Strain

Within a given species, there are likely to be sizable strain variations in response to any specific carcinogen. In the more than 10 strains of rats that have been tested with N-2-fluorenylacetamide, the response in a given target organ varied from zero to almost 100 percent, depending on the strain. Similarly, ethionine causes liver tumors in some strains of rats but not in others; a single oral dose of 7, 12-dimethylbenz[a]anthracene leads to a high incidence of mammary tumors in Sprague-Dawley-derived virgin female rats and none in some other strains. Mouse strains also exhibit considerable variation in their response to ethyl carbamate and other carcinogens (28).

The spontaneous incidence of tumors of particular organs varies with the strain of animal used for the test. This factor will determine the number of animals required for a meaningful assay. Strains with a high spontaneous incidence of tumors may be particularly sensitive to exposure to test compounds, a characteristic that will also affect the numbers of animals needed for the assay. Species variation in spontaneous tumor incidence does not, however, predict sensitivity to a specific agent.

Before initiating any bioassay, thorough study of the literature is needed to select the proper strain of animal for the types of compounds under test.

Sex

There are appreciable differences in the response of male and female animals to some known carcinogens. Examples are the higher incidence of skin tumors in male mice after painting with 7, 12-dimethylbenz[a]anthracene and the greater number of liver tumors in male rats after feeding 2-diacetylaminofluorene. With o-aminoazo-toluene, however, female mice were affected more than males. The differences may reside in the role sex hormones play in determining the levels of certain activating enzymes.

Male mice of many strains fight among themselves, causing skin wounds and deaths. The males of such strains should not be used for dermal assays unless they are individually housed or acclimated to each other when young.

Age

In routine tests, animals that are a few weeks' post-weaning are preferred so that they may be exposed to the test agent for the major part of the life span. If the animals are too old when the tests begin, they may die of other causes before tumors have time to develop.

Neonatal animals are more susceptible to many carcinogens than are young adults. A striking example is the induction of liver tumors

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in mice treated on day 1–7 of life by aflatoxin $B_1(AFB_1)$; much larger doses of AFB_1 administered to weanlings or young adult mice did not induce liver tumors (25). Similar results were noted with vinyl chloride (12). However, the difficulties in using neonatal animals are such that this method is hardly used for routine testing of compounds.

Diet

Both the total calories available from the diet and the type of diet influence the outcome of carcinogenicity studies. Restriction in calories may decrease not only the incidence of spontaneous tumors in animals but also the response to a carcinogen (20, 24). Diets deficient in protein, vitamins, or other essential factors may enhance the action of certain carcinogens (11). On the other hand, high levels of some vitamins increase the activity of detoxifying enzymes, thus depressing or inhibiting a carcinogenic effect. High levels of fats enhance the action of certain carcinogens (14, 19); indications are that high fat levels lead to production of bile acids (17), which may have a cocarcinogenic effect.

Adventitious dietary factors that may affect carcinogenesis assays include traces of nitrosamines, mycotoxins, and pesticides. Many nitrosamines and some mycotoxins are highly active carcinogens. Traces of pesticides may induce enzymes that activate or detoxify carcinogens. Similarly, vegetable material, usually a component of the processed rodent diets sold in pellet form, and antioxidants act as enzyme inducers and may influence the outcome of carcinogenicity trials.

Spontaneous Tumor Incidence

Since many experiments will extend over most of the lifespan of the experimental animals, it is necessary to know what spontaneous tumors might be expected. The many literature references on tumors in various rat or mouse strains should be consulted (5, 7, 16, 21, 27). These furnish background information on spontaneous tumor incidence that allows the researcher to avoid a strain with a very high tumor incidence that may complicate the interpretation and evaluation of bioassay data. However, tumor incidence in an inbred strain may shift over a period of years. Furthermore, specific laboratory conditions such as feed, water, lighting, housing, and handling procedures may affect the "spontaneous" tumor incidence. Adequate numbers of untreated control animals must be included in the experimental design.

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Immune Status

The immune status of animals influences their response to the carcinogenic action of viruses or ultraviolet radiation (*1, 10, 18, 23*). The same may be true for chemical carcinogens. Although immuno-suppression increases the likelihood of tumor development or successful transplantation (*9*), even from allogeneic tumors, few carcinogenicity studies have been done on immunosuppressed animals.

Procedures

Planning

Any long-term bioassay must be thoroughly planned. Consideration should be given to delineating responsible personnel and their specific duties, obtaining and analyzing the test substance, selecting the animal species and strain, and deciding on dose, route of administration, length of exposure, animal group size, randomization, what observations should be made, animal husbandry, data acquisition, processing, storage and retrieval, data analysis or statistical methods, diet, safety measures, working protocol, and quality control measures (*8, 15, 26*).

Conduct of Experiments

During the actual conduct of the experiment, the following points should be considered: quarantine of newly received animals; surveillance for disease; proper caging, general environment, lighting, temperature, ventilation, and handling; health monitoring of test animals; clinical examination; biochemical studies of blood, urine, and feces; proper necropsy procedures; histopathological techniques, diagnosis, and statistical analysis; and report preparation (*3,8*).

Such attention to detail, although costly, is necessary to avoid discrepancies that may compromise or invalidate the results of the study.

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EXPERIMENTAL CARCINOGENESIS WITH TOBACCO SMOKE

Introduction

Tobacco carcinogenesis exemplifies a meaningful and successful interaction between epidemiology and laboratory studies. The impetus for the development of experimental tobacco carcinogenesis came from large-scale epidemiologic studies between 1950 and 1960 (2, 46, 64, 120, 201) that indicated a causal association between cigarette smoking and cancer (see the Part in this Report on biomedical evidence).

The Physicochemical Nature of Tobacco Smoke

During the last three decades, major progress has been achieved in our knowledge about tobacco smoke, its formation, its physicochemical nature, and its composition. This new knowledge has contributed significantly to biologists in their study of the pharmacology, toxicity, and carcinogenicity of tobacco smoke.

The composition of tobacco smoke is a function of the physical and chemical properties of the leaf or of the tobacco blend, the wrapper, and the filter, as well as the way the tobacco is burned. A variety of chemical and physical processes occur in the oxygen-deficient, hydrogen-rich environment of the burning cone of the cigarette at temperatures up to 950°C. The majority of the more than 3,600 smoke components are formed in a pyrolysis-distillation zone just behind the heat-generating combustion zone (6, 61). The smoke is called mainstream smoke if it is generated during a puff and exits from the butt end and is called sidestream smoke if it arises mainly from the passive burning of the tobacco product and is released into the environment.

