] *Clinica Chimica Acta* 95(2): 337-345, 1979.

(108) LONGO, L.D. The biological effects of carbon monoxide on the pregnant woman, fetus, and newborn infant. *American Journal of Obstetrics and Gynecology* 129(1): 69-163, September 1, 1977.

(109) LOUGH, J. Cardiomyopathy produced by cigarette smoke. Ultrastructural observations in guinea pigs. *Archives of Pathology and Laboratory Medicine* 102(7): 377-330, July 1978.

(110) MAGEE, P.N., MONTESANO, R., PREUSSMANN, R. N-Nitroso compounds and related carcinogens. In: Searle, C.E. (Editor). *Chemical Carcinogens.* American Chemical Society Monograph 173, 1976.

(111) MARSHALL, M., HESS, H., STAUBESAND, J. Experimentelle Untersuchungen uber den Risikofaktor Rauchen. [Experiments on the risk factor smoking.] *Vasa; Zeitschrift Gefaesskrankheiten* 7(4): 389-397, 1978.

(112) MARTELL, E.A. Radioactivity of tobacco trichomes and insoluble cigarette smoke particles. *Nature* 349(5454): 215-217, May 17, 1974.

(113) MCCOY, G.D., WYNDER, E.L. Etiological and preventive implications in alcohol carcinogenesis. *Cancer Research* 39(7): 2844-2850, July 1979.

(114) MCGILL, H.C., JR., ROGERS, W.R., WILBUR, R.L., JOHNSON, D.E. Cigarette smoking baboon model: Demonstration of feasibility. *Proceedings of the Society for Experimental Biology and Medicine* 157(4): 672-676, April 1978.

(115) MCLEMORE, T.L., MARTIN, R.R., PICKARD, L.R., SPRINGER, R.R., WRAY, N.P., TOPPELL, K.L., MATTOX, K.L., GUINN, G.A., CANTRELL, E.T., BUSBEE, D.L. Analysis of aryl hydrocarbon hydroxylase activity in human lung tissue, pulmonary macrophages, and blood lymphocytes. *Cancer* 41(6): 2292-2300, June 1978.

(116) MCMILLAN, G.C. Evidence for components other than carbon monoxide and nicotine as etiological factors in cardiovascular disease. In: Wynder, E.L., Hoffmann, D., Gori, G.B. (Editors). Proceedings of the Third World Conference on Smoking and Health, New York, June 3-5, 1975. Volume I. *Modifying the Risk for the Smoker*. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Cancer Institute, DHEW Publication No. (NIH) 76-1221, 1976, pp. 363-367.

(117) MEURMAN, L.O., KIVILUOTO, R., HAKAMA, M. Combined effect of asbestos exposure and tobacco smoking on Finnish anthophyllite miners and millers. In: Selikoff, I.J., Hammond, E.C. (Editors). Health Harards of Asbestos Exposure. *Annals of the New York Academy of Sciences* 330: 491-495, 1979.

(118) MILLER, E.C. Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. *Cancer Research* 38(6): 1479-1496, June 1978.

(119) MIZUSAKI, S., OKAMOTO, H., AKIYAMA, A., FUKUHARA, Y. Relation between chemical constituents of tobacco and mutagenic activity of cigarette smoke condensate. *Mutation Research* 48(3/4): 319-325, July 1977.

(120) MORIE, G.P. Selective filtration of tobacco smoke components: A review. Proceedings of American Chemical Society Symposium, 173rd American Chemical Society Meeting, Agricultural and Food Chemistry Division, New Orleans, 1977. *Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke,* 1977, pp. 553-583.

(121) MORIE, G.P., SLOAN, C.H. Determination of N-nitrosodimethylamine in the smoke of high-nitrate tobacco cigarettes. *Beitrage zur Tabakforschung* 7(2): 61-66, June 1973.

(122) MOROSCO, G-J., GOERINGER, G.C. Pancreatic elastase and serum a_1-antitrypsin levels in beagle dogs smoking high- and low-nicotine cigarettes: Possible mechanism of pancreatic cancer in cigarette smokers. *Journal of Toxicology and Environmental Health* 5(5): 879-890, September 1979.

