Nicotine Reduction in Tobacco and Tobacco Smoke

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In addition to the selection of low-nicotine varieties of tobacco, several approaches have been used, or suggested for use to reduce the nicotine in tobacco smoke by alteration of the alkaloid content of leaf. In a general way, these approaches can be divided into three categories: physical methods, such as heat, steam, or solvent extraction; chemical techniques utilizing oxidants or other reactive chemical agents; and biological methods, such as fermentation.

PHYSICAL METHODS

The basic dissociation constants for nicotine (*1*) are 7.94×10^{-12} and 4.90×10^{-7} (for the pyridyl and *N*-methylpyrrolidinyl moieties, respectively), and the *p*H values of domestic cigar and cigarette tobaccos are within the range of 4.90–7.25 (*2*). Thus, nicotine in most tobaccos exists primarily in the salt form, although the presence of this alkaloid and related bases bound in high-molecular weight pigments with unknown linkages has been reported recently (*3*). Therefore, physical methods for reducing nicotine in leaf usually require alkalinization before treatment to release the free bases. The latter may then be removed by water, organic solvents, or passage of moist air through the tobacco with or without heat (*4*). This general approach suffers from several economic and technical disadvantages, including undesirable alteration of the flavor, aroma, and combustibility of the treated tobacco. However, several brands of domestic cigarettes marketed over 30 years ago contained tobacco denicotinized by such processes (*5*). At present, one brand of U.S. cigarettes employs a physical method to reduce the nicotine; since commercial practices are confidential, details of the process are unavailable.

Another physical method involves the quick heating of tobacco to 100°C with concurrent passage of air through the tobacco and is believed to act by thermal dissociation of the nicotine salts (*4*). Although this method was employed in Austria in the past, the costs involved in the process apparently mitigated against its widespread use. A method for nicotine reduction involving electrolysis has also been reported (*4*).

CHEMICAL APPROACHES

In the chemical approach, several agents have been used experimentally to reduce nicotine levels in leaf. Among these are compounds which oxidize nicotine to derivatives having lower toxicity than the parent alkaloid, *e.g.*, oxynicotine. Hydrogen peroxide and ozone have been employed in this manner. However, as expected, other leaf constituents also react with these oxidants, and the aroma and certain physical properties of the tobacco are affected deleteriously (*4*). Treatment of experimental tobaccos with nitrous oxide, carbon monoxide, and sulfur dioxide has given variable results with relatively small reductions of nicotine (about 5 to 20%) in some cases (*6*).

Of the various chemical agents suggested for use in nicotine reduction, ethylene and propylene oxides have created the most interest. Of the two, ethylene oxide has been studied more extensively and has been employed commercially as a fumigant and fermentation accelerator in some European countries. This oxide is an alkylating agent which acts by ring fission, forming a 2-hydroxyethyl radical that alkylates nucleophiles. In the case of nicotine, the major reaction product is *N*-(2-hydroxyethyl)-3-(2′-*N*′-methylpyrrolidinyl)pyridine, which occurs in leaf as the salt (*7*). The same product is obtained from the dihydrochloride and monoacetate of nicotine, but a second monoalkylated compound formed by reaction with the pyrrolidine nitrogen of nicotine is also produced from the monoacetate (*8*). In addition, *N*-methylaminoethanol may be formed from reactions between the oxide and methylamine or other aliphatic amines in tobacco when extremely high concentrations of ethylene oxide are employed (*9*). In treating tobacco with ethylene oxide, concentration of gas, proportion of gas to tobacco, reaction time, reaction temperature, and moisture content of tobacco are some important variables (*10*, *11*). The oxide also reacts with other tobacco constituents and may influence flavor deleteriously when some conditions of treatment are employed (*10*). In one study (*10*), about 40–50% of the leaf nicotine was alkylated in this reaction without altering the flavor of the smoke, but higher levels of bound nicotine were accompanied by the development of undesirable organoleptic properties.

Data on the thermal stability of leaf nicotine bound in this way are sparse. Pyrolytic experiments on the alkylated reaction products indicate that cleavage occurs easily, yielding nicotine, pyridine, and other basic compounds (*8*). In the case of ethylene oxide-treated cigarettes, reductions

of 25% of the nicotine in smoke have been claimed (*11*) and only traces of nicotine in smoke have been reported when all the leaf nicotine reacted with the oxide (*7*). Other data indicate that about 30% of the alkylated nicotine in leaf is cleaved, releasing the alkaloid and related products into the smoke (*8*). The thermolability of the alkylated nicotine may not be unexpected, since a thermal cleavage superficially similar to the classical Hofmann degradation can be visualized easily.

More recent work in Austria has shown that treatment of tobacco with ethylene oxide under conditions used in commercial practice has little effect on tobacco aroma and flavor and results in only small reductions (usually less than 5% in nicotine, dry smoke condensate, and benzo[*a*]pyrene contents of smoke and nicotine levels of leaf for most tobacco types (*12*).

It is believed that the use of ethylene oxide in U.S. commercial practices is very limited, if not nil. Fumigants are probably not used to any large extent for tobaccos grown domestically and intended for domestic use, but when such agents are required, methyl bromide may be employed. Although this halide is also an alkylating agent, there is apparently no significant binding of leaf nicotine into methyl derivatives of high thermal stability.

BIOLOGICAL APPROACHES

Perhaps the most practical processing approach for reducing nicotine in tobacco leaf is biological. Generally, cigarette tobaccos in the United States are subjected to a period of prolonged storage (1.5–3.0 years) before being manufactured into cigarettes. This "aging" process involves the packing of leaf strips with relatively low moisture contents (10–13% for flue-cured) into large hogsheads and storage in unheated warehouses at ambient temperatures (*2*, *13*) resulting in subtle chemical changes that are reflected in improved flavor and aroma (*14*). The available information on these chemical changes (*13–15*) indicates that small losses (1–15%) of nicotine usually occur during aging, but reduction as high as 34% has been reported for Burley tobacco (*15*). Also, a decrease in *p*H (from about 5.25–4.80) may occur during aging. Cigar tobaccos and snuff are subjected to a more vigorous process ("fermentation") involving a series of steps in which high levels of leaf moisture (up to 50%), high humidity, and high temperature (up to 50°C) are employed for shorter periods than aging. Fermentation extensively alters cellular constituents, including nicotine, and increases *p*H. As much as 90% of the nicotine may be transformed during fermentation (*13*), but lower values (50% of less) are frequently obtained. Some of the loss is due to mechanical leaching with the added water, but most of it is a result of enzymatic and/or microbial transformation of nicotine into volatile or nonvolatile products, with the latter predominating. The major nonvolatile products are oxynicotine, cotinine, nicotinic acid, nicotinamide, *N*-methylnicotinamide, alkyl pyridyl ketones, and polymeric pyridine compounds (*16*) which may be related to the com-

plex brown leaf pigments (*3*). About 75% of the nicotine transformed during fermentation is found in these products (*4*), most of which are pharmacologically less active than nicotine (*17*). To date, cigarette tobaccos have not been fermented industrially in the United States, but some European commercial practices include a mild fermentation of sun-cured and other tobacco types.

NICOTINE ABSORPTION FROM SMOKE

Although a correlation exists between the total nicotine levels of leaf and smoke, a similar relationship is not found between the nicotine level absorbed by the smoker and the total nicotine concentration in smoke. This effect is probably due in part to the influence of *p*H on the partitioning of nicotine between the particulate and vapor phases of smoke. High *p*H favors release of the more volatile free bases from the particulate phase and a displacement of the particulate-vapor equilibrium of nicotine toward the vapor phase. Flue-cured, air-cured, and cigar tobaccos show progressively higher *p*H in the smoke produced therefrom, and progressively larger proportions of nicotine in the vapor phase would be expected in this series. Laboratory studies have shown that a direct relationship does exist between the *p*H of smoke and the degree of nicotine absorption in simulated saliva (*18*). Approximately fivefold increases in absorption are apparent in tobacco smoke varying in *p*H from 5.0–8.0. Clinical data confirming this pattern have also been obtained (*19*). Of course, other factors may affect the nicotine levels of smokers, such as degree of inhalation and puff volume, which in turn may also be influenced by *p*H through effects on the irritative properties of the smoke.

CURRENT AND FUTURE INVESTIGATIONS

From the above, it is apparent that one may theoretically reduce the free nicotine absorbed by the smoker by increasing the acidity of the smoke. One way to do this would be through increasing the acidity of the leaf. In studies conducted almost 40 years ago, acidification of tobacco by the addition of citric acid, oxalic acid, and sulfuric acids was claimed to increase the bound nicotine in leaf (*5*), but these findings require confirmation. The use of citric acid in this manner is known to increase the mildness of cigarette smoke (*20*) and is claimed to inhibit in part the ciliostatic activity of smoke (*21*). The effect of such acidification on the partitioning of nicotine between particulate and vapor phases of smoke has not been determined. However, since essentially all the nicotine in smoke from domestic cigarettes is believed to be in the particulate matter, it is doubtful whether further acidification of the tobacco would affect the vapor-particulate equilibrium significantly.

Another possible avenue of exploiting the *p*H effect may involve filtration. There are indications that selective filtration of nicotine (presumably from the vapor phase) may be achieved (*22*). If the *p*H of the smoke were raised to increase the nicotine in the vapor phase and a selective filter used to remove the alkaloid, the free nicotine available for absorption might be reduced. The total quantity of nicotine that would have to be removed from smoke under such conditions would be substantial. Presently used filters have limited capacities and may not be capable of removing all the nicotine, phenol, and gaseous components that would be potentially filterable from smoke having large proportions of nicotine in the vapor phase.

Although there are many domestic patents for special filters that claim to remove nicotine from smoke (*23*), including ion exchange resins, none of these has apparently been employed commercially.

The use of additives in cigarettes to alter combustion patterns and chemical composition of smoke is another possible route to nicotine reduction. The use of sodium nitrate in this way has produced some slight selective reduction of nicotine in addition to a decrease in benzo[*a*]pyrene (*24*).

A potential complicating factor in the entire problem has been the recent finding that nicotine and related alkaloids and bases occur in smoke as moieties in high-molecular weight pigments (*25*, *26*). These pigments comprise at least 3–5% of smoke condensate and must be considered major constituents. Although studied incompletely, available data indicate that the alkaloids are firmly linked within the pigment molecule and are not hydrolyzed by conventional acidic hydrolysis but are released during experimental pyrolysis (*27*). Although it has not been possible to obtain analytical values for the total nicotine contents of the pigments thus far, it appears that their contribution to the total alkaloid content of smoke may be relatively small. The *in vivo* fate of these pigments is obviously unknown.

SUMMARY

At present, the most practical commercial approach for reduction of nicotine in smoke is that used most widely in the United States: selection of low-nicotine tobacco varieties and leaves. Almost all the available physical and chemical methods to reduce the nicotine in leaf have economic or technical shortcomings that mitigate against their commercial adoption. However, promising avenues for further research are available, including the use of ethylene oxide to bind leaf nicotine and the possibility of fermentation of cigarette tobaccos. Also, alteration of nicotine levels in smoke by the use of tobacco additives or a combination of selective filtration and *p*H modification should be investigated more extensively.

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Mechanical Filtration: A Review of Filtration Mechanisms Pertinent To Cigarette Smoke

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IN discussing the various aspects of mechanical filtration of cigarette smoke, it is pertinent to first consider some of the characteristics of this aerosol. Cigarette smoke is a mixture of gases and particulate matter produced by the combustion of tobacco and paper. During the combustion process, peak temperatures can exceed 1000°C, pyrolizing and distilling many of the chemical components ahead of the fire zone. Because of the large temperature gradient, many of these materials condense and deposit on the tobacco close to the fire zone. This process is repeated many times during the burning of the cigarette, thus building up "tar" in the tobacco near the end of the cigarette.

Close to the fire zone, the mass mean size of the tiny "tar" particles comprising the particulate phase is close to 0.3 μ. However, because of their extremely high concentration, the particles coagulate to the extent that the mass median diameter of those leaving the cigarette increases to approximately 1.0 μ, with a number median diameter of approximately 0.3 μ (*1*, *2*). The concentration of these smoke particles is about 4×10^9 particles/ml of smoke. Thus, for each average puff of 35 ml of smoke, the cigarette filter is challenged with approximately 1.4×10^{11} particles as well as a variety of gases. The removal of total particulate matter ("tar") in present-day commercial cigarette filters ranges up to 50%. The removal of the gaseous components by selective filtration is a problem in itself and will not be covered in this presentation.