Smoking Conditions

The composition of the mainstream and sidestream smoke depends greatly on the smoking conditions and the methods of collection and analysis. This has long been realized; more than 20 years ago, standardized smoking conditions were established for machine measurements of cigarette smoke (199). Since then, the Federal Trade Commission (FTC), research institutions, and the U.S. cigarette industry have used the same standardized parameters for cigarette smoking (9, 152): one 2-second puff per minute with a volume of 35 ml and a butt length of 23 mm. For filter cigarettes, the butt length is given by the length of the filter tip plus overwrap plus 3 mm. For the analysis of sidestream smoke, a cigarette is placed in a water-cooled glass vessel with a free inner volume of 250 ml. The cigarette is smoked under the standard conditions applied for the

analysis of the mainstream smoke, but for the collection of the sidestream smoke, an air flow of 1.5 liters per minute is sent through the glass vessel (28).

The standard cigarette smoking conditions reflect the average smoking habits of a male smoker of nonfilter cigarettes as determined 25 years ago (32). Today, however, fewer than 10 percent of all U.S. smokers appear to follow this pattern (130). The average smoking parameters recently recorded for filter cigarette smokers were one puff of 1.94 to 2.06 seconds duration, repeated every 26.9 to 30.0 seconds, with a puff volume of 35.9 to 47.8 ml (75). Nevertheless, FTC-standard cigarette smoking conditions continue to be used for comparisons of tar and nicotine yields in the smoke of present cigarettes and for comparisons between present cigarettes and those made years and even decades ago. The values discussed in this introduction were obtained under the standard smoking conditions, except where otherwise noted.

For cigar smoking, the following conditions have been widely used: a 1.5-second puff every 40 seconds, a puff volume of 20 ml, and a butt length of 33 mm (99a). The conditions used for sidestream smoke collection of cigars are the same as those for cigarettes (28). Conditions for pipe smoking have not been standardized, although conditions of a 2-second puff every 18 seconds and a puff volume of 50 ml have been repeatedly used (134).

Temperature Profiles

The temperature profiles of the burning cigarette are affected by the length and circumference of the cigarette, the nature of the tobacco type or blend, the amount and nature of the processed tobacco "stems," the width of the tobacco shreds, the packing density and the moisture content of the tobacco, the porosity and ingredients of the cigarette paper, and the design of the filter (including the filter material and plasticizer, draw resistance, construction, and perforation). During smoking, the temperature of the burning cone reaches up to 950°C; hot spots on the periphery of the burning zone may reach 1050°C (148, 202). In a cigarette with paper of medium porosity, the temperature falls from 800°C to 40°C over the 30 mm of the tobacco column adjacent to the burning cone (185). The highest temperatures of cigars may reach slightly above 900°C and those of pipes may go slightly above 800°C; however, the temperature gradient away from the burning cone is not as steep as that in cigarettes, primarily because of the larger diameter of the burning cone and the very low porosity of the cigar wrapper and of the pipe bowl (202).

On the basis of the temperature profiles, three zones are defined in a burning cigarette during puffing: the high temperature zone (900–600°C), which is very low in free oxygen and contains up to 8 volume

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percent of hydrogen and 15 volume percent of carbon monoxide; the oxygen-depleted pyrolysis-distillation zone (600–100°C); and the low temperature zone ($<100°C$), with up to 12 volume percent of oxygen. The actual generation of mainstream smoke occurs in these three zones by hydrogenation, pyrolysis, oxidation, decarboxylation, dehydration, reactions between freshly generated chemical species, distillation, and sublimation. The exit temperature of the mainstream smoke ranges from 25° to 50°C, depending on the butt length. The previously cited temperature profiles do not apply to cigarettes with perforated filters. In this case, the smoke is diluted by air drawn through the filter wrapper. This lowers the velocity of the air drawn through the burning cone. The result is a more complete combustion of the tobacco.

Smoke Analyses

About 30 percent of the total weight of the mainstream smoke originates from the tobacco; the remainder comes from the air drawn into the cigarette. Five to eight percent by weight of the total effluent from a nonfilter cigarette is made up of moist particulate matter; about 55 to 65 percent are nitrogen, 8 to 14 percent are oxygen, and the remainder consists of other gas phase components generated during smoking (107). Undiluted cigarette smoke, as it leaves the mouthpiece, contains up to 5×10^9 heterogeneous particles per ml, with round and spheric forms ranging in diameter between 0.2 and 1.0 μ and a median particle size of about 0.4 μ (36, 107). In the case of filter cigarettes, the median particle size of the smoke is somewhat smaller (between 0.35–0.4 μ). For cigarettes with perforated filter tips, the number of particles generated is significantly lower than for unfiltered cigarettes (36).

The smoke particles that are inhaled are slightly charged with about 10^{12} electrons per gram of smoke (equivalent to two or three cigarettes). Since the smoke is partially generated in the oxygen deficient zone, the aerosol leaving the mouthpiece has reducing activity that increases with the number of puffs drawn and that disappears completely only minutes after smoke generation (166). Thus, freshly generated tobacco smoke as inhaled may affect the redox balance of respiratory tract tissues.

The pH of tobacco smoke is of major significance since it influences its inhalability by the smoker and the availability of unprotonated nicotine (3). Figure 1 depicts the percentage of diprotonated, monoprotonated, and unprotonated nicotine in aqueous solution at various pH. For a blended U.S. cigarette, the pH of the mainstream smoke varies between 5.5 and 6.2; cigarettes made exclusively from Burley or black tobacco, and cigars yield mainstream smoke with pH ranges between 6.5 and 8.5, reaching the highest pH with the last
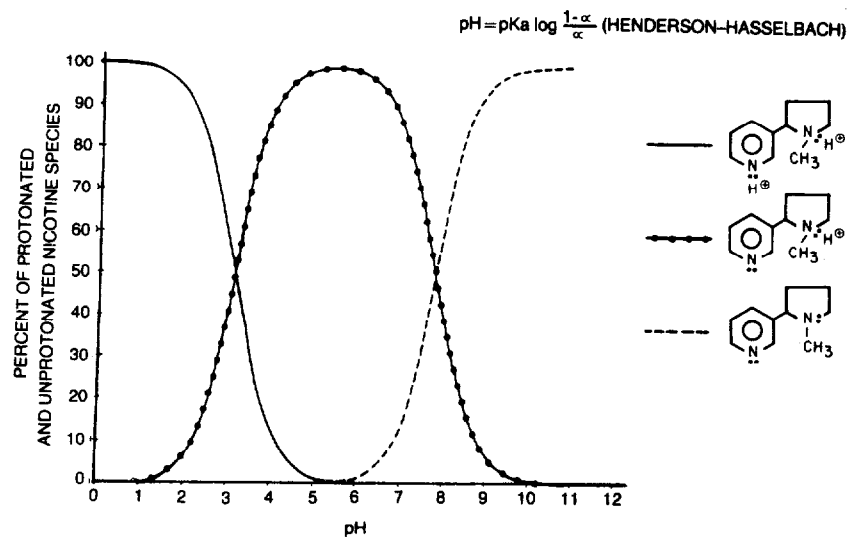
$$pH = pKa \log \frac{1-\alpha}{\alpha} \text{ (HENDERSON-HASSELBACH)}$$