(123) MOROSCO, G.J., GOERINGER, G.C. Lifestyle factor and cancer of the pancreas: A hypothetical mechanism. *Medical Hypotheses* 6(9): 971-985, September 1980.

(124) NICHOLAS, H., DAVIES, P., SORNBERGER, C., HUBER, G. Alterations in lung parenchyma following experimental chronic inhalation of tobacco smoke. *Chest* 72(3): 409, September 1977.

(125) NICOLOV, I.G., CHERNOZEMSKY, I.N. Tumors and hyperplastic lesions in Syrian hamsters following transplacental and neonatal treatment with cigarette smoke condensate. *Journal of Cancer Research and Clinical Oncology* 94(12): 249-256, 1979.

(126) NORMAN, V. The effect of perforated tipping paper on the yield of various smoke components. *Beitrage zur Tabakforschung* 7(5): 282-287, September 1974.

(127) NYLUND, L., LUNELL, N.O., PERSSON, B., FREDHOLM, B.B., LAGERCRANTZ, H. Acute metabolic and circulatory effects of cigarette smoking in late pregnancy. *Gynecologic and Obstetric Investigation* 10(1): 39-45, 1979.

(128) PAIGEN, B., GURTOO, H.L., MINOWADA, J., HOUTEN, L., VINCENT, R, PAIGEN, K., PARKER, N.B., WARD, E., HAYNER, N.T. Questionable relation of aryl hydrocarbon hydroxylase to lung-cancer risk. *New England Journal of Medicine* 297(7): 346-350, August 18, 1977.

(129) PATRIANAKOS, C., HOFFMANN, D. On the analysis of aromatic amines in cigarette smoke. *Journal of Analytical Toxicology* 3(4): 150-154, July-August 1979.

(130) PELKONEN, O., JOUPPILA, P., KARKI, N.T. Effect of maternal cigarette smoking on 3,4-benzpyrene and N-methylaniline metabolism in human fetal liver and placenta. *Toxicology and Applied Pharmacology* 23(3): 399-407, November 1972.

(131) PELKONEN, O., KARKI, N.T., KOIVISTO, M., TUIMALA, R, KAUPPILA, A. Maternal cigarette smoking, placental aryl hydrocarbon hydroxylase and neonatal size. *Toxicology Letters* 3(6): 331-335, June 1979.

(132) PETITTI, D.B., WINGERD, J., PELLEGRIN, F., RAMCHARAN, S. Risk of vascular disease in women. Smoking, oral contraceptives, noncontraceptive estrogens, and other factors. *Journal of the American Medical Association* 242(11): 1159-1154, September 14, 1979.

(133) PILLSBURY, H.C., BRIGHT, C.C., O'CONNOR, K.J., IRISH, F.W. Tar and nicotine in cigarette smoke. *Journal of the Association of Official Analytical Chemists* 52(3): 458-462, May 1969.

(134) POUR, P., KRUGER, F.W., CHEM, D., ALTHOFF, J., CARDESA, A., MOHR, U. Cancer of the pancreas induced in the Syrian golden hamster. *American Journal of Pathology* 76(2): 349-354, August 1974.

(135) PRIETO, F., ENGLISH, M.J., COCHBANE, G.M., CLARK, T.J.H., RIGDEN, B.G. Spirometry in healthy men: A correlation with smoking and with mild symptoms. *Thorax* 33(3): 322-327, June 1978.

(136) QUIGLEY, M.E., SHEEHAN, K.L., WILKES, M.M., YEN, S.S.C. Effects of maternal smoking on circulating catecholamine levels and fetal heart rates. *American Journal of Obstetrics and Gynecology* 133(6): 685-690, March 15, 1979.

(137) RADFORD, E.P., JR., HUNT, V.R. Polonium-210: A volatile radioelement in cigarettes. *Science* 143(3603): 247-249, January 17, 1964.

(138) RAEDER, E.A., BURCKHARDT, D., PERRUCHOUD, A., BLUM, P., AMREIN, R., HERZOG, H. Effects of smoking and inhalation of carbon monoxide on systolic time intervals and blood pressure. Differences between two types of cigarettes and a cigar. *Chest* 75(2): 136-140, February 1979.