For an aerosol having such characteristics, practical filter design depends on the ability to control the collection mechanisms at high efficiency while maintaining pressure drop across the filter at a figure low enough to ensure consumer acceptance.

THEORY OF FILTRATION

The basic practical theory of mechanical filtration was developed in 1942 by Langmuir (*3*). This theory is based on collection on isolated fibers. The velocity field around an isolated fiber is used to calculate the deposition due to diffusional, direct interception, and inertial impaction effects. The influence of neighboring fibers is taken into consideration by use of an empirically derived correction figure, and the final calculation yields a filtration efficiency for the whole filter bed.

Langmuir's theory assumes that gravitational forces are unimportant, that there are no electrostatic effects, that all aerosol collisions with fibers result in complete capture of the particles, that the collected aerosol particles utilize only a small portion of the filter surface, and that the velocity through the filter is restricted. In calculations based on this theory, each of the three applicable mechanical filtration mechanisms—diffusion, direct interception, and inertial impaction—dominates a different air-velocity regimen. On this basis, Langmuir theorizes there should be one aerosol particle size that will exhibit an optimum penetrating power.

An optimum penetration size has also been theorized by other experimenters. However, there has been some recent evidence contrary to these findings indicating that penetration power continues to increase as particle size decreases. Pich recently reviewed most of the theoretical studies of aerosol filtration and prepared an excellent discussion (*4*) that includes all areas pertinent to filtration.

FILTRATION MECHANISMS

There are five mechanisms of interest relative to the collection of particulate matter in a fibrous filter, although to date the first three have been considered significant in the design of cigarette filters: 1) interception, 2) inertial impaction, 3) diffusion, 4) gravitational forces, and 5) electrostatic effects. Each will be discussed briefly. Also discussed are the implications of mechanism combinations and fiber interference.

1) *Interception.*—The flow conditions within a filter for low face velocities (volumetric flow rate divided by face area of filter) can be considered wholly viscous. Thus, an aerosol particle passing around a fiber tends to follow a streamline course. Particles contained in a streamline that pass the fiber at a distance which is less than the particle radius will be intercepted. Interception becomes important when the particle diameter is within an order of magnitude of the fiber diameter. Using standard potential-flow equations, Ranz (*5*) showed that the collection efficiency due to interception for an isolated fiber is:

$$\eta_o = (1 + R) - \frac{1}{1 + R} \qquad 1]$$

where

η_o = collection efficiency of isolated fiber,
R = interception parameter = d_p/d_f,
d_p = diameter of particle,
d_f = diameter of fiber.

Langmuir (*3*) derived the following efficiency equation:

$$\eta_o = \frac{1}{2[2.00 - lnN_{Re}]}\left[2(1 + R)ln(1 + R) - (1 + R) + \frac{1}{1 + R}\right], \qquad [2]$$

where

N_{Re} = Reynolds number based on upstream velocity for an isolated cylinder (such as a fiber).

Torgeson (*6*) derived the following expression for interception efficiency:

$$\eta_o = 0.0518\left(\frac{C_D N_{Re}}{2}\right)R^{3/2}, \qquad [3]$$

where

C_D = drag coefficient
$= \frac{8\pi}{N_{Re}(2 - lnN_{Re})}$.

For a 1 μ diameter particle and a 27 μ diameter filter fiber, the collection efficiency calculated from [1] is 0.073, [2] is 0.00072, and [3] is 0.00195. The actual efficiency probably falls between the values predicted by [2] and [3].

2) *Inertial impaction.*—A particle of suitable mass and inertia may follow an airstream until the stream bends. At this point the particle's inertia carries it out of the airstream in the original direction of motion. Increases of either particle mass or air-flow velocity favor the impaction of particles in filter materials.

Davies (*7*) developed what is probably the most suitable impaction theory. In this theory it is necessary to add to the impaction effect the interception effect, since any particle whose trajectory comes within $d_p/2$ of the collection surface will be caught. In the collection theory based on inertia and interception effects, collection is a function of Ψ, R, and N_{Re}. Davies' results are presented in the following equation:

$$\eta_{im} = 0.16R + 0.16[(0.5 + 0.8R)\Psi - 0.1052R\Psi^2]. \qquad [4]$$

Torgeson (*6*) modified Davies' equation to the following:

$$\eta_{im} = \eta_o[] + R^{-3R\Psi}(0.5 + 0.8R)], \qquad [5]$$

where

η_o = interception efficiency = $0.0518\left(\frac{C_D N_{Re}}{2}\right)R^{3/2}$,
C_D = drag coefficient,
Ψ = inertia parameter = $\frac{1}{18}\frac{\rho_p d_p^2}{\mu d_f}$,
ρ_p = density of particle,
μ = coefficient of viscosity,
V = filter face velocity.

Many investigators believe that inertial impaction plays an important role in the filtration of cigarette smoke. At a flow rate of 17.5 ml/second (a 35 ml puff in 2 sec), the average velocity through a typical cigarette filter is approximately 40 cm/second. For a 30 μ filter fiber, the inertial and interception collection efficiencies for 0.1, 0.5, and 1.0 μ particles, which are representative of cigarette smoke, are 6.7×10^{-5}, 1.2×10^{-3}, and 4.5×10^{-3}, respectively.

3) *Diffusion.*—Brownian movement, the erratic motion of particles brought about by molecular bombardment of very fine particles, is responsible for the removal of some particles that are intercepted by the fibers of a cigarette filter. Contrary to the situation with larger particles, a decrease in the air velocity through the filter increases the probability of deposition of small particles, because the particles remain within the filter configuration longer and have more opportunities for interception by the fibers.

Stern and co-workers (*8*), using a diffusion-boundary-layer concept, developed an expression for diffusional collection efficiency as follows:

$$\eta_o = 0.75 \left(\frac{C_D N_{Re}}{2}\right)^{0.4} Pe^{-0.6}, \qquad [6]$$

where

C_D = drag coefficient,

Pe = peclet number = $\frac{V d_f}{D}$.

D = diffusion coefficient.

For a 27 μ diameter cigarette-filter fiber, the diffusional collection efficiency for 1.0, 0.5, and 0.1 μ particles is 0.8×10^{-3}, 1.2×10^{-3}, and 4.6×10^{-3}, respectively.

4) *Gravitational forces.*—In accordance with Stoke's Law, heavier particles in an airstream may settle out on the fibers of a filter because of gravitational forces. Such removal is a function of the particles' settling velocity in relation to the particles' stream velocity and the horizontal area of the filter. However, because essentially no smoke particles are larger than 2 μ, gravitational forces are minimal in cigarette-smoke filtration.

5) *Electrostatic effects.*—Electrostatic effects have largely been ignored in studies of aerosol filtration. Despite the fact that naturally occurring aerosols are known to have a distribution of charges, and generated aerosols are often highly charged, nearly all theories of aerosol filtration assume the effects of electrostatic charges are negligible. However, much work has been done on the deposition of charged particles on single cylinders, *e.g.*, that of Natanson (*9*), Gillespie (*10*), and Torgeson (*6*); but, except in the studies of Rossano and Silverman (*11*), Gillespie (*10*), Lundgren and Whitby (*12*), and Goyer *et al.* (*13*), the effects on overall filter efficiency have been somewhat neglected.

Combined Filtration Mechanisms

The determination of fiber collection efficiency with respect to any single mechanism is a straightforward procedure. However, when an attempt is made to calculate collection efficiency in terms of combinations of mechanisms, problems arise. In a general sense, the problem becomes one of predicting interactions between mechanisms. Most theoretical studies have been limited to a single mechanism or to combinations of two mechanisms, although Davies (*7*), Torgeson (*6*), and Dorman (*14*) have considered impaction, interception, and diffusion simultaneously. It has not been shown conclusively whether any of these theories is applicable over a wide range of conditions such that different mechanisms become controlling, although each has been proved adequate in certain regimens (*3*, *15–17*). It is, therefore, important that experiments be performed over a wide range of conditions to determine the effects of interactions between deposition mechanisms.

Fiber Interference

The theoretical analyses of single-fiber efficiencies are limited in application to calculation of filter efficiencies through their dependence on interference effects among fibers. These interference effects occur when a fiber is embedded in a filter mat. In most cases, it has been necessary to use experimental data on filter efficiencies to obtain the information essential for the use of calculations of single-fiber efficiency.

Chen (*15*) and Davies (*7*) presented correlations expressing the pressure drop through a filter and the interference effects in terms of the packing density or fiber volume fraction, α. Clarenburg and Van der Wal (*17*) used a simplified form of Davies' correlation for the same purpose over a narrow range of fiber volume fractions. Fuchs and Stechkina (*18*), for the case of diffusional deposition, used the flow models of Kuwabara (*19*) and Happel (*20*), which take into account the presence of other fibers and thus eliminate the need for accounting for interference effects through an experimental correlation. However, the Kuwabara-Happel flow model is based on an average packing and does not account for statistical variations in filter construction. Like the correlations with single-fiber efficiencies, the calculations based on the Kuwabara-Happel flow model give efficiencies lower than those measured experimentally (*18*).

The discrepancies between the correlations accounting for fiber interactions can be illustrated as follows. Filter efficiency can be expressed according to Chen (*16*) as:

$$\eta = 1 - exp\left[-\frac{d_f \Delta P \eta_o (1 + 4.5\alpha) ln(k_5 \alpha^{-0.5})}{V \mu k_4}\right], \qquad [7]$$

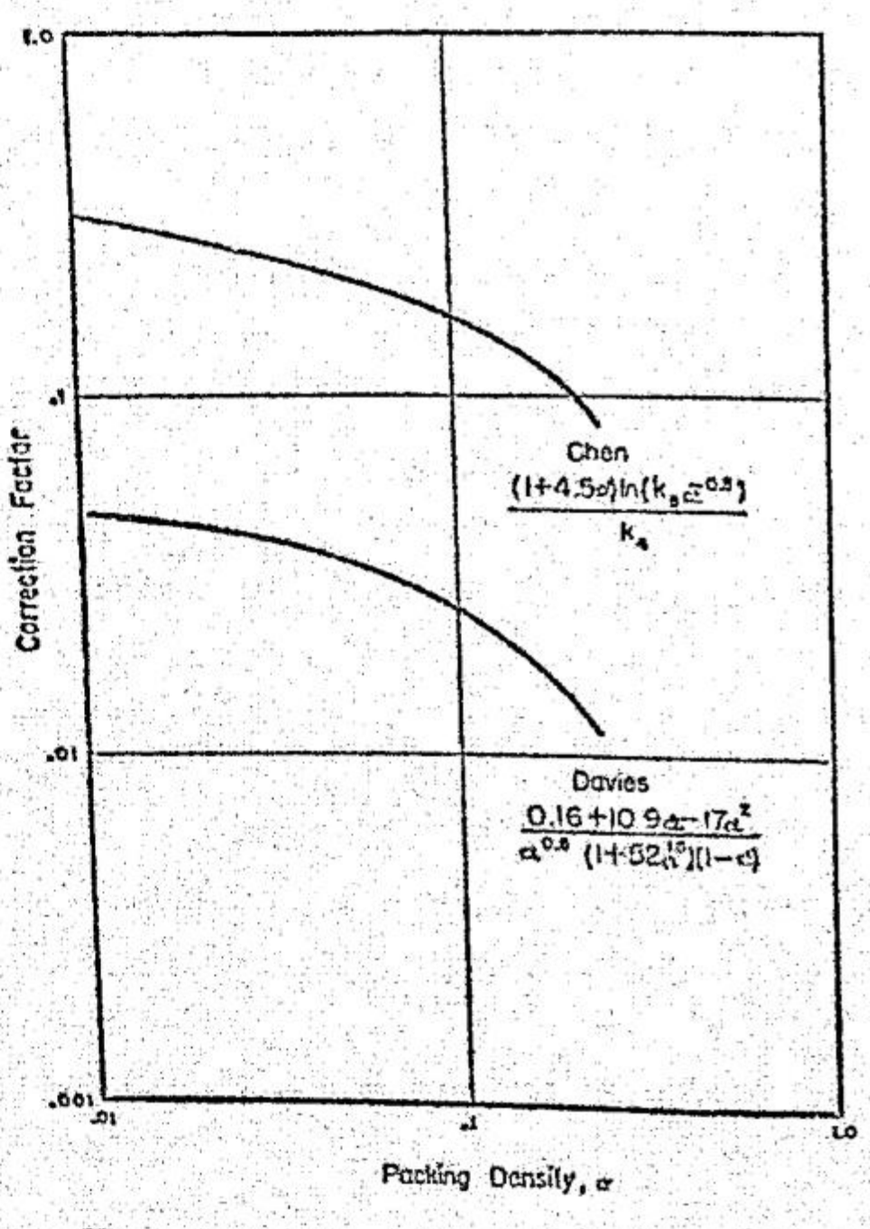
and according to Davies (*7*) as:

$$\eta = 1 - exp\left[-\frac{d_f \Delta P \eta_o (0.16 + 10.9\alpha - 17\alpha^2)}{V \mu 17\pi \alpha^{0.5}(1 + 52\alpha^{1.5})(1 - \alpha)}\right], \qquad [8]$$

where

η = filter efficiency,
η_o = single-fiber efficiency,
ΔP = pressure drop,
d_f = fiber diameter,
V = gas velocity upstream,
μ = gas viscosity,
α = fiber volume fraction in filter (packing density),
k_4 = constant ($\simeq$ 6.1),
k_5 = constant ($\simeq$ 0.64).