FIGURE 1.—Protonation of nicotine
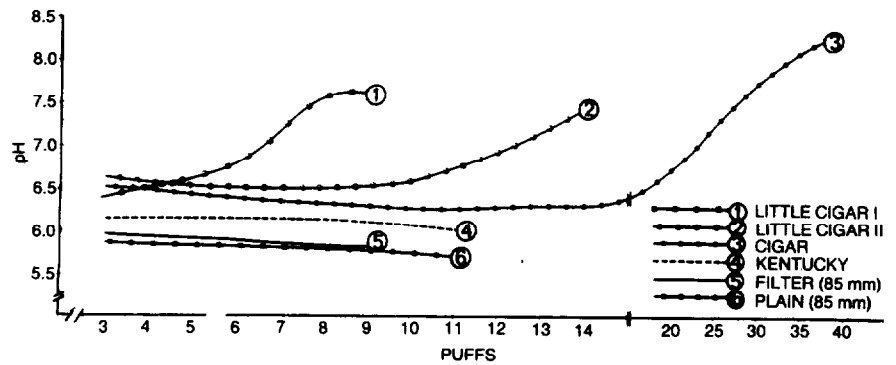
**FIGURE 2.—pH of individual puffs of total mainstream
smoke of various tobacco products**

puffs (28). Figure 2 shows the pH of individual puffs of the mainstream smoke of some tobacco products (6).

Bioassays

Inhalation Studies

Ideally, a suspected carcinogen should be tested using the route of administration corresponding to the exposure of humans. The experimental induction of respiratory cancer with tobacco smoke is

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beset with major difficulties because of toxicity introduced by high carbon monoxide concentrations (generally 3.5 to 5 volume percent), and high levels of nicotine. Furthermore, laboratory animals are not willing to inhale aerosols very deeply and are especially reluctant to inhale tobacco smoke. Inhalation studies have been explored by training Rhesus monkeys and baboons to smoke cigarettes. This approach does not produce respiratory neoplasms because of insufficient exposure time and because of the tendency of the animals merely to puff rather than to inhale (102, 156a).

Invasive and noninvasive bronchoalveolar tumors developed in several of 78 dogs that were trained to smoke through a tracheostoma and that smoked cigarettes daily for about 2¹/₂ years. In a group of 24 dogs that smoked nonfilter cigarettes, 2 animals developed early invasive squamous cell carcinoma in the bronchi (4). However, this observation has not been repeated so far (137).

A number of inhalation studies have been conducted with rats. Recently they have yielded tumors of the respiratory tract (43, 137). In 1980, investigators at the Oak Ridge National Laboratory succeeded in obtaining tumors of the respiratory tract of rats using a highly developed smoke inhalation device (43, 126). On 5 days each week over their entire lifespan, 80 rats were exposed to air-diluted smoke (10 percent) of seven cigarettes (one cigarette per hour). At the end of the experiment, a large number of rats had developed hyperplasia or metaplasia in the epithelium of the nasal system, the larynx, or the trachea. Seven of the eighty smoke-exposed rats had tumors of the respiratory tract, including five animals with pulmonary adenomas, two with alveologenic carcinomas, one with a squamous carcinoma of the lung, and one with adenocarcinoma and squamous cell carcinoma in the nasal cavity. One alveologenic carcinoma was observed in 30 sham-exposed control rats; no respiratory tract tumors were seen in 63 untreated control rats (43).

At present, the most promising animal for tobacco smoke inhalation studies appears to be the Syrian golden hamster. This animal is more resistant to respiratory infections than are mice and rats and is also more tolerant of cigarette smoke (52). Dontenwill et al. developed the first smoke inhalation device and bioassay methodology for the chronic exposure of hamsters to cigarette smoke (51). For 5 days per week and for the duration of their lifetime, the hamsters were exposed once, twice, or three times daily for 10 minutes to air-diluted cigarette smoke (1:15). In the 3 groups of 80 hamsters, 11.3, 30, and 30.6 percent of the animals developed pre-invasive carcinoma, and 0.6, 10.6, and 6.9 percent had invasive carcinoma of the upper larynx (51). Laryngeal tumors were not observed in the control group nor in the animals exposed only to the gas phase of cigarette smoke. Trachea and bronchi of all animals were free of neoplastic growth. Tumors that developed in other organs of the exposed

hamsters were not different from those in the control group. This inhalation assay represents the first reproducible method for the induction of tumors in the respiratory tract of animals exposed to tobacco smoke. Dontenwill and his group have successfully applied this method to the evaluation of the carcinogenic potential of experimental cigarettes with and without reduced activity as measured in mouse skin bioassays (48).

Bernfeld et al. (11) improved the inhalation model primarily by using an inbred hamster strain that is susceptible to carcinogenic inhalants. The smoking schedule called for exposure for 59 to 80 weeks to a 22 percent cigarette smoke aerosol twice daily for 12 minutes with cigarettes made entirely from flue-cured tobacco, such as those used in the United Kingdom. This induced carcinoma of the larynx in 27 out of 57 hamsters at risk ($\cong$47 percent). Three of the animals developed papilloma of the trachea; none had tumors of the lung. In tests with an 11 percent smoke aerosol, only 3 out of 44 hamsters at risk (7 percent) developed laryngeal carcinoma, indicating a possible dose-response for the induction of carcinoma of the larynx with cigarette smoke. Thus, it appears that this hamster inhalation model is a promising bioassay system for estimating the relative carcinogenic potential of total, unaged smoke of various cigarettes.

Why these inhalation experiments with hamsters did not induce carcinoma of the lung remains to be elucidated. Two investigations have examined this question using tracer studies with decachlorobiphenyl (DCBP) (11,86). In one study, DCBP was added to cigarettes and the concentration of the tracer in the mainstream smoke was determined for the appropriate exposure for each animal. DCBP is not volatile and is, therefore, not found in the gas phase, but rather is an integral part of the smoke particulate phase. Bernfeld et al. (11) determined that 160 µg tar[3] reached the lung of a hamster and that 15 µg tar were deposited in the larynx after each exposure of a hamster to DCBP-spiked mainstream cigarette smoke. In another study with a different smoke inhalation device, 88 µg tar were found to reach the lungs and 2.8 µg tar were traced to be deposited in the larynx (86). Considering the relative surface area of both larynx (0.1 to 3.0) and lung (1,000), Bernfeld et al. calculated that, per surface area unit, 300 to 900 times more tar is deposited in the larynx than in the lungs. In the other study (86), the relative deposition per surface area unit was calculated to range from 110:1 to 320:1. This high density of tar deposits in the larynx suggests an explanation of the occurrence of a high yield of laryngeal cancers in hamsters exposed to cigarette smoke but a lack of lung tumors in the same experiments.

[3]Throughout this section the term "tar" is used as a descriptive noun only; it is realized that the terms "smoke particulates" or "smoke condensates" are often more correct.