(139) RAWBONE, R.G., MURPHY, K., TATE, M.E., KANE, S.J. The analysis of smoking parameters, inhalation and absorption of tobacco smoke in studies of human smoking behavior. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences.* New York, Churchill Livingstone, 1978, pp. 171-194.

(140) RICE, J.M. Prenatal effects of chemical carcinogens and methods for their detection. In: Kimmel, C.A., Buelke-Sam, J. (Editors). *Developmental Toxicity,* in press.

(141) RICKERT, W.S., ROBINSON, J.C., YOUNG, J.C. Estimating the hazards of "leas hazardous" cigarettes. I. Tar, nicotine, carbon monoxide, acrolein, hydrogen cyanide, and total aldehyde deliveries of Canadian cigarettes, *Journal of Toxicology and Environmental Health* 6(2): 351-365, 1980.

(142) ROBINSON, J.C., YOUNG, J.C., FORBES, W.F. Low-tar cigarettes can produce long-term reduction in nicotine exposure. *Canadian Medical Association Journal* 123: 889-891, November 8, 1980.

(143) RODRIGUEZ, R.J., WHITE, RR., SENIOR, R.M., LEVINE, E.A. Elastase release from human alveolar macrophages: Comparison between smokers and nonsmokers. *Science* 198(4314): 313-314, October 21, 1977.

(144) ROMANSKI, B., BRODA, S., SWIATKOWSKI, M., ZBIKOWSKA, M. The immunologic response to tobacco antigens in smokers. III. Type III hypersensitivity skin reactions and specific serum precipitins to four different tobacco extracts in patients suffering from coronary artery disease. *Allergologia et Immunopathologia* 7(3): 187-196, May-June 1979.

(145) RUSSELL, M.A.H. The case for medium-nicotine, low-tar, low-carbon monoxide cigarettes. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 297-310.

(146) RUSSELL, M.A.H., JARVIS, M., IYER, R., FEYERABEND, C. Relation of nicotine yield of cigarettes to blood nicotine concentrations in smokers. *British Medical Journal* 280: 972-976, April 5, 1980.

(147) RUSSELL, M.A.H., SUTTON, S.R., FEYERABEND, C., COLE, P.V. Addiction Research Unit nicotine titration studies. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences.* New York, Churchill Livingstone, 1978, pp. 336-348.

(148) SAKABE, H. Lung cancer due to exposure to bis (chloromethyl) ether. *Industrial Health* 11(3): 145-148, 1973.

(149) SCHACHTER, S. Pharmacological and psychological determinants of smoking. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences.* New York, Churchill Livingstone, 1978, pp. 208-228.

(150) SCHULZ, W., SEEHOFER, F. Smoking behaviour in Germany-The analysis of cigarette butts (KIPA). In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and and Influences.* New York, Churchill Livingstone, 1978, pp. 259-276.

(151) SELKE, W.A. Reconstituted tobacco sheet. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe* Cigarette? Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 205-214.

(152) SEPPANEN, A., UUSITALO, A.J. Carboxyhaemoglobin saturation in relation to smoking and various occupational conditions. *Annals of Clinical Research* 9(5): 261-268, October 1977.

(153) SHAW, H.M., MILTON, G.W., MCCARTHY, W.H., FARAGO, G.A., DIG WORTH, P. Effect of smoking on the recurrence of malignant melanoma. *Medical Journal of Australia* 1(6): 208-209, March 24, 1979.

(154) SOBOL, B.J., VAN VOORHIES, L., EMIRGIL, C. Detection of acute effects of cigarette smoking on airway dynamics: A critical and comparative study of respiratory function tests. *Thorax* 32(3): 312-316, June 1977.

(155) STEDMAN, RL. The chemical composition of tobacco and tobacco smoke. *Chemical Reviews* 68(2): 153-207, April 1968.

(156) TACHMES, L., FERNANDEZ, R.J., SACKNER, M.A. Hemodynamic effects of smoking cigarettea of high and low nicotine content, *Chest* 74(3): 243-246, September 1978.