Constants k_4 and k_5, experimentally derived for a particular filter, are a function of fiber orientation and will be different for filters of different construction. The correction factors derived by Chen and Davies and their dependence on packing density or fiber volume fraction are compared in text-figure 1. It is evident that there is little agreement in the magnitudes of these correction factors, although the manners in which they vary with fiber volume fraction are in good agreement. Interestingly, Clarenburg and Van der Wal (*17*) report that their results indicate agreement with Davies' interference correction regarding the dependence on α, but do not agree with Chen's. Fuchs and Stechkina (*18*) report that Chen's (*16*) correction factor gives the correct dependence on α.



TEXT-FIGURE 1.—Comparison of dependence on packing density or fiber volume fraction.

Because the effects of fiber interference and filter structure are not well defined, some efforts directed toward elucidation of these effects are needed. A more representative filter model used in conjunction with the Kuwabara-Happel flow model should provide an improved theoretical representation of filtration. Well-designed experiments should complement the theoretical treatment and provide information necessary to establish the effects of filter structure on filtration efficiency.

EFFECTS OF PARAMETERS AND INDIVIDUAL VARIABLES

Because of the overall complexity of the filtration process, it is useful to single out individual factors for evaluation of their effect on filtration efficiency. This is often a practical approach because it allows examination of the variables or factors that can be controlled to improve filter performance or that are problem areas in filter design. Three important factors are discussed in the following paragraphs: the effect of velocity, the most penetrating particle size, and the effect of pressure drop across a filter.

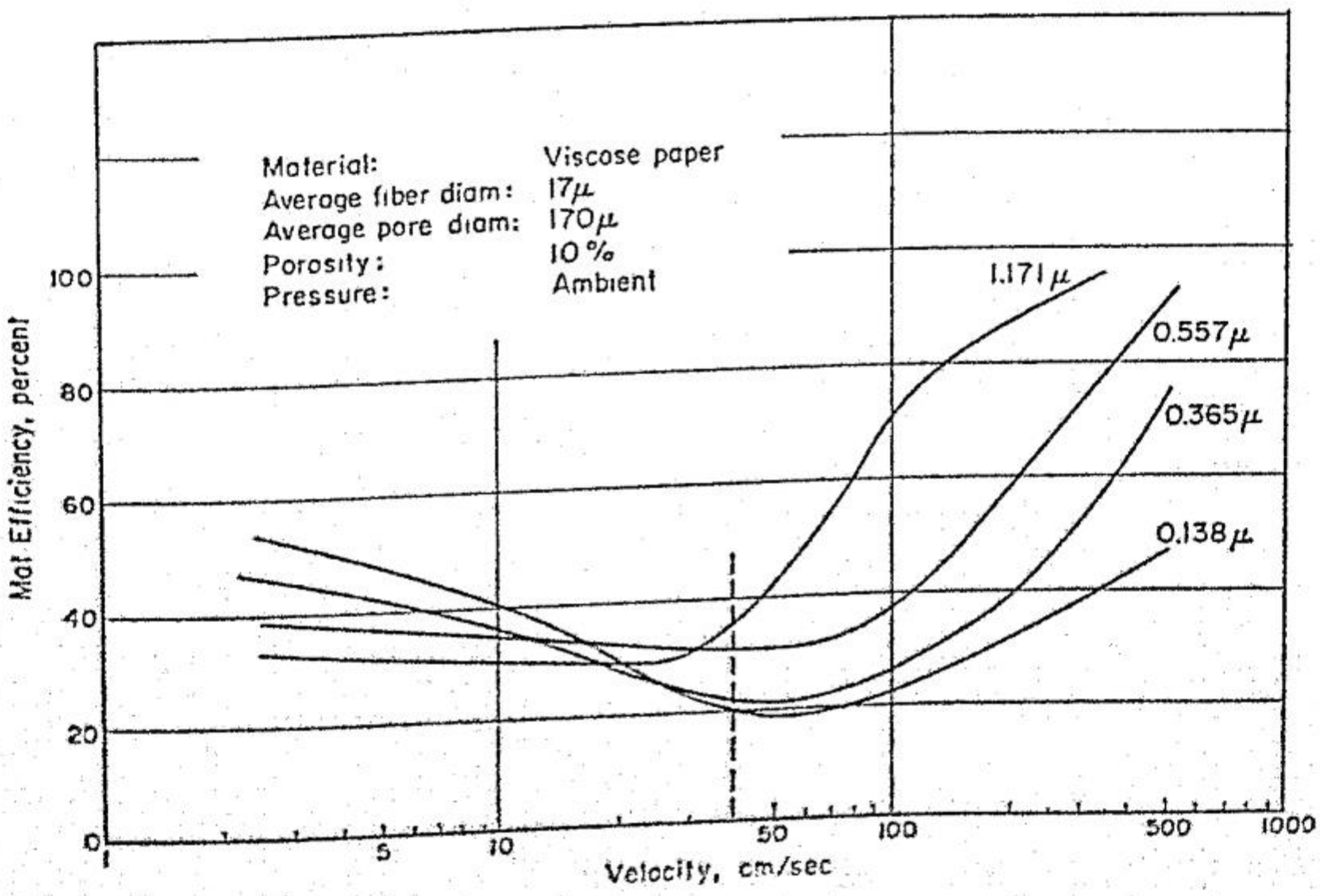
Effect of Velocity

According to most of the filtration theories, penetration through a filter increases as velocity is increased out of the diffusion regimen. The penetration reaches a maximum and then decreases as the increasing velocity causes inertial effects to become more important. The velocity for peak penetration is greater as the size of the particles decreases.

Text-figure 2 is a plot of experimental results obtained by Stern *et al.* (*15*) for monodispersed polystyrene latices of the same order of size as cigarette smoke. This curve shows the shift in maximum penetration as the particles decrease in size. The curve also shows that the face velocity for a cigarette (40 ml/sec) is not the ideal for efficient filter design.

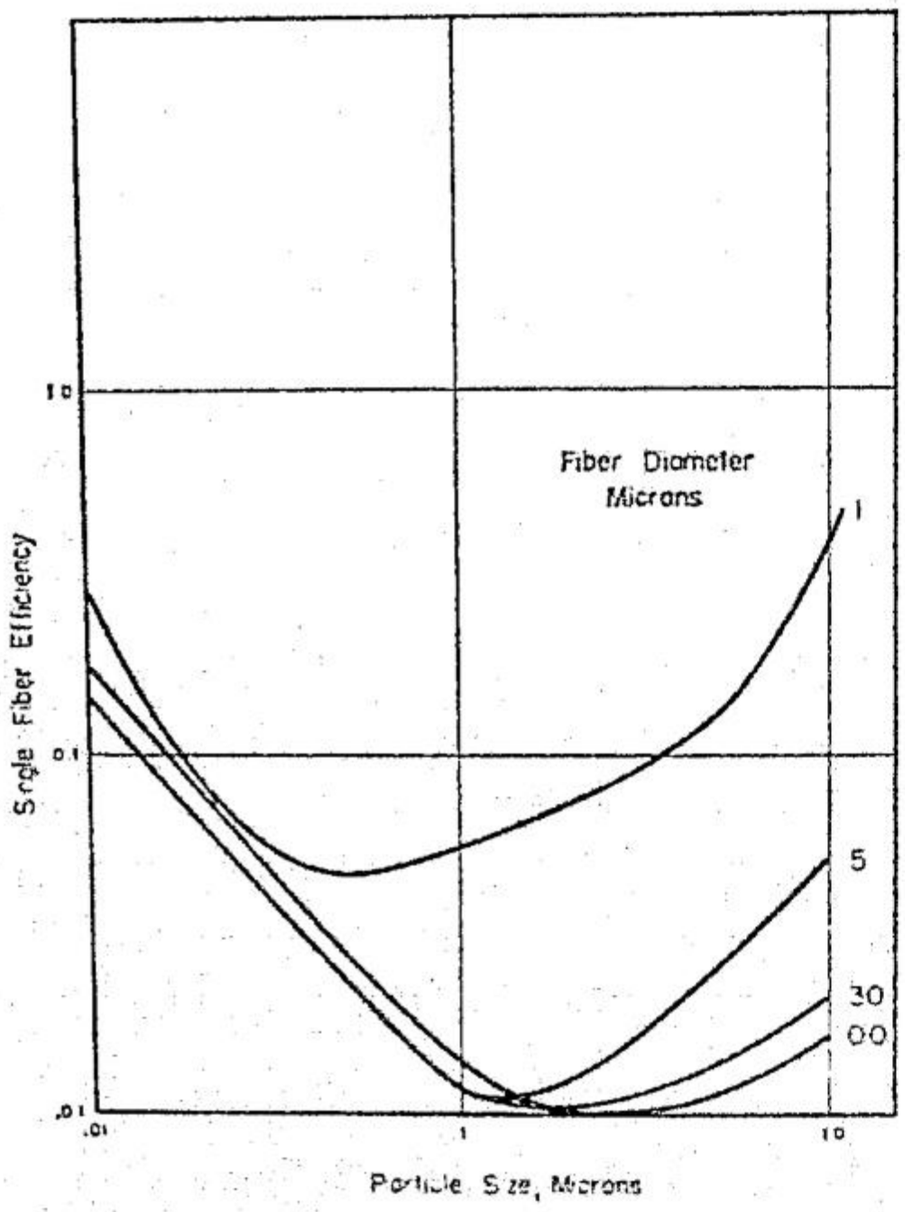
Most Penetrating Particle Size

The theories of Langmuir (*3*), Davies (*7*), Torgeson (*6*), and Friedlander (*21*) predict there will be a particle size that is least effectively col-



TEXT-FIGURE 2.—Experimental collection efficiency obtained by Stern *et al.* (*15*) for removal of spherical polystyrene beads.

lected in a fibrous filter. An example of the theoretical predictions by Torgeson is given by the computer-calculated efficiencies presented in text-figure 3. Minima in the efficiency curves occur between 0.04 and 0.3 μ and depend on the diameter of the fibers forming the filter. These minima also depend on velocity and on void fraction $(1-\alpha)$ in the filter. These were assumed to be 35 ml/second and 0.9, respectively, for this particular case.



TEXT-FIGURE 3.—Single-fiber efficiency as a function of particle size as calculated on the basis of Torgeson's theory (*6*).

Although the theories of filtration predict a most penetrating particle size, there is little agreement as to the size of the particles that is most penetrating. Of more concern, however, are experimental studies indicating that penetration continues to increase as particle size decreases. This behavior, indicating the absence of a most penetrating size, was noted by LaMer (*22*), Ramskill and Anderson (*23*), and Stern *et al.* (*15*). However, Gillespie (*10*) and Thomas and Yoder (*24*) did observe a most penetrating size.

These conflicting results need to be resolved. The most convincing evidence would be obtained by means of a thorough experimental study under carefully controlled conditions. This work should be supplemented by the development of analytical models for describing the particle behavior.

Effect of Pressure Drop Across a Filter

One of the most important parameters in the design or use of a filter is its pressure drop. Although it is not difficult to prepare a filter having a collection efficiency of nearly 100%, the high pressure drop would make

any such filter impractical. Considerable work has been done on the measurement and on the calculation of the pressure drop for filters by use of both the channel and drag theories. However, the porosity of cigarette filters is too great to correlate pressure drop with the channel theory.