Assays With Smoke Particulates

The gaseous phase of tobacco smoke does not induce tumors of the respiratory tract in laboratory animals (51, 202), except for lung adenomas in certain sensitive strains of mice (119). This suggests that the carcinogenic activity of smoke requires the particulate phase. Benign and malignant tumors have been induced with tobacco tar in the skin and ear of rabbits, in the connective tissue of rats, and by intratracheal instillation, in the bronchi of rats (137, 202). However, the most widely used methodology for the induction of tumors in epithelial tissues has been topical application to mouse skin. Detailed studies have shown that the effect of a tumor initiator is irreversible, but promoter activity will cease upon termination of treatment (193, 195). It appears likely that the metabolically activated form of a tumor initiator is bound to the DNA of a target cell, but the promoter effect is not directly linked with cellular DNA damage and can, therefore, be repaired. Single applications of a low dose of 7,12-dimethylbenz[a]anthracene (DMBA) or benzo[a]pyrene (BaP) have served as initiators in chemical carcinogenesis studies that demonstrate initiation and promotion as two successive stages. Most model experiments utilize repeated application of 2.5 μg or lower doses of tetradecanoyl phorbol acetate (TPA) as a promoter (192). In another setting, mouse skin is treated 10 times with a very low dose of BaP or another tumor initiator and is subsequently treated with TPA (72, 116). A cocarcinogen is defined as an agent that potentiates the activity of a carcinogen when both substances are coadministered. The cocarcinogen by itself may exert little or no carcinogenic activity.

The merit of the mouse skin assay lies in its sensitivity and reproducibility as a method for the identification of tumor initiators, tumor promoters, and cocarcinogens in tobacco smoke. By definition, a tumor initiator is an agent that does not elicit a significant tumor response in mouse skin or in other epithelial tissue, but suffices to bring about benign and malignant tumors when its application is followed by repeated treatments with a tumor promoter. Reversal of the order of application produces few tumors. The mouse skin assay has been employed to establish a clear dose response for carcinogenicity of tars. It has been most useful in evaluating the relative potential for the induction of benign and malignant tumors by contact carcinogens. The relative activity of the smoke particulate matter of commercial and experimental cigarettes has been compared on mouse skin (50, 202), and the response was found to be in good agreement with results from the bioassays in which inhalation of tobacco smoke led to carcinoma of the larynx in hamsters (48, 49).

The mouse skin assay has been helpful in evaluating the relative tumorigenic potential of the smoke particulates of cigarettes made from different tobacco varieties, reconstituted tobacco sheets, lami-

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na, stems, and tobacco substitutes (*88, 143*). Bioassays conducted with standardized methods on the same strain of mice have indicated a gradual decline of the carcinogenic potential of the smoke particulates of a leading U.S. cigarette brand during the last 20 years. This reflects the changes in the makeup of commercial cigarettes (*188*).

Fractionation Experiments

Assessments have been made for the materials derived primarily from two major separation schemes employed for the identification of tumorigenic agents. One system begins with fractionation of the smoke particulates into neutral, acidic, basic, and insoluble portions, followed by column chromatographic subfractionation schemes for further delineation of tumorigenic constituents (*17, 90*). The other system consists of the partitioning of the particulates with solvent systems and of the subsequent chromatographic separations (*59*). Both methods have clearly established that the tar subfractions, which contain the bulk of polynuclear aromatic hydrocarbons (PAH), are the only portions that elicit carcinoma on mouse skin when applied in high concentrations. These subfractions harbor the majority of the tumor initiators. Intratracheal instillation in rats also led to carcinomas only with those subfractions that were highly enriched in PAH. However, the PAH subfractions also contain neutral cocarcinogens. These are non-carcinogenic PAH, which nevertheless potentiate the activity of carcinogenic PAH. The chemical identification of still other cocarcinogens in these neutral subfractions points to nonvolatile ketones and tobacco terpenes (*165*).

The weakly acidic portion of smoke particulates and its subfractions have also been shown to contain tumor promoters as well as important cocarcinogens, including phenolic compounds and catechols (*18, 67*).

Transplacental Carcinogenesis

In the 1979 report *Smoking and Health: A Report of the Surgeon General*, several questions were raised in respect to transplacental effects of cigarette smoking (*189*). Activation of enzymes that induce metabolic activation of benzo[a]pyrene (BaP) in the foreskin of human newborns of smoking mothers has been interpreted as one indication of possible transplacental migration of smoke constituents (*41, 123*).

Several experimental studies suggest that tobacco smoke has transplacental carcinogenic effects. Intraperitoneal injections of tobacco tar in olive oil during the 10th to 14th day of gestation of Syrian golden hamsters led to tumors in 2 of 58 females and to benign and malignant tumors in 17 of 51 transplacentally exposed offspring, within 15 to 25 months of observation. The tumors in the

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offspring were primarily located in the adrenal glands, pancreas, female sex organs, and liver. Untreated control animals, or those whose mothers were injected with olive oil alone, did not develop any tumors during the course of this experiment.

This experiment should be repeated, in order to establish the reproducibility of the transplacental effects. Its results are in line with general observations of transplacental carcinogenesis. These include pronounced prenatal susceptibility, expressed in a far higher lifetime tumor yield in the offspring, as compared with their mothers (156).

In that direct-acting alkylating agents are generally the most effective transplacental carcinogens, the high tumor incidence in the offspring of hamsters treated with tobacco tar is remarkable. Compounds requiring metabolic activation to ultimate active forms of carcinogenic species, however, are also transplacental carcinogens, though of a lesser potency than direct alkylating carcinogens. Enzymes necessary for activation are known to exist in the fetus only at low levels, if at all, until just prior to birth (110). A number of tobacco smoke constituents, which need metabolic activation in order to acquire carcinogenic properties, are known transplacental carcinogens. Among these are volatile N-nitrosamines, BaP, o-toluidine, ethyl carbamate, and vinyl chloride (156).

The role of nicotine in regard to possible transplacental effects of tobacco smoke also requires further elucidation, since its transplacental migration into the animal fetus has long been known (184). A smoker of 20 cigarettes daily is exposed to 20 to 30 mg of nicotine, and in a pregnant woman it is to be expected that some of this nicotine reaches the fetus. Enzymatic oxidation to cotinine in the fetus is very slow, because of low enzyme activities. Thus, nitrosamine formation from the unmetabolized nicotine may occur. Such considerations suggest the need for further experimental studies of the transplacental effects of tobacco products.

Syncarcinogenesis: Occupational Carcinogens and Smoking

In the United States, cigarette smoking is generally more prevalent among blue-collar workers than among the white-collar work force (42). Thus, smokers are more likely to be in occupational environments with chemicals, dusts, and fumes than are their nonsmoking counterparts (56). This indicates the need to examine the role of smoking as a confounding variable to occupational exposure and raises the question whether tobacco smoke acts synergistically with other factors in respiratory tract carcinogenesis.