(157) TAGER, I.B., WEISS, S.T., ROSNER, B. SPEIZER, F.E. Effect of parental cigarette smoking on the pulmonary function of children. *American Journal of Epidemiology* 110(1): 15-26, July 1979.

(158) TAYLER, R., PIPER, D.W. The carcinogenic effect of cigarette smoke. The effect of cigarette smoke on human gastric mucosal cells in organ culture. *Cancer* 39(6): 2520-2523, June 1977.

(159) THOMAS, C.E. Automated method for FTC tar of low delivery cigarettes baaed upon fluorescence of TPM extracts. *Tobacco Science* 24: 64-68, 1980.

(160) TRAP-JENSEN, J., CARLSEN, J.E., SVENDSEN, T.L., CHRISTENSEN, NJ. Cardiovascular and adrenergic effects of cigarette smoking during immediate non-selective and selective beta adrenoceptor blockade in humans. *European Journal of Clinical Investigation* 9(3): 181-183, June 1979.

(161) TSO, T.C. Tobacco. *Encyclopedia of Chemical Technology* 20: 503-527, 1969.

(162) TSO, T.C. Limited removal of Po^{210} and Pb^{210} from soil and fertilizer by leaching. *Agronomy Journal* 62(5): 663-664, September-October 1970.

(163) TSO, T.C. Manipulation of leaf characteristics through production-Role of agriculture in health-related tobacco research. *Journal of the National Cancer Institute* 48(6): 1811-1819, June 1972

(164) TSO, T.C. The potential for producing safer cigarette tobacco. *Agricultural Science Review* 10(3): 1-10, Third Quarter, 1972

(165) TSO, T.C. Modification through agricultural techniques for developing a safer tobacco. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 181-190.

(166) TSO, T.C. Progress in producing tobacco for less harmful cigarettes. *World Smoking and Health* 5(1): 30-36, Spring 1980.

(167) TSO, T.C., CHAPLIN, J.F. *Simple Correlation and Multiple Regression Among Leaf Characteristics, Smoke Components and Biologic Activity of Bright Tobacco.* U.S. Department of Agriculture Technical Bulletin 1551, May 1977.

(168) TSO, T.C., HALLDEN, N.A., ALEXANDER, L.T. Radium-226 and Polonium-210 in leaf tobacco and tobacco soil. *Science* 146: 1043-1045, November 20, 1964.

(169) TSO, T.C., LOWE, R., DEJONG, D.W. Homogenized leaf curing. I. Theoretical basis and some preliminary results. *Beitrage zur Tabakforschung* 8(1): 44-51, January 1975.

(170) TSO, T.C., SIMS, J.L, JOHNSON, D.E. Some agronomic factors affecting N-dimethylnitrosamine content in cigarette smoke. *Beitrage zur Tabakforschung* 8(1): 34-38, January 1975.

(171) TSO, T.C., STEFFENS, G.L., FERRI, E.S. Agronomic factors affecting Po-210 and Pb-210 levels in tobacco. I. Soil and fertilizer. *Agronomy Journal* 60(6): 647-649, 1968.

(172) TUYNS, A.J. Epidemiology of alcohol and cancer. *Cancer Research* 39(7): 2840-2843, July 1979.

(173) U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE. *Smoking and Health: A Report of the Surgeon General.* U.S. Department of Health, Education, and Welfare, Public Health Service, Office of the Assistant Secretary for Health, Office on Smoking and Health, DHEW Publication No. (PHS) 79-50066, 1979, 1136 pp.

(174) U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE. *The Health Consequences of Smoking for Women: A Report of the Surgeon General.* U.S. Department of Health, Education, and Welfare, Public Health Service, Office of the Assistant Secretary for Health, Office on Smoking and Health, 1980, 359 pp.

(175) U.S. PUBLIC HEALTH SERVICE. *The Health Consequences of Smoking: A Reference Edition.* U.S. Department of Heath, Education, and Welfare, Public Health Service, Center for Disease Control, HEW Publication No. (CDC) 78-8357, 1976, 657 pp.

(176) VAN DUUREN, B.L. Carcinogens, cocarcinogens, and tumor inhibitors in cigarette smoke condensate. In: Gori, G.B., Bock, F.G. (Editors). *Banbury Report 3--A Safe Cigarette?* Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1980, pp. 105-112.