Several theories have been derived which neglect the pressure drop caused by interference effects between neighboring fibers. Davies, however, combined dimensional analysis and experimental results to include fiber interference effects and obtained the following equation for filters with packing densities greater than 0.02:

$$\Delta P = \frac{70VL\mu\alpha^{1.5}(1 + 52\alpha^{1.5})}{d_{fe}^2}, \qquad [9]$$

where

L = filter length,
d_{fe} = effective fiber diameter.

Chen (*16*) also derived a pressure drop equation summing the drag forces on the fibers in a unit volume of a filter. This sum represents the pressure drop across a unit thickness of the filter. Chen assumed that the neighboring fibers act like a boundary around a given fiber, and the ratio of interfiber distance to the fiber diameter will determine the drag coefficient for a fiber in a filter, particularly at low Reynolds number. He found that the drag coefficient (C_D) is inversely proportional to the Reynolds number (N_{Re}). His final equation for pressure drop, based on correlations with experimental results, is as follows:

$$\Delta P = \frac{4}{\pi} \frac{k_4}{lnk_5\alpha^{-0.5}} \frac{\alpha}{1 - \alpha} \frac{\mu VL}{d_f^2}, \qquad [10]$$

where

k_4 = constant ($\simeq$ 6.1),
k_5 = constant ($\simeq$ 0.64),
L = filter length.

CHARACTERIZATION OF FILTERS

Chen (*16*) also derived a relationship for judging the performance of a filter This relationship is as follows:

$$\gamma = \frac{ln(1 - \eta)}{\Delta P} = \frac{\eta_o(d_f)(1 + 4.5\alpha)ln(k_5\alpha^{-0.5})}{k_4\mu V}. \qquad [11]$$

It shows that the higher the value of γ, the better the filter. Also, it shows that when d_f, d_p, and V are constant, the lower the value of α, the higher the value of γ. When d_p, V, and α are constant: 1) At low velocities diffusion is important, and the larger the filter size, the higher the value of γ; 2) at high velocities, inertia is important and γ remains fairly constant; and 3) at medium velocities, similar to those in a cigarette filter, interception is important, and the smaller the fiber size, the larger the value of γ. Also, at

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Reconstituted-Tobacco-Leaf Technology: A Tool for Tobacco-Smoke Modification

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IN previous communications (*1*, *2*), we discussed reconstituted-tobacco-leaf technology and the economic bases for the utilization of reconstituted leaf as wrapper and binder in cigars and as filler in cigarettes. Since the cost of tobacco for cigarettes is about 60% of the cost of the final article, there is the economic incentive to improve the utilization of the tobacco purchased at auction. Reconstituted-tobacco-leaf technology provides the means by which manufacturing fines and tobacco stems can be given a physical form suitable for incorporation in cigarettes.

The use of reconstituted tobacco leaf in cigarettes has increased rapidly during the past 15 years to a current annual rate exceeding 175 million pounds. For the cigar industry, in addition to the economics of more complete tobacco utilization, reconstituted leaf technology has been the key to labor savings and automation of cigar manufacture. Current usage of reconstituted leaf tobacco in cigars is more than 30 million pounds annually.

The 5 major processes for manufacturing reconstituted tobacco sheet from tobacco scrap, fines, and stems which have been developed and are in commercial use have been described previously (*1*, *2*). By their very nature, all these processes result in products with a degree of physical and chemical uniformity substantially superior to natural tobaccos.

In recent communications (*3*, *4*), the structural differences among reconstituted tobacco leaves, made by the different commercial processes, were described. Varying the conditions in a given process can result in a considerable range of structural modifications of the reconstituted leaf.

Accordingly, one may conclude that reconstituted sheet technology is a means for uniform control and modification of the physical structure of tobacco to a degree which cannot be accomplished with tobacco in leaf form. It follows, then, that the structural modifications which can be effected by reconstituted sheet technology could result in differences in the burn properties of these products, which could be reflected by quantitative

and/or qualitative differences in the smoke. Preliminary data were given which indicated that reconstituted tobacco could differ from natural tobacco leaf in the yield of particulate matter, nicotine, and carbonyl compounds in the smoke (*3*, *4*).

In addition to structural modifications, the reconstitution process permits uniform incorporation of burn-modifying additives, resulting in many possible combinations for smoke modification and representing another dimension in tobacco technology. As contrasted to the economic considerations given before, the possibilities for smoke modification by this technology are perhaps even more profound.

This paper presents results which expand on the utility of reconstituted leaf technology as a tool for the modification of the chemical nature of the smoke. A single type of tobacco (a 70/30 blend of Bright leaf/stem)—a range of structurally different reconstituted leaves made from this tobacco type with and without nontobacco additives—made by one of the commercial processes (slurry process) will be discussed.

The range of physical as well as quantitative and qualitative differences in cigarette smoke obtained from a single type of tobacco via reconstituted leaf technology is compared to the natural variability in physical and combustion properties which exists between different lots of this same tobacco type before reconstitution. The data obtained on smoke-condensate yields as well as on smoke composition indicate that expanded research activity in this area could be fruitful.

We believe that reconstituted leaf technology is a basic tool for smoke modification.

MATERIALS AND METHODS

All experimental reconstituted tobacco sheets were prepared by the slurry process on Microflake tobacco sheet-forming equipment from the American Machine and Foundry Company (AMF). A constant tobacco type was used in 2 natural-tobacco control runs and 8 experimental reconstituted tobacco runs. This tobacco type was a 70/30 (weight/weight) blend of top-quality uncased Bright strip and flue-cured Bright stems. Two different lots of this tobacco type were employed in these trials, with 1 natural-tobacco control and 4 of the experimental reconstituted samples being prepared from each lot. The 2 lots of this tobacco type were perhaps as different as might be encountered within the framework of a single blend of tobacco. This was desirable, since comparison of the 2 natural-leaf controls will give some measure of the variability in physical and combustion properties which can occur without the reconstitution process. This is a useful point of reference in considering the range of variability which one can produce through reconstituted leaf technology.

One lot of Bright leaf/stem blend was from the South Carolina 1962 crop and had a nicotine content of 2.26% (dry basis). This lot was used in reconstituted sheet Samples A, B, C, and D, as well as in the natural-leaf

control Sample E. The second lot of the 70/30 Bright leaf/stem blend was from the South Carolina 1964 crop, had a lower nicotine content (1.37% dry basis), and was used in reconstituted sheet Samples F, G, H, and J, as well as in the natural-leaf control Sample K. The stem portion of the tobacco blend was purchased as rolled stems, and was used in the natural-leaf controls E and K by shredding in the conventional manner. When used in the experimental reconstituted sheets, the rolled stems were either ground to a minus 120-mesh U.S. Standard sieve size or physically modified to contribute functional properties to the sheet. The Bright leaf portion of the tobacco blends was shredded conventionally for the natural-tobacco controls E and K, and ground to minus 120 mesh dust for use in the experimental reconstituted sheet types.

Cigarettes were prepared on an AMF "Chico" maker, Model CCM, with the use of nonporous cigarette paper. Cigarette length, circumference, and diameter were 70 mm, 25.8 mm, and 8.20 mm, respectively. Cigarettes were equilibrated at 68% RH and 72°F before they were sorted by weight. Sorting into 0.05 gram weight-range groups was accomplished automatically with a modified AMF Automatic Cigarette Weigher Model 3-151. Pressure drop (draw resistance) was measured on the cigarettes from each weight group under such conditions that 17.5 ml of air per second was being drawn through each cigarette.

Cigarettes for determination of particulate matter and nicotine were selected from each 0.05 gram weight-range group to be within ± 20 mg of the average weight of the group and to exhibit the mean draw resistance for the group (5% maximum deviation permissible). Wet particulate matter was determined by the Cambridge filter method of Ogg (*5*). Cigarettes were smoked on an automatic smoking machine at a rate of 1 puff per minute with a puff volume of 35 ml and a puff duration of 2 seconds. The water content of the particulate matter was determined by gas chromatography according to Schultz and Spears (*6*), and the results were used to calculate the dry particulate matter values reported in this study.

The nicotine content of the natural tobacco blends and of the experimental reconstituted sheets was determined by the standard steam distillation, colorimetric method described in (*7*). The nicotine content of the particulate matter obtained from combustion of the control and experimental cigarettes was determined by use of a gas chromatographic procedure found to be in agreement with the steam distillation method.

Static (free) burn rate was measured in cigarettes that had been selected for weight and draw resistance. The cigarettes were marked at 10 and 62 mm with a soft pencil. Ignition was carried out with a controlled puff by an automatic smoking apparatus, and each burning cigarette was then horizontally mounted on 1 of 10 pegs, protruding 30–40 mm from a vertical board. The pegs were nails less than 1 mm in diameter. Each cigarette was positioned so that the nail extended 20 mm into the center of the tobacco column, but a distance of at least 10 mm between the cigarette and the plane of the peg board was maintained.

The static burning rate is given by the time of free burning between the marks at 10 and 62 mm and by the tobacco weight corresponding to the length of the cigarette. It is expressed in terms of milligrams of tobacco consumed per second. The average value may be determined from 10 cigarettes with an experimental deviation of less than ±5%.

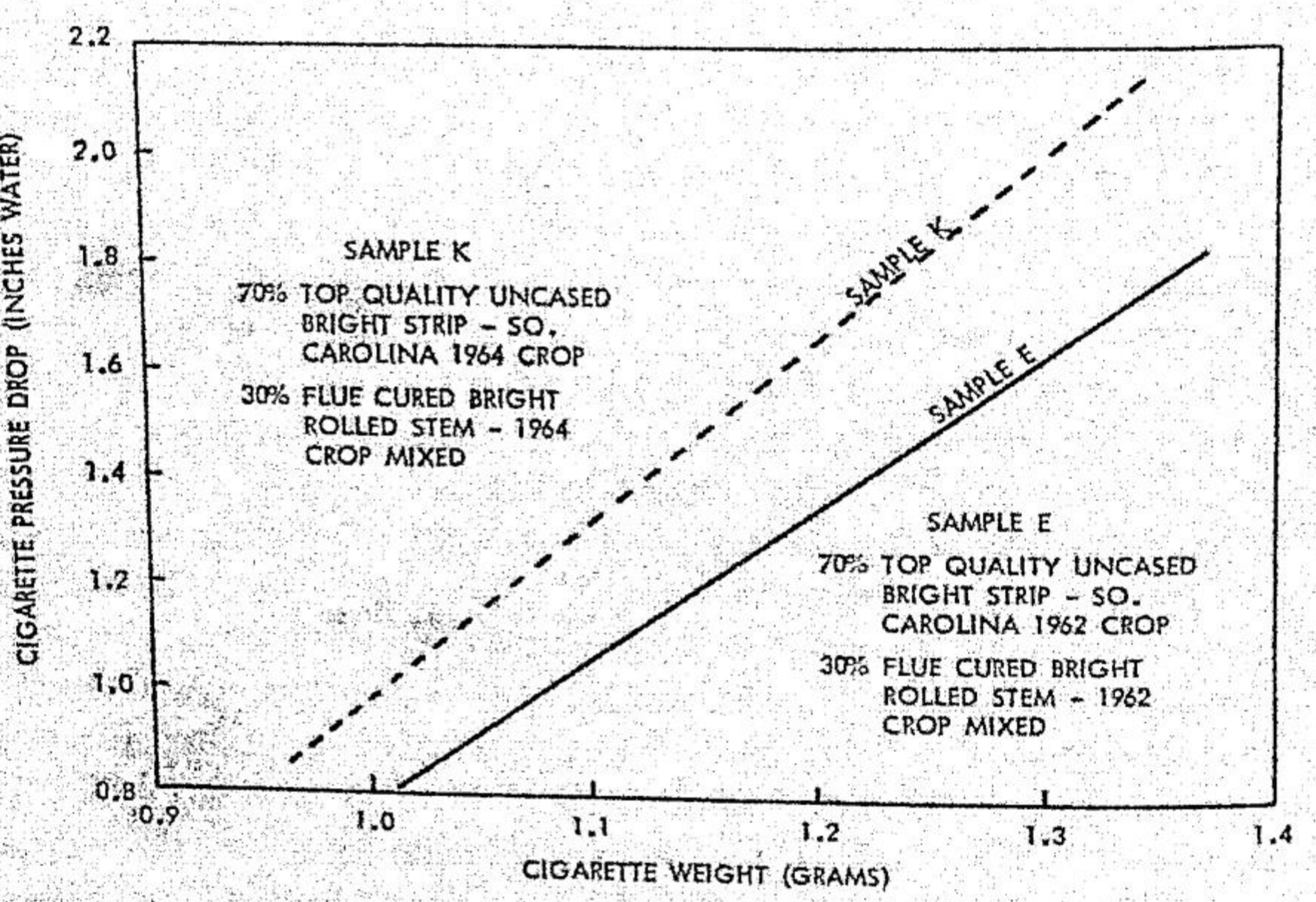
Benzo[*a*]pyrene and phenol in particulate matter were analyzed by methods reported by Hoffmann and Wynder (*8–10*). Carbon monoxide in the gaseous phase of cigarette smoke was analyzed by an infrared analytical procedure. Acrolein was determined by the method of Newsome *et al.* (*11*).