In 1979, Hammond et al. (65) evaluated the smoking history relating to 276 deaths from lung cancer among asbestos workers. The calculated mortality ratios (the ratio of death rates in smokers compared with death rates in nonsmoking men of a similar age

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distribution) for lung cancer were 87.36 for workers who smoked more than 20 cigarettes per day, 50.82 for those who smoked less than 20 cigarettes per day, and 5.33 for asbestos workers who had never smoked regularly. The authors also reported that exposure to asbestos dust in the absence of smoking may have little or no influence on death rates from cancer of the esophagus, larynx, pharynx, or buccal cavity.

Several carcinogenesis experiments were designed to measure the combined effects of tobacco smoke and the various types of asbestos fibers (189). In one such study, 500 µg of asbestos were instilled in the trachea of hamsters, prior to exposure to diluted cigarette smoke, 10 times weekly over a period of 18 months. Since no more than about 1 percent of the smoke particulates reached the hamsters' lungs in such experiments, the smoke exposure alone did not produce tumors in the lower respiratory tract, nor did it potentiate the subthreshold dose of the carcinogenic asbestos (51). In contrast, synergistic action of tobacco smoke and asbestos were indicated when asbestos fibers were first incubated with cigarette tar and then added to human lymphocyte cultures. This resulted in significantly increased induction of aryl hydrocarbon hydroxylase (AHH) compared with the enzyme induction in the lymphocyte cultures with either agent alone (171). This finding suggests that a surface (and chemical) interaction between asbestos and cigarette smoke may have occurred with formation of a product having higher carcinogenic activity than is inherent in either agent alone. An elucidation of the mechanisms involved in syncarcinogenic effects of tobacco smoke and asbestos fibers requires further experimental studies.

A substantial excess of lung cancer has been reported among uranium miners who smoke cigarettes (189). Archer et al. (2) calculated that the lung cancer rate for U.S. uranium miners who smoked was 42.2 per 10,000 persons/years compared with 4.4 for nonminers who smoked two or more packs of cigarettes a day. There is also some evidence that cigarette smoking may change the latent period for lung cancer development following radiation exposure among uranium miners (2). As will be discussed later, polonium 210 (^{210}PO) is present in tobacco and cigarette smoke (0.03 to 1.0 pCi/cigarette); however, it is unlikely that these traces represent a major risk for the smoker.

Beagle dogs were exposed to radon daughters in uranium ore dust (group 1) or to the same uranium ore dust, together with cigarette smoke (group 2). After more than 40 months, all dogs showed areas of epithelial changes, including large areas of adenomatosis, and squamous metaplasia of the alveolar epithelium with atypical cells. After more than 50 months of exposure, lungs from 50 percent of the dogs in groups 1 and 2 contained large cavities within the parenchyma surrounded by bands of hyperplastic adenomatous epithelial

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cells. These changes were not seen in dogs exposed only to cigarette smoke (178).

Little and his group (124) tested the hypothesis that ^{210}Po α-radiation acts synergistically with polynuclear aromatic hydrocarbons (PAH) present in cigarette smoke. Syrian golden hamsters were given intratracheal instillations of low levels of both ^{210}Po and BaP simultaneously or in sequence. Upon simultaneous intratracheal instillation of ^{210}Po and BaP on ferric oxide, the induction of peripheral lung tumors was simply additive. Sequential application of a single dose of ^{210}Po (0.04 μCi) and repeated dosage of BaP (0.3 mg x 7 weeks), however, produced syncarcinogenic effects. Among 139 animals at risk in the group receiving a single dose of ^{210}Po, only 1 animal (0.7 percent) had a lung tumor. The sequential application of ^{210}Po and BaP to 135 animals induced lung tumors in 23 of them (17 percent), and BaP alone gave tumors in less than 4 percent of the hamsters (132).

Although other occupational environments may provide additional cancer risk factors for workers who smoke, epidemiological and experimental studies have not documented such occurrences to date. It has been suggested that synergistic carcinogenic effects may occur in cigarette smokers who work in factories producing or handling chloromethyl ether (59), vinyl chloride (34), nickel (47), or 2-naphthylamine (189).

Alcohol and Tobacco Products

Epidemiological data have indicated that the combination of chronic alcohol and tobacco consumption greatly increases the risk for cancer of the oral cavity, esophagus, and larynx, but not of the lung (157, 189). Several possible mechanisms have been proposed in regard to synergistic effects of tobacco smoke and alcohol. Alcohol serves as a solvent for tobacco carcinogens, or it alters the liver metabolism of tobacco carcinogens and, thus, has an indirect influence on tobacco carcinogenesis at distant organs. Chronic alcohol consumption sometimes leads to deficiencies in essential micronutrients, making the target cells more susceptible to carcinogens. Also, alcohol induces changes in metabolism of the tobacco carcinogens in target tissues.

It has been shown in the experimental setting that alcohol, as a solvent, increases the carcinogenic effect of PAH, which are the major tumor initiators in smoke (177) and of the distillation residues of alcoholic spirits that contain carcinogens (114). Chronic alcohol consumption, among other effects, enhances the drug metabolism capabilities of liver microsomes in both men and animals (136). The metabolism in the liver of the tobacco carcinogen N-nitrosopyrrolidine (NPYR), for example, was enhanced in ethanol-consuming

hamsters (*137*). Excessive alcohol consumption is also known to lead to various other cellular injuries that influence carcinogenesis (*136*).

Vitamin A deficiency, which frequently accompanies alcohol abuse, increases susceptibility to carcinogens of the PAH type in laboratory animals (*175*). Vitamin B$_2$ deficiency has been shown to potentiate effects of carcinogens in mouse skin (*37*). Rats on a zinc-deficient diet are more susceptible to the esophageal carcinogen, N-nitrosobenzylmethylamine (*55*). The carcinogenicity of NPYR in Syrian golden hamsters is enhanced when the animals are on a high alcohol diet, yet this enhancement has not been observed for the tobacco-specific N'-nitrosonornicotine (*131*). Further studies of biochemical changes and bioassays with coadministration of alcohol and tobacco smoke or its constituents may provide a better understanding of the increased cancer risk of consumers who use both alcohol and tobacco.

Tumorigenic Agents In Tobacco Products

Vapor Phase Components

The definition of the vapor phase components is arbitrary and does not represent the true physicochemical conditions prevailing in tobacco smoke. In carcinogenesis, the tobacco chemist's definition has been widely accepted. For the purposes of this discussion the term "vapor phase component" includes all smoke constituents of which more than 50 percent pass through a Cambridge glass fiber filter. Collecting smoke from a single cigarette on a filter pad yields fairly reproducible data. More than 90 percent of the total weight of mainstream smoke is made up of vapor phase components, of which nitrogen and oxygen constitute more than 70 percent. Carbon dioxide and carbon monoxide make up 15 to 20 percent by weight of the total effluents of most cigarettes, unless the cigarette filter tip contains unblocked perforations that reduce this percentage.

Carbon monoxide in cigarette smoke, although not a carcinogen, may contribute to respiratory carcinogenesis because of its inhibiting effect on the mucus clearance mechanism of the respiratory tract (*10*). Its most important toxic effect, however, lies in its burden on the circulatory system because it combines with hemoglobin of the blood to form carboxyhemoglobin.