(177) VAN DUUREN, B.L., GOLDSMITH, B.M., KATZ, C., LANGSETH, L., MERCADO, G., SIVAK, A. Alpha-haloethers: A new type of alkylating carcinogen. *Archives of Environment Health* 16(4): 472-476, April 1968.

(178) VAUGHT, J.B., GURTOO, H.L., PARKER, N.B., LEBOEUF, R., DOCTOR, G. Effect of smoking on benzo(a)pyrene metabolism by human placental microsomes. *Cancer Research* 39(8): 3177-3183, August 1979.

(179) VOGT, T.M., SELVIN, S., WIDDOWSON, G., HULLEY, S.B. Expired air carbon monoxide and serum thiocyanate as objective measures of cigarette exposure. *American Journal of Public Health* 67(6): 545-549, June 1977.

(180) WALD, NJ., IDLE, M., BOREHAM, J., BAILEY, A. Inhaling habits among smokers of different types of cigarette. *Thorax,* in press.

(181) WATSON, I.D. Rapid analysis of nicotine and cotinine in the urine of smokers by isocratic high-performance liquid chromatography. *Journal of Chromatography* 143(2): 203-206, March 1, 1977.

(182) WEISBURGER, J.H., WILLIAMS, G.M. Chemical carcinogens. In: Doull, J., Klaasen, C.D., Amdur, M.O., (Editors). *Casarett and Doull's Toxicology: The Basic Science of Poisons.* Second edition. New York, Macmillan Publishing Co., 1980, pp. 34-138.

(183) WESNES, K., WARBURTON, D.M. The effects of cigarette smoking and nicotine tablets upon human attention. In: Thornton, R.E. (Editor). *Smoking Behavior, Physiological and Psychological Influences.* New York, Churchill Livingstone, 1978, pp. 131-147.

(184) WHITE, JR., FROEB, H.F. Small-airways dysfunction in nonsmokers chronically exposed to tobacco smoke. *New England Journal of Medicine* 302(13): 720-723, March 27, 1980.

(185) WHITE, R., WHITE, J., JANOFF, A. Effects of cigarette smoke on elastase secretion by murine macrophagea. *Journal of Laboratory and Clinical Medicine* 94(3): 489-499, September 1979.

(186) WILLIAMS, G.M. Review of in vitro test systems using DNA damage and repair for screening of chemicai carcinogens. *Journal of the Association of Official Analytical Chemists* 62(4): 857-863, July 1979.

(187) WOOD, A.J.J., VESTAL, R.E., WILKINSON, G.R., BRANCH, RA., SHAND, D.G. Effect of aging and cigarette smoking on antipyrine and indocyanine green elimination. *Clinical Pharmacology and Therapeutics* 26(1): 16-20, July 1979.

(188) WYNDER, E.L., HOFFMANN, D. *Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis.* Academic Press, 1967, 730 pp.

(189) WYNDER, EL, HOFFMANN, D. The epidermis and respiratory tract as bioassay systems in tobacco carcinogenesis. *British Journal of Cancer* 24(3): 574-587, September 1970.

(190) WYNDER, E.L., HOFFMANN, D. Tobacco and health: A societal challenge. *New England Journal of Medicine* 300(16): 894-903, April 19 ,1979.

(191) YAMAGIWA, K., ICHIKAWA, K. Experimental study of the pathogeneais of carcinoma *Journal of Cancer Research* 3(1): 1-29, January 1918.

(192) YAMASAKI, E., AMES, B.N. Concentration of mutagens from urine by adsorption with the nonpolar resin XAD-2: Cigarette smokers have mutagenic urine. *Proceedings of the National Academy of Sciences of the United States of America* 74(8): 3555-3559, August 1977.

(193) YOUNG, J.C., ROBINSON, J.C., RICKERT, W.S. *How Good Are the Numbers for Cigarette Tar at Predicting Deliveries of Carbon Monoxide, Hydrogen Cyanide and Acrolein?,* in preparation.

(194) YOUNG, J.C., ROBINSON, J.C., RICKERT, W.S. A Study of Chemical Deliveries as a Function of Cigarette Butt Length, in preparation.