EXPERIMENTAL RESULTS

As indicated before, the objective of this work was to determine the degree to which tobacco smoke could be modified quantitatively and qualitatively by reconstituted-tobacco-leaf technology, with the use of the variability that occurs between lots of the same blend of natural tobacco as a point of reference.

Variability of Natural Tobacco

One of the tobacco characteristics believed to influence the combustion of cigarettes is the relationship between cigarette weight and pressure drop. This relationship was determined for the 2 natural-leaf tobacco controls, Samples E and K. The results are plotted in text-figure 1. Sample K (70/30 Bright leaf/stem blend; 1.37% nicotine content) cigarettes had



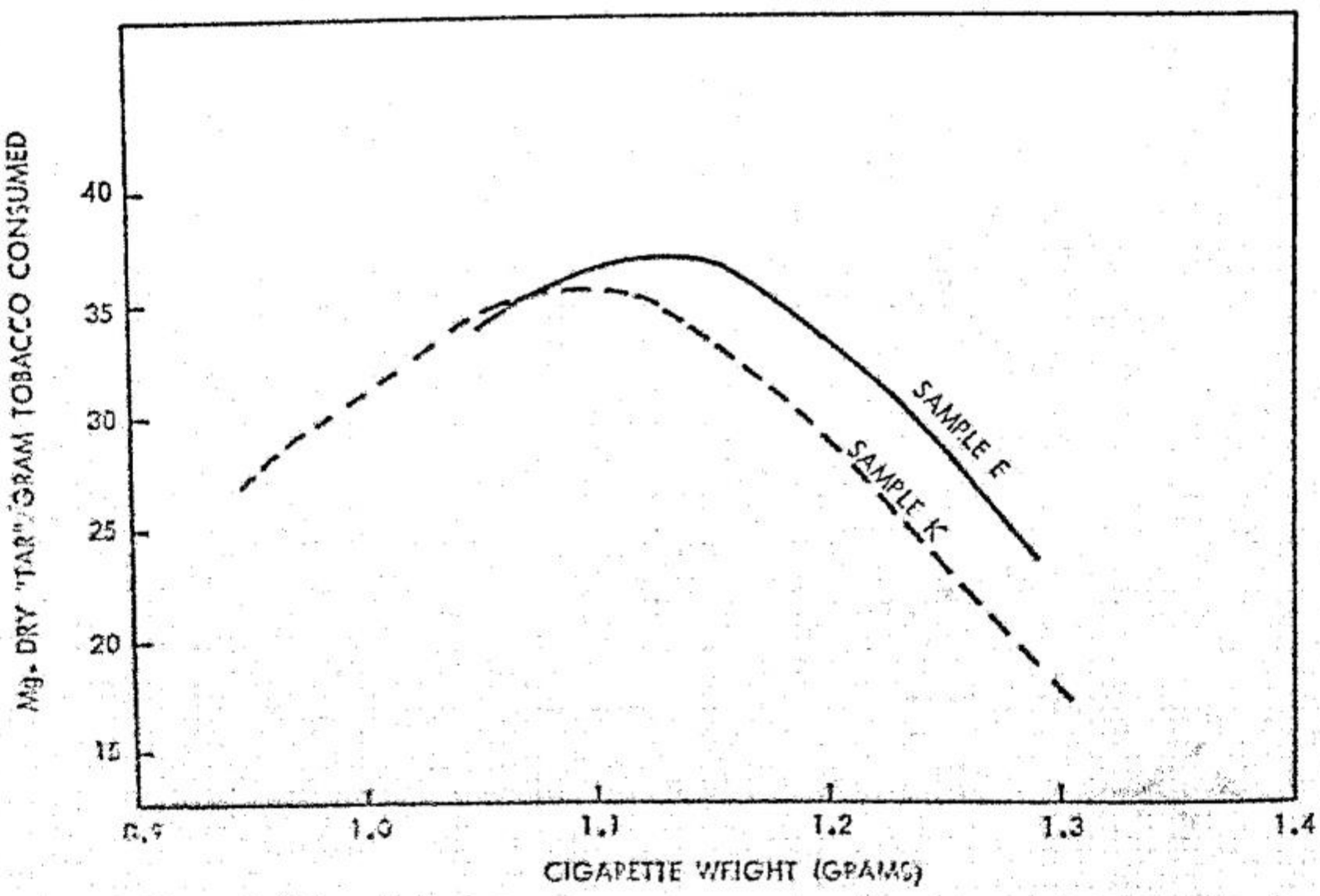
TEXT-FIGURE 1.—Cigarette weight versus pressure drop relationship for one blend of uncased natural tobaccos.

pressure drops 0.2–0.4 inches water higher than those of Sample E (70/30 Bright leaf/stem; 2.26% nicotine content) at comparable cigarette weights. Experience with natural Bright tobaccos indicated that this is probably as great a difference in the pressure-drop-versus-weight characteristic as would be encountered between 2 different lots of the same tobacco blend.

The variability of particulate matter ("tar") yield between natural-tobacco control Samples E and K was determined. For each sample, the particulate matter was determined on a number of different weight groups. Results for particulate matter were corrected to milligrams of dry particulate matter "tar"/gram of tobacco consumed, in which form all results of this type will be reported.

The results for particulate matter as a function of cigarette weight for Samples E and K are plotted in text-figure 2. The particulate matter at first increased with cigarette weight, then reached a peak, and decreased with further increases in cigarette weight. This appears to occur in most weight-versus-particulate-matter plots reported herein. Possibly, as cigarette weight increased, the particulate matter delivery in the mainstream smoke resulted from the two opposing factors. The first factor may be poorer burning due to the increased packing of tobacco shreds which results in higher "tars." The second factor may be a filtration effect by the butt, which increases with increasing weight and causes less particulate matter in the mainstream smoke. This hypothesis suggests that, as cigarette weight is increased, poorer burning is initially the dominant factor until a weight is reached at which greater filtration becomes more important.

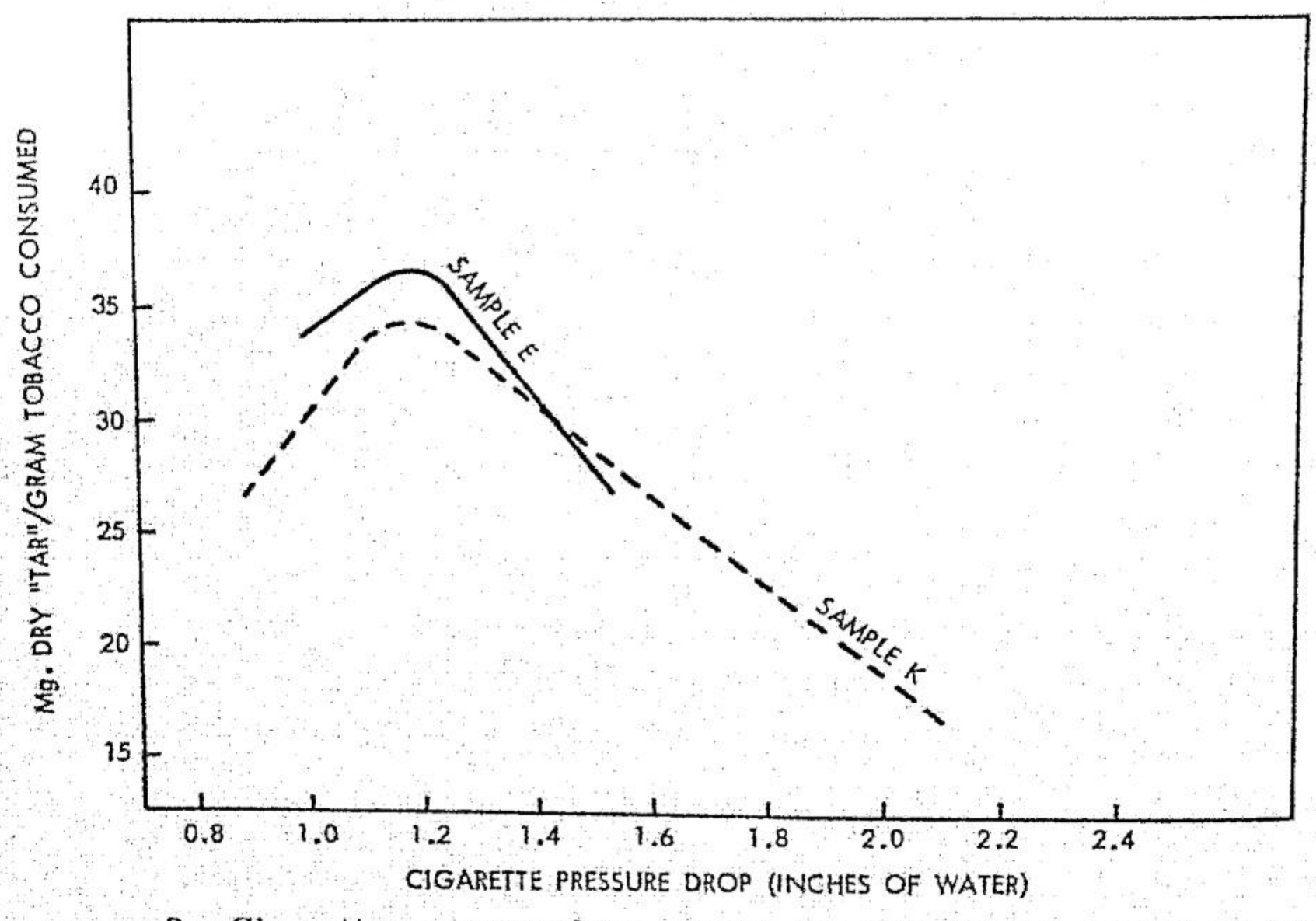
In text-figure 2, it is seen that dry particulate matter varied by as much as 5 mg between Samples E and K at comparable cigarette weights.



TEXT-FIGURE 2.—Cigarette weight versus dry "tar"/gram consumed for one blend of uncased natural tobaccos.

TOWARD A LESS HARMFUL CIGARETTE

However, part of this difference was due to the fact that the pressure drops of the 2 samples were somewhat different, at comparable weights, as was seen in text-figure 1. Therefore, to separate that part of the difference in "tar" delivery due to pressure drop differences, one may plot dry particulate matter versus pressure drop, as is shown in text-figure 3. The maximum difference in "tar" yield between Samples E and K then would be only 3 mg, which may be considered the maximum variability between the 2 lots of this particular blend after differences in pressure drop have been eliminated.