The plain cigarette and the conventional filter cigarette contain 2 to 7 volume percent of carbon monoxide per puff, with the concentration increasing with the later puffs. The total carbon monoxide in the smoke of these cigarettes in the United States in 1980–1981 amounts to 3 to 5 volume percent or 13 to 26 mg/cigarette. However, air dilution of the smoke from cigarettes with a perforated filter tip reduces carbon monoxide to 0.5 to 13 mg/cigarette (*27,191*). It is estimated that more than 50 percent of

the cigarettes currently sold on the U.S. market have perforated filter tips. The smoke of cigars and little cigars contains carbon monoxide values up to 11 volume percent (27).

In the 1979 report *Smoking and Health: A Report of the Surgeon General,* carbon dioxide, nitrogen oxide, ammonia, hydrogen cyanide, and volatile sulfur compounds and nitriles have been discussed in addition to carbon monoxide (189). Since that time no significant new information has been published in respect to the contribution of these vapor phase components to the overall toxicity and carcinogenicity of tobacco smoke. It should be noted that the gradual reduction of tar and nicotine was accompanied by a gradual decrease of most vapor phase components in the smoke of the sales-weighted average U.S. cigarette (89). This reduction does not apply to the level of nitrogen oxides (NO_x), of which more than 95 percent are nitric oxide (NO). The NO_x content of the smoke of the sales-weighted average U.S. cigarette has remained at a level of 270 to 280 µg per cigarette (89). One reason for this appears to be the use of increasing percentages of Burley tobacco and of "stems" in the cigarette blend. Burley tobacco and "stems" are richer than Bright tobacco in nitrate, a main precursor for NO_x in the smoke. A major reduction in smoke NO_x can be achieved by high smoke dilution (146). As discussed before, these observations apply to the smoke generated by standard machine smoking schedules and do not allow for the fact that many smokers of low tar cigarettes smoke more intensely.

It has been demonstrated that a high percentage of the ciliatoxic agents, which inhibit the lung clearance, are present in the vapor phase (10,44). These are chiefly hydrogen cyanide (280 to 550 µg/cig), acrolein (10 to 140 µg/cig), ammonia (10 to 150 µg/cig), nitrogen dioxide (0 to 30 µg/cig), and formaldehyde (20 to 90 µg/cig). Squamous cell carcinomas were induced in the nasal cavities of rats exposed in chambers for 30 hours a week to 15 ppm of formaldehyde for 18 months (182). The mechanism of its action is unknown; metabolically, it is rapidly oxidized further to formic acid.

The vapor phase, i.e., that portion of the smoke passing through a glass fiber filter, does not by itself induce tumors in laboratory animals, except in certain strains of mice (119). The carcinogenic effects of low levels of volatile smoke constituents may currently escape detection by means of bioassays because of the low doses used and the low sensitivity of models available at present (100). Table 1 lists the major components of the vapor phase and whether the agent is reported to be toxic or tumorigenic. The volatile N-nitrosamines are largely retained by the smoke particulates in the glass fiber filters and will be discussed in the section on organ-specific carcinogens. In general, our understanding of the mechanisms of carcinogenesis by other volatile smoke components is scanty.

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

Agent	Biologic activity[a]	Concentration/cigarette	
		Range reported	U.S. cigarettes[b]
Carbon monoxide	T	0.5 - 25 μg	17 μg
Nitrogen oxides (NO$_x$)[c]	T	16 - 600 μg	350 μg
Hydrogen cyanide	CT, T	28 - 550 μg	110 μg
Formaldehyde	CT, C	20 - 90 μg	30 μg
Acrolein	CT	10 - 140 μg	70 μg
Acetaldehyde	CT	18 -1,400 μg	800 μg
Ammonia	T?[d]	2.5 - 250 μg	10 μg
Hydrazine	C	24 - 43 ng	32 μg
Vinyl chloride	C	1 - 16 ng	12 μg
Urethane	C	10 - 35 μg	30 μg
2-Nitropropane	C	0.73 - 120 μg	1.2 μg
Quinoline	C	0.8 - 2.0 μg	1.7 μg

*Volatile nitrosamines are listed in Table 4.

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

[a] T notes toxic agent; CT, cilia toxic agent; and C, carcinogenic agent.

[b] 85 mm cigarettes without filter tips.

[c] NO$_x$ >95% NO; rst NO$_2$.

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

SOURCE: Hoffmann et al. (87,90).

Hydrazine or its salts are most effective as carcinogens in mice. Metabolic transformation of hydrazine in some animals yields acetyl and diacetyl derivatives, although ammonia is formed in dogs (40). Numerous studies on the toxicity and carcinogenicity of hydrazine have been reported (125), but few on its metabolic transformation and the mechanism of its action. Indications are that hydrazine may disrupt normal methylation processes in the organism, since methylated guanines were noted in liver DNA after exposure.

The cytochrome P-450 enzyme system forms a halogenated epoxide from vinyl chloride (8, 205). In turn, this epoxide may yield halogenated aldehydes or alcohols through rearrangement. Contrary to the situation with the nucleic acid adducts of most other activated carcinogenic intermediates, the epoxide from vinyl chloride ethylenates or adds across the N-1 and N-6 of adenosine or the N-3 and N-4 of cytidine, forming new rings in these particular bases (21). The presence of these additional structures would probably interfere in the normal base pairing between adenosine-thymidine and guanosine-cytidine.

Urethane is not a potent carcinogen, in terms of dose, except in neonatal mice. Although it is metabolized to N-hydroxyurethane, which acylates cytosine (144), there still remains a question whether urethane or N-hydroxyurethane is the active material (135).

Tumor Initiators

The carcinogenic activity of the particulate matter of tobacco smoke in epithelial tissues of laboratory animals is greater than the sum of the effects of the known carcinogens present. Large scale fractionation studies in a number of laboratories have shown that the total carcinogenic activity also results from the effects of tumor initiators, tumor promoters, and cocarcinogens in the tar.

Large-scale tar fractionation studies in a number of U.S. and foreign laboratories have shown that the tumor initiators reside in those neutral subfractions in which the polynuclear aromatic hydrocarbons (PAH) are enriched (87). So far, at least two dozen PAH and a few neutral aza-arenes have been identified to serve as tumor initiators at the dose levels found in tobacco tar. It is likely that the PAH concentrates of smoke particulates contain additional tumor initiators that may yet be identified by detailed capillary GC-MS analysis (172). All of these PAH tumor initiators are formed during smoking by similar pyrosynthetic mechanisms (5, 87). More recent observations showed, surprisingly, that tumor initiators are also found among dimethylated or polymethylated three-ring aromatic hydrocarbons in which the formation of bay region dihydrodiol epoxides is favored, but the detoxification to phenols is reduced. An example is 1,4-dimethylphenanthrene (117). These methylated three-ring aromatic hydrocarbons may be present in tobacco smoke in much higher concentrations than the corresponding parent PAH. Table 2 lists tumor-initiating PAH and aza-arenes identified in tobacco smoke.