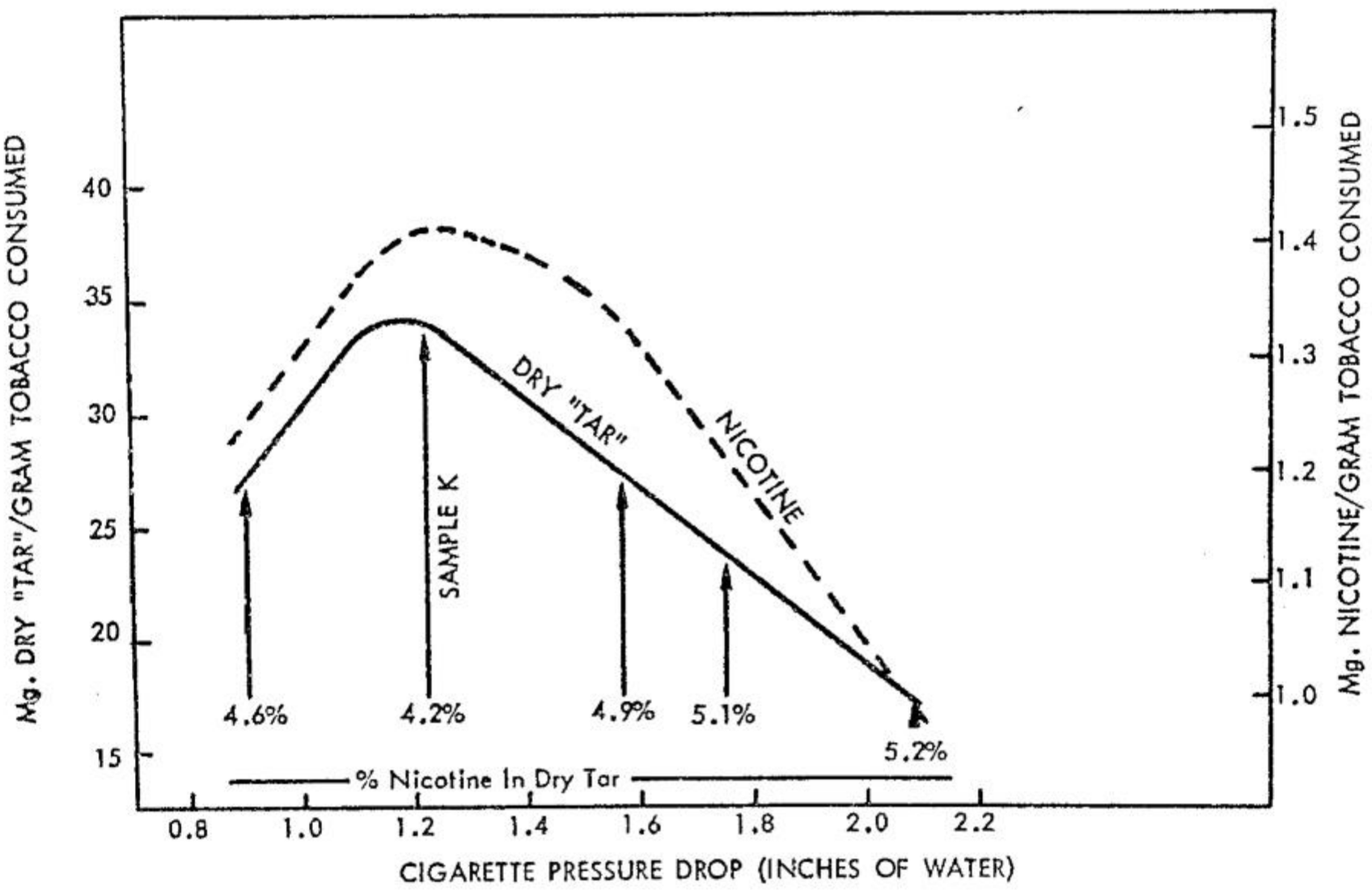


TEXT-FIGURE 3.—Cigarette pressure drop versus dry "tar"/gram consumed for one blend of uncased natural tobaccos.

The values for nicotine per gram of tobacco consumed were determined on each of the weight-sorted groups, and plotted in text-figure 4 for Sample K. The shape of the nicotine-pressure-drop curve is similar to that for particulate matter. An important factor illustrated in text-figure 4 is that the nicotine content of "tar" holds fairly constant as the pressure drop varies, despite the fact that the nicotine and "tar" yields are changing substantially. Although quantitative reductions in nicotine yield can be accomplished strictly by increasing the pressure drop, if a selective nicotine reduction has occurred, it must be evidenced by a decrease in the percentage of nicotine in the particulate matter.

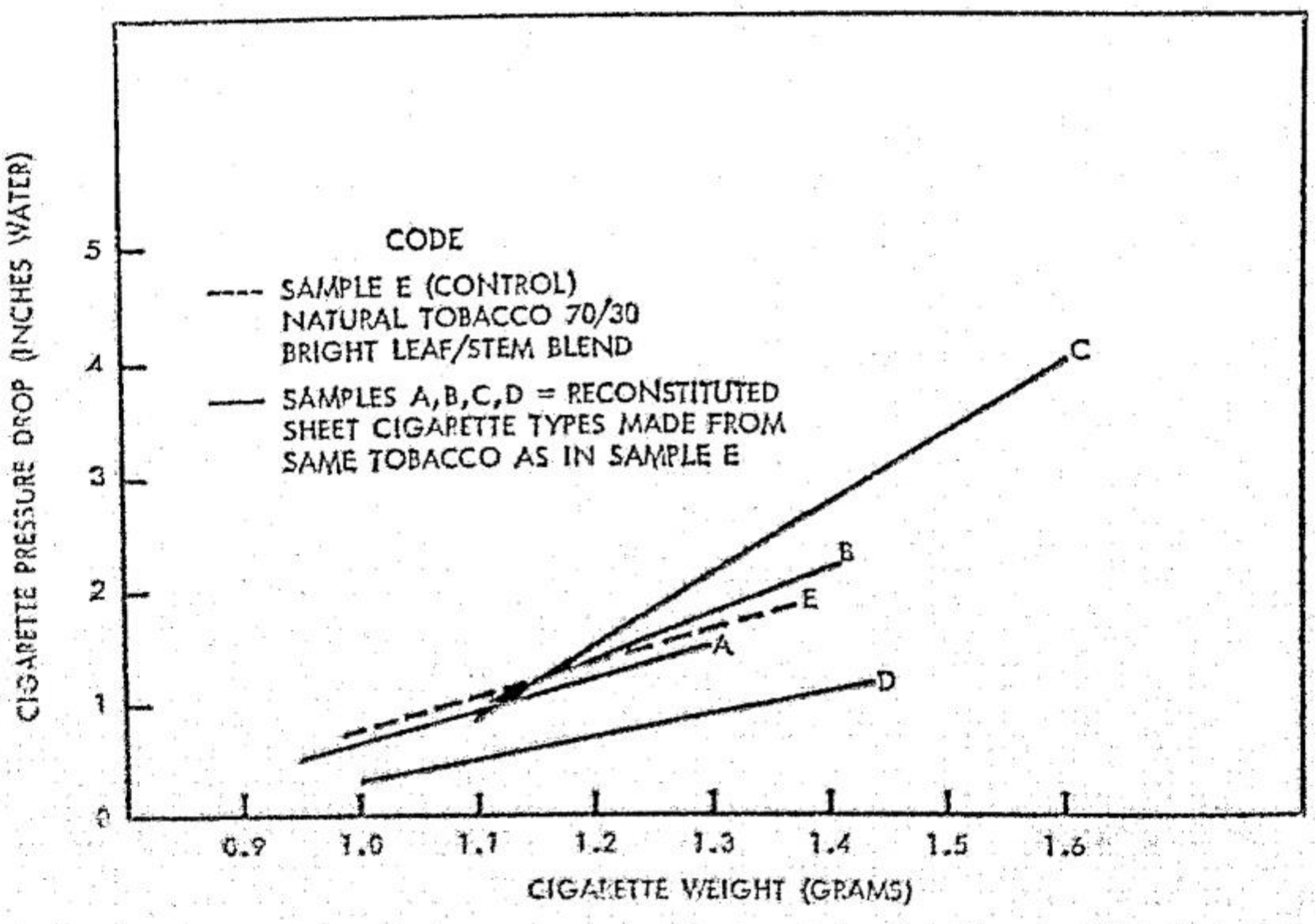
Variability of Reconstituted Tobacco Leaf

The variability in physical and combustion properties occurred upon conversion of the 2 natural-tobacco lots used in controls E and K into 8 types of reconstituted tobacco leaf (4 types from the tobacco used in Sample E and 4 types from that used in Sample K).

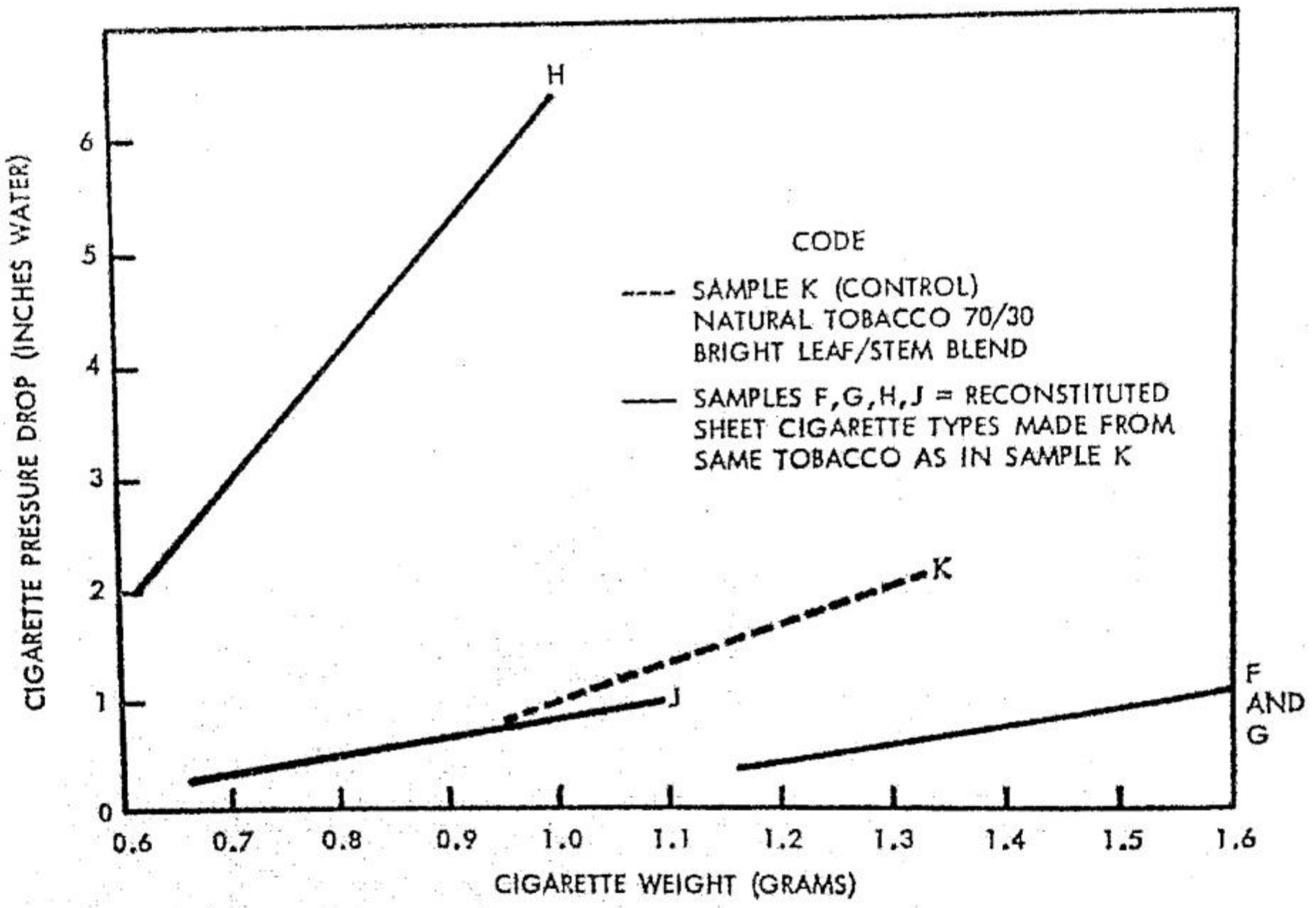


TEXT-FIGURE 4.—Nicotine-"tar" relationship as a function of pressure drop for one blend of uncased natural tobacco.

The characteristics of cigarette-weight-versus-pressure-drop for reconstituted Samples A, B, C, and D and their natural-leaf control, Sample E, are plotted in text-figure 5. The reconstituted sheet samples may have a pressure drop which is either higher or lower than the control tobacco at constant weight. The reconstituted sheets varied in pressure drop, at a given cigarette weight, to a greater degree than was observed previously in the 2 lots of natural-leaf tobacco cigarettes. The pressure drops of recon-

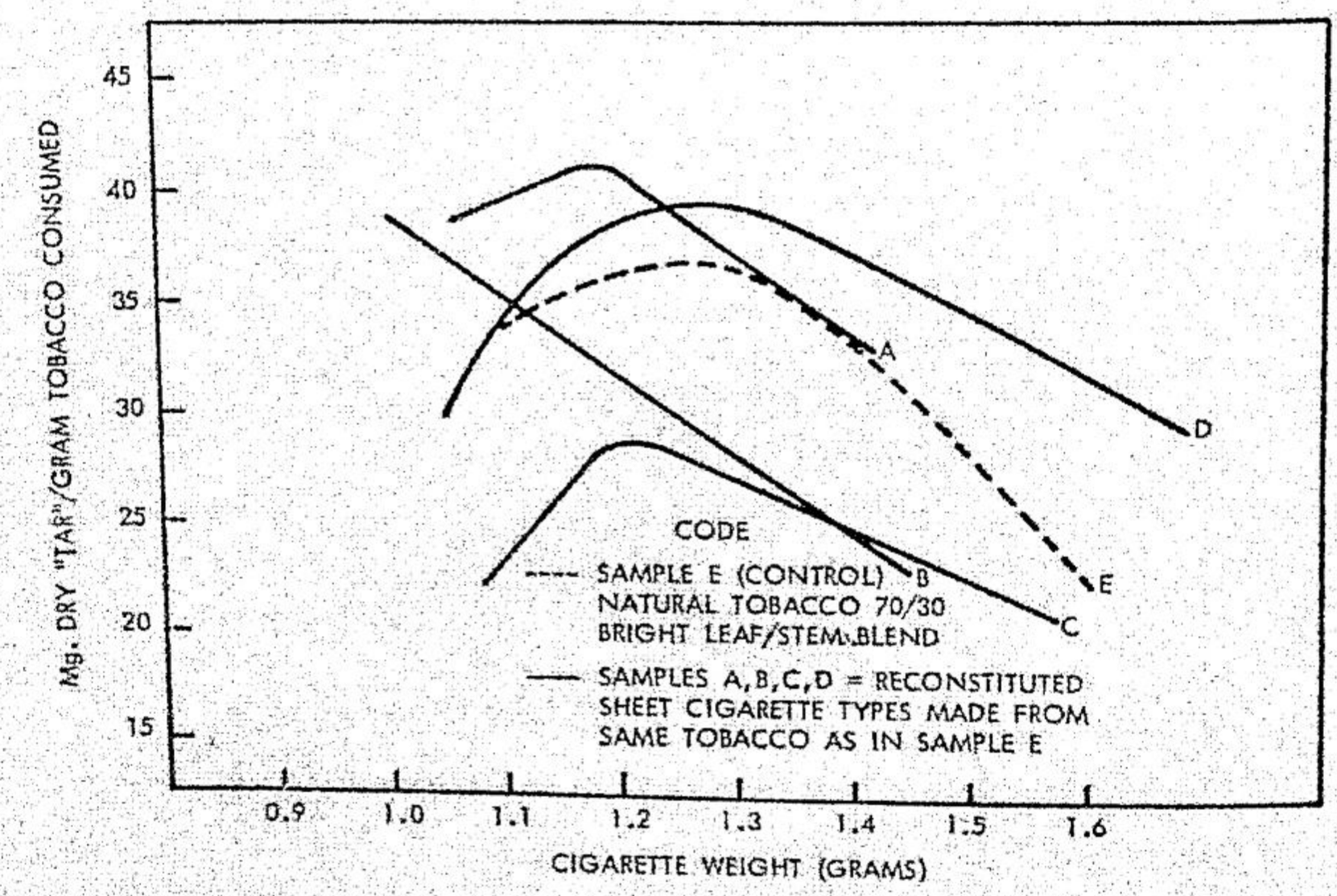


TEXT-FIGURE 5.—Cigarette weight versus pressure drop for reconstituted sheet cigarettes in Samples A–E.

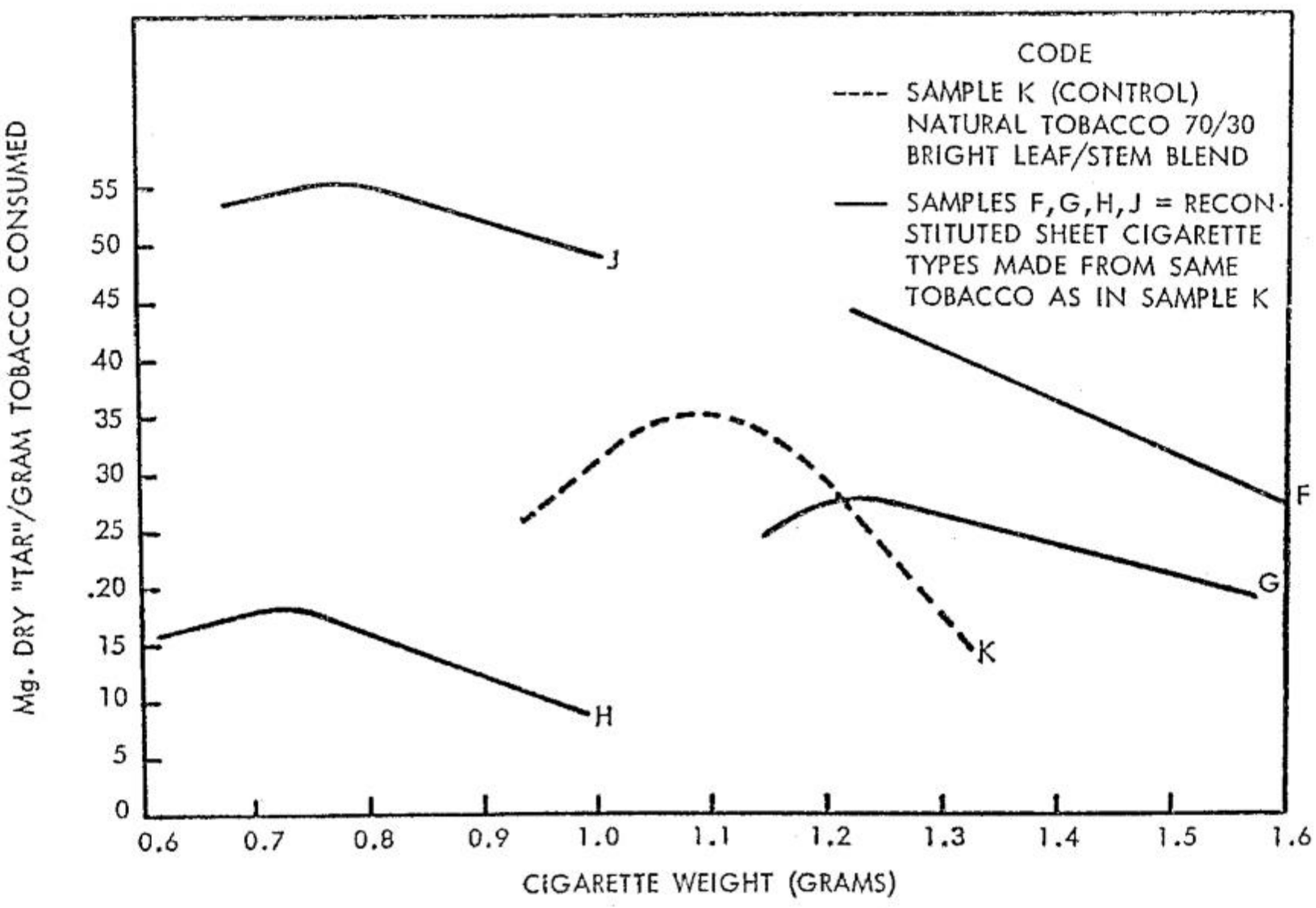


TEXT-FIGURE 6.—Cigarette weight versus pressure drop for reconstituted sheet cigarettes in Samples F–H, J, and K.

stituted-tobacco-sheet cigarettes C and D varied by as much as 1.8 inches of water, as compared to the 0.4 inch maximum pressure-drop differential observed for Samples E and K. More pronounced differences in pressure drop between reconstituted-tobacco-sheet cigarettes are apparent in text-figure 6, which shows the relationship of cigarette weight and pressure drop for reconstituted-tobacco-leaf Samples F, G, H, and J with their natural-tobacco control, Sample K. The maximum pressure-drop differ-



TEXT-FIGURE 7.—Cigarette weight versus dry "tar"/gram consumed for reconstituted sheet cigarettes in Samples A–E.



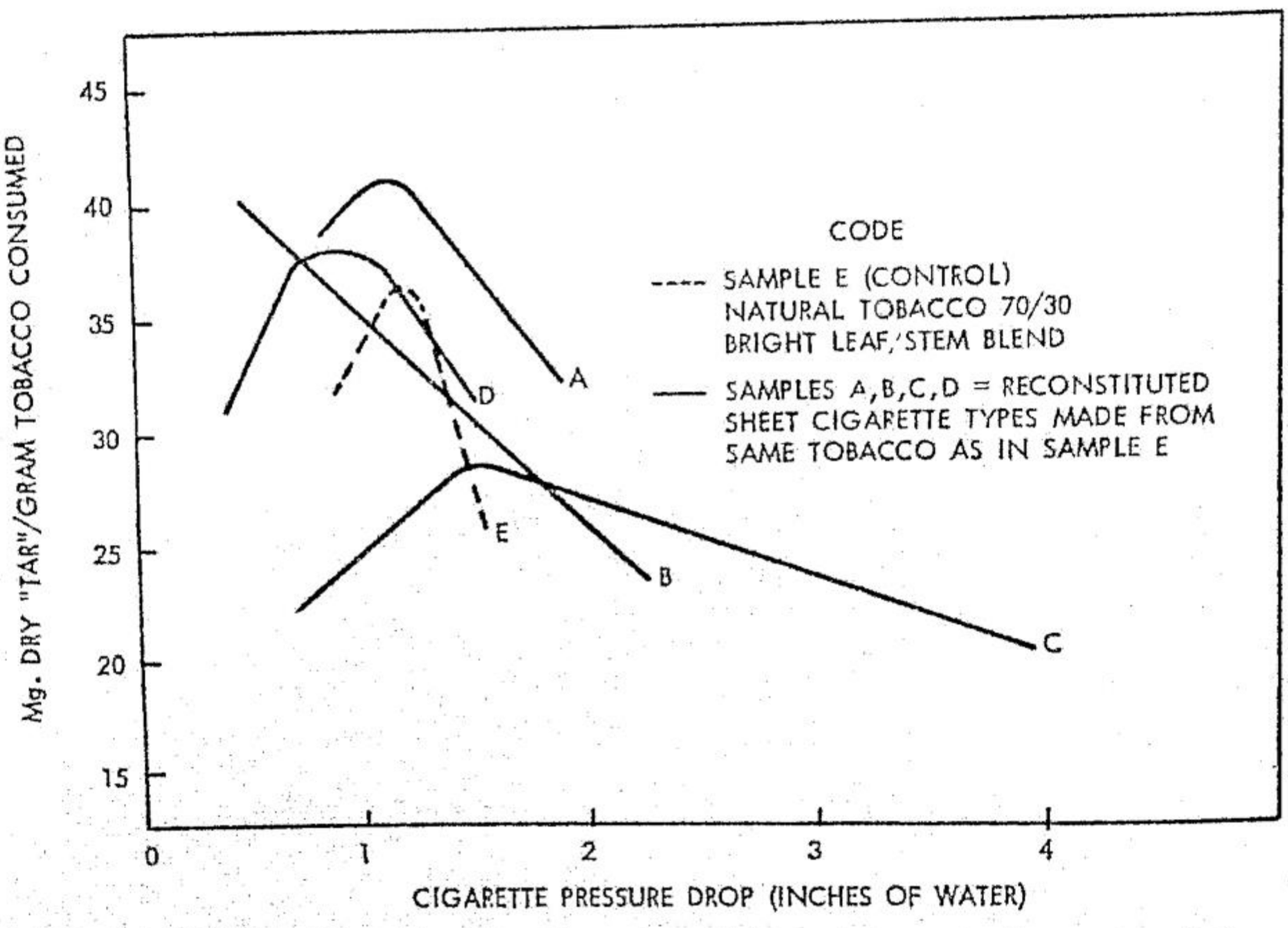
TEXT-FIGURE 8.—Cigarette weight versus dry "tar"/gram consumed for reconstituted sheet cigarettes in Samples F–H, J, and K.

ential was between reconstituted-leaf-Samples H and J, and was 5.6 inches water. One may then conclude that the relationship between cigarette weight and pressure drop can be varied over a wide range by reconstituted sheet technology, as compared to the range obtained with the natural-tobacco counterparts.