These compounds are secondary or procarcinogens since they require metabolism to show an effect. Metabolic activation is generally mediated through the mixed-function oxidase system of enzymes. The metabolic activation of polycyclic aromatic hydrocarbons, as typified by benzo[a]pyrene (BaP), has been reviewed within the past 2 years (58). In brief, BaP is metabolized by means of the mixed-function oxidase system to the 2,3-, 4,5-, 7,8-, and 9,10-epoxides, of which only the 4,5-epoxide is stable enough to permit isolation and thus to exist in the environment. The various epoxides can be converted to phenols, which in turn may be conjugated through glucuronyl transferase or sulfotransferase to water-soluble glucuronides or sulfates.

The phenols may also be oxidized to quinones such as the 1,6-, 3,6-, and 6,12-quinones derived from BaP. The original epoxides are good substrates for the glutathione-S-transferase system that forms glutathione conjugates and premercapturic and mercapturic acids from the epoxides. In addition, the epoxide hydrolase system converts the epoxides to dihydrodiols with the (-)-*trans* configuration.

However, an additional activation step is required, i.e., the further oxidation of the dihydrodiols, also mediated by the mixed-function

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TABLE 2.—Tumor-initiating agents in the particulate phase of tobacco smoke[1]

Compound	Relative activity as complete carcinogen[2]	ng/cig
Benzo(a)pyrene	+ + +	10–50
5-Methylchrysene	+ + +	0.6
Dibenz(a,h)anthracene	+ +	40
Benzo(b)fluoranthene	+ +	30
Benzo(j)fluoranthene	+ +	60
Dibenzo(a,h)pyrene	+ +	pr[3]
Dibenzo(a,i)pyrene	+ +	pr[3]
Dibenz(a,j)acridine	+ +	3–10
Indeno(1,2,3-cd)pyrene	+	4
Benzo(c)phenanthrene	+	pr[3]
Benz(a)anthracene	+	40–70
Chrysene	+ ?	40–60
Benzo(e)pyrene	+ ?	5–40
2- and 3-Methylchrysene	+ ?	7
1- and 6-Methylchrysene	-	10
2-Methylfluoranthene	+	34
3-Methylfluoranthene	?	40
Dibenz(a,c)anthracene	(+)	pr[3]
Dibenz(a,h)acridine	(+)	0.1
Dibenzo(c,g)carbazole	(+)	0.7

[1] Incomplete list; all listed compounds are active as tumor initiators on mouse skin.

[2] Relative carcinogenic activity on mouse skin as measured in our laboratory on Swiss albino (Ha/ICR/Mil) mice;

?: Carcinogenicity unknown; (+): not tested in own laboratory.

[3] pr: present, but no quantitative data given.

SOURCE: Hoffmann et al. (88).

oxidase system. For BaP, the *trans* isomer of the 8,8-dihydrodiol-9,10-expoxide thus formed appears to be the active intermediate, capable of reacting with nucleic acids, proteins, and other cellular constituents. In the nucleic acid adducts, the 10-position of the diol epoxide was linked to the amino group in the 2-position of guanosine, although some reaction with the phosphates of the DNA backbone also occurred.

Various enzymatic and radioimmunoassays have been devised to measure the level of the BaP-DNA adduct in biological materials (93). Although the actual biological consequences resulting from the BaP-DNA adduct have not been exactly delineated, there are indications that the adduct can interfere in elongation of the nucleic acid during replicative processes.

No studies on the mechanism of carcinogenesis by metabolic products of polycyclic heterocyclics have been reported. On the premise that they may be activated through a similar mechanism as the polycyclic aromatic hydrocarbons, some of the dihydrodiols of benz[a]- and [c]acridine have been synthesized as model compounds (161). The possible metabolic transformation to N-oxides should also be considered.

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Tumor Promoters

The water extract of processed tobacco and the particulate matter of tobacco smoke contain tumor-promoting agents (16, 20). Pretreating mouse skin with 125 µg of DMBA, Bock and collaborators (19) found that the tumor-promoting activity of tobacco extracts requires the concurrent presence of two agents, one of large molecular weight (LM), insoluble in organic solvents, and the other of small molecular weight (SM), soluble in organic solvents. They suggest that the SM agent could be nicotine (20). Bock and Clausen (15) fractionated the portion with the LM agent by dialysis. A subfraction with a presumptive molecular weight greater than 13,000 exhibited the highest copromoting activity when tested together with nicotine. It appears likely that the LM fraction with the highest activity consists of tobacco leaf pigments (14).

Certain compounds used or suggested as sucker control agents or pesticides were active as tumor promoters on mouse skin when tested in concentrations between 0.3 and 1.0 percent. Certain fatty acid esters and fatty alcohols proposed as agricultural chemicals were also tumor promoting agents in concentrations of 3 percent or greater. Among the active tumor promoters were a 0.3 percent solution of dodecyldimethylamine, suggested as a sucker growth inhibitor; Tween 20 and Tween 80, used as surfactants; 1 percent of the insecticides DDD and DDT; and 3 percent mixtures of fatty acid esters and fatty alcohol proposed as sucker growth inhibitors (20). The very small residual amounts of these agricultural chemicals found in tobacco make it unlikely that they are of consequence in the tumor-promoting activity of tobacco extract or tar.

The total smoke condensates of cigarettes, cigars, and pipes act as tumor promoters. The active agents are found primarily in the weakly acidic portion and in certain neutral subfractions. Certain fatty acids, especially oleic acid, and phenols have been identified as weakly acidic tumor promoters. Tumor promoters in the neutral subfractions were DDD, DDT and its major pyrolysis product 4,4'-dichlorostilbene, and N-methylated indoles and carbazoles (165, 189). The majority of the tumor promoters in tobacco tar remain to be identified. These include certain high molecular weight components in the most polaric neutral fraction or in the insoluble portion.

Cocarcinogens

Fractionation studies of tobacco smoke particulates have shown that coadministration of the neutral and weakly acidic portions raises the tumor yield in mouse skin experiments significantly above the number of tumors obtained from each fraction alone (67, 87, 203). Benzo[a]pyrene (BaP) at 0.005 percent concentration applied together with a 5 and 10 percent solution of the weakly acidic portion of tobacco smoke particulates also yields tumors in greater proportion

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than expected on the basis of the additive effects of the individual materials. Some subfractions of the weakly acidic portion are inactive when tested alone, yet they potentiate the carcinogenic activity of 0.003 percent BaP when coadministered with the carcinogen. Van Duuren et al. (194) were the first to demonstrate that catechol, the major phenolic compound in tobacco smoke (20 to 460 µg/cigarette), is a powerful cocarcinogen. Systematic fractionation studies monitored with bioassays have illustrated that the catechols are in fact a major group of cocarcinogens in cigarette smoke (67). A considerable number of other components have been identified in the cocarcinogenic weakly acidic subfractions. None of these, however, are known cocarcinogens (67, 163). They are either inactive or not as yet tested. The levels of the catechols alone cannot account for the cocarcinogenic activity observed for the weakly acidic fraction, but catechol values serve as a fairly reliable indicator of the cocarcinogenic potential of this portion of the smoke particulates. The polyphenols of the leaf apparently serve as important precursors for the catechols (35, 162).

Subfractions of the neutral portion that contain concentrates of PAH are also active as cocarcinogens in studies on mouse skin (165). So far, a number of methylated naphthalenes, indoles, carbazoles, and PAH that have no tumor initiator activity have been identified as cocarcinogens in neutral subfractions (165, 196, 200, 202). Further fractionations and bioassays have demonstrated that both PAH-containing and PAH-free subfractions have cocarcinogenic activity (165). The PAH-free material was shown to contain several unsaturated hydrocarbons as well as oxygenated terpenes, which remain to be bioassayed as potential individual cocarcinogens.

In model studies, C_{10}–C_{14} paraffin hydrocarbons as vehicles for carcinogenic PAH are potent cocarcinogens (13, 92). However, the normal paraffinic and the iso-paraffinic hydrocarbons in tobacco and tobacco smoke are waxy solids with chain lengths of C_{25}–C_{34} and with n-$C_{31}H_{64}$ as the predominant paraffin (174). The neutral subfraction that consists primarily of paraffin hydrocarbons has no demonstrable cocarcinogenic activity. In mouse skin bioassays of cigarette smoke condensates mixed with BaP, increased paraffin levels of the smoke condensates apparently inhibited tumor development (202).

The basic fraction of cigarette tar contains 60 to 80 percent nicotine and other alkaloids. Since nicotine is highly toxic, only the nicotine-free basic portion has been assayed for tumorigenic activity and has been found to be inactive (90, 202). However, when nicotine is given in low doses together with TPA and BaP, it acts as a cocarcinogen. Such cocarcinogenic activity is not found for cotinine and nicotine-N'-oxide, the two major metabolites of nicotine. In fact, nicotine-N'-oxide inhibits the cocarcinogenic activity of TPA (14, 188). The concept of nicotine as a cocarcinogen in tobacco products is

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TABLE 3.—Cocarcinogenic agents in the particulate matter of tobacco smoke[1]

Compound[2]	Cocarcinogenic activity[3]	Ng/cig
I. Neutral Fraction		
Pyrene (-)	+	50–200
Methylpyrenes (?)	?	50–300
Fluoranthene (-)	+·	100–260
Methylfluoranthenes (+;?)	?	180
Benzo(ghi)perylene (-)	+	60
Benzo(e)pyrene (+)	+	30
Other PAH (+)	?	?
Methylnaphthalenes (-)	+	360–6300
1-Methylindoles (-)	+	830
9-Methylcarbazoles (-)	+	140
4 and 4'-Dichlorostilbene (-)	+	1500 [4]
Other or unidentified neutral compounds (?)	?	?
II. Acidic Fraction		
Catechol (-)	+	40,000–350,000
3-Methylcatechol (-)	+	11,000–20,000
4-Methylcatechol (-)	+	15,000–21,000
4-Ethylcatechol (-)	+	10,000–24,000
4-n-Propylcatechol (?)	?	≈ 5,000
Other or unidentified catechols and phenols (?)	?	?
Other or unidentified acidic agents (?)	?	?

[1] Incomplete list.
[2] In parenthesis complete carcinogenic activity on mouse skin; (?) unknown.
[3] + = active; ? = unknown.
[4] Value from 1966 U.S. cigarettes; today's values will be lower, because DDT and DDD decreased in the U.S. tobaccos.
SOURCE: Hoffmann et al. (88).

supported by the observation that the concentration of the alkaloids is closely correlated with the carcinogenic activity of the tested tars in four large-scale mouse skin bioassays (14, 143). More research is needed to elucidate the cocarcinogenic activity of nicotine, especially since it may also be correlated with the risk of tobacco chewers and snuff dippers for cancer of the oral cavity (189, 200).

Table 3 lists the identified cocarcinogens and their concentrations in cigarette smoke. Although certain PAH and catechols represent two major groups of tobacco cocarcinogens, others may be identified.

Organ-Specific Carcinogens

Cigarette smokers have an increased risk of cancer of the esophagus, pancreas, kidney, and urinary bladder (189). Since cigarette smoke does not directly come in contact with these organs, except for the esophagus, mechanisms other than contact carcinogenesis are involved in the pathogenesis of these cancers. Several hypotheses can be postulated for such mechanisms. Cigarette smoke contains organ-specific carcinogens and also agents that give rise to in vivo formation of carcinogens (189). Cigarette smoking may also

shift the metabolism of dietary components toward *in vivo* formation of carcinogenic metabolites (*109*), or may induce enzymes that convert environmental carcinogens to their ultimate active forms (*41*). Another concept relates to the presence in cigarette smoke of cocarcinogens that potentiate the activity of trace amounts of the carcinogens from environmental sources or of those formed *in vivo* (*189*).

Epidemiological and experimental studies have documented the occurrence of organ-specific carcinogens in certain occupational settings. Classic examples for these are 2-naphthylamine, 4-aminobiphenyl, and benzidine in dye factories (*149*); vinyl chloride in the chemical industry is a more recent example (*98*). Tobacco smoke, as a plant-combustion product containing more than 3,600 compounds (*61*), also contains organ-specific carcinogens which have been identified and studied by a number of groups.

N-Nitrosamines

N-Nitrosamines are formed *in vitro* and *in vivo* by nitrosation of amines. More than 50 of the approximately 100 N-nitrosamines which have been tested have various degrees of carcinogenic potency in laboratory animals (*127*). There is a lack of direct evidence that these compounds are also human carcinogens. Nonetheless, many scientists concur with the International Agency for Research on Cancer (*97*) that, for practical purposes, these nitrosamines should be regarded as carcinogenic in humans.

Tobacco and tobacco smoke contain three types of N-nitrosamines; namely, volatile nitrosamines (VNA), nitrosamines derived from residues of agricultural chemicals on tobacco, and the tobacco-specific nitrosamines (TSNA). These compounds are formed during tobacco processing and during smoking from precursors such as primary, secondary, and tertiary amines and quaternary ammonium salts (*97*), reacting with N-nitrosating agents such as nitrogen oxides, nitrite, and some C-nitro compounds (*149, 195*). It is also possible that the oxidation of certain amines can lead to nitrosamine formation (*147*).

Volatile N-Nitrosamines

A number of volatile N-nitrosamines (VNA) are present in tobacco products and tobacco smoke. Practically all of the VNA appear to be retained by the respiratory system upon inhalation of cigarette smoke (*38*). N-nitrosodimethylamine (NDMA) and N-nitrosopyrrolidine (NPYR) occur in the highest concentrations (Table 4) (*97, 158*). NDMA, N-nitrosoethylmethylamine, and N-nitrosodiethylamine (NDEA) are among the most potent environmental carcinogens in this class of compounds (*97*). Tumors of the respiratory tract were