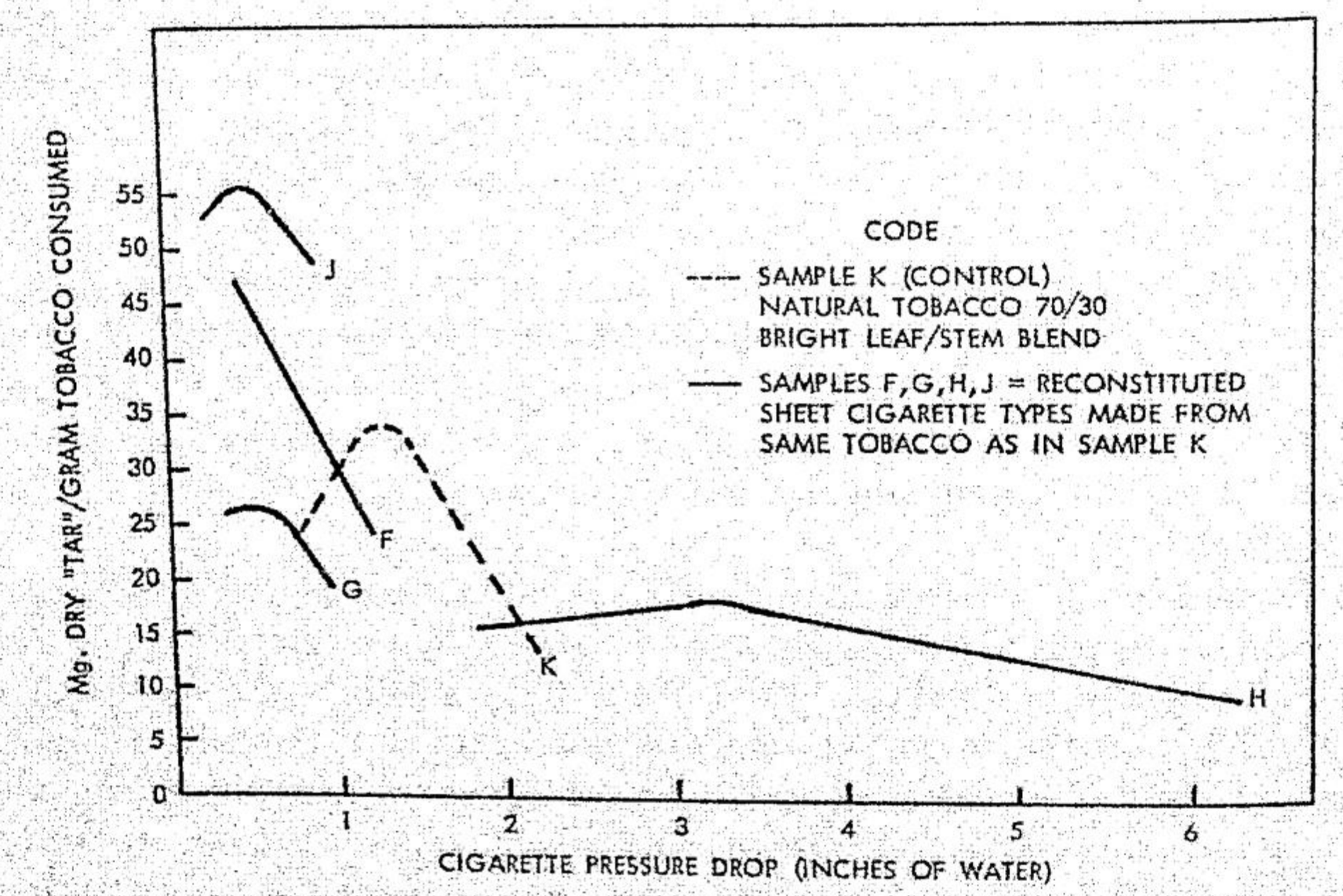
The relationship between cigarette weight and particulate matter of the 8 reconstituted-tobacco-leaf cigarettes and their 2 controls is shown in text-figures 7 and 8. As compared to the variability of 5 mg in particulate matter, at constant cigarette weight, between natural-tobacco controls E and K, the reconstituted-tobacco-leaf types varied up to 37 mg in particulate matter.

To eliminate the possible contribution of differences in pressure drop to the yield of particulate matter, the particulate-matter-versus-pressure drop for Samples A–E were plotted in text-figure 9 and for Samples F–H J, and K in text-figure 10. At constant pressure drop, Samples G and J varied in yield of particulate matter by 30 mg/g tobacco consumed. The reconstituted-tobacco-leaf samples may yield either higher or lower amounts of particulate matter than their natural-leaf controls. Table 1 compares the "tar" yield of Samples A–E at a constant pressure drop of 1.3 inches water. One may conclude that reconstituted sheet technology offers a means of varying particulate matter yield over a wide range, independent of the contribution caused by pressure-drop differentials.

The nicotine in smoke from reconstituted tobacco was plotted versus weight and pressure drop (text-fig. 11) for one reconstituted sheet, Sample F. As in text-figure 4, for the natural-leaf control, the nicotine yield on combustion of this reconstituted sheet cigarette closely followed the shape of the "tar"-yield curve, falling off substantially with increasing weight or



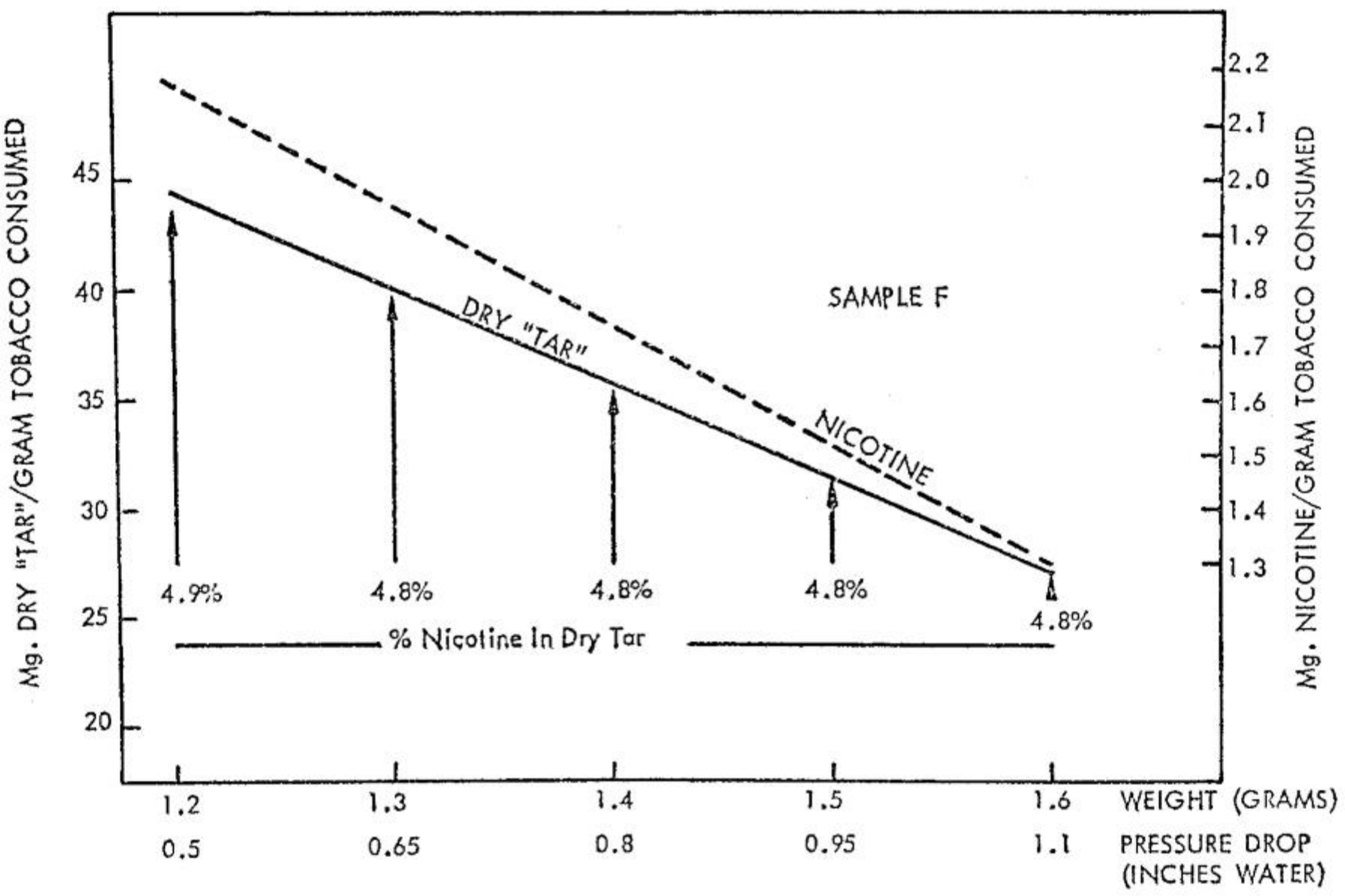
TEXT-FIGURE 9.—Cigarette pressure drop versus dry "tar"/gram consumed for reconstituted sheet cigarettes in Samples A–E.



TEXT-FIGURE 10.—Cigarette pressure drop versus dry "tar"/gram consumed for reconstituted sheet cigarettes in Samples F–H, J, and K.

TABLE 1.—Comparison of cigarettes of equal pressure drop (1.3 inches)

Sample No.	Cigarette wt. (g)	Dry "tar" gram consumed (mg)
A	1.23	39.9
B	1.18	32.4
C	1.17	27.3
D	1.50	34.5
E	1.18	35.5



TEXT-FIGURE 11.—Nicotine-"tar" relationship as a function of weight or pressure drop for one type of reconstituted tobacco sheet.

pressure drop. The percentage of nicotine in the "tar," however, remained quite uniform over the entire weight range, indicating that the reduction of nicotine as a function of increasing cigarette weight was not selective. The average nicotine content of the dry "tars" from the reconstituted-tobacco-leaf and control cigarettes, Sample A–H, J, and K, is listed in table 2. The "tars" of Samples A–E are generally higher in nicotine content than the "tars" of Samples F–H, J, and K, due to the higher nicotine content of the tobacco blend in the former series.

TABLE 2.—Average percentage of nicotine in dry "tar" for reconstituted tobacco sheets

Sample No.	Average % nicotine in dry "tar"
A	5.7
B	6.2
C	4.3
D	5.9
E (Natural-tobacco control)	6.2
F	4.8
G	4.1
H	2.6
J	2.3
K (Natural-tobacco control)	4.8

Although samples H and J appeared to have significantly reduced nicotine content in the "tars," each contained 20% of nontobacco additives, and, accordingly, the nicotine content in the reconstituted sheet was 20% lower than that of the control, Sample K. However, since the reductions in nicotine in the "tars" from Samples H and J were both in the range of 50%

TABLE 3.—Percentage of consumed nicotine of tobacco in mainstream smoke

	A	B	D*	E (control)	F	G*	H*	J*	K (control)
% nicotine in natural tobacco (containing 12% moisture) used to make reconstituted sheets and controls	2.01	2.01	2.01	2.01	1.20	1.20	1.20	1.20	1.20
% nicotine in reconstituted sheets and controls (containing 12% moisture)	1.90	2.04	2.02	2.01	1.17	1.07	0.95	0.95	1.20
Comparisons at pressure drop of 1.3 inches water									
Cigarette weight (g)	1.23	1.18	1.50	1.18	1.70	—	—	—	1.09
mg nicotine in 47 mm of cigarette length available for combustion	15.7	16.2	20.3	15.9	13.3	—	—	—	8.8
mg nicotine/cigarette in mainstream smoke	1.9	1.6	2.1	1.7	1.2	—	—	—	1.2
% of consumed nicotine of tobacco in mainstream smoke	12.1	9.9	10.3	10.7	9.0	—	—	—	13.6
Comparisons at pressure drop of 2.0 Inches water									
Cigarette weight (g)	—	—	—	—	—	—	0.62	—	1.30
mg nicotine in 47 mm of cigarette length available for combustion	—	—	—	—	—	—	4.0	—	10.5
mg nicotine/cigarette in mainstream smoke	—	—	—	—	—	—	0.17	—	0.75
% of consumed nicotine of tobacco in mainstream smoke	—	—	—	—	—	—	4.2	—	7.2

*Contained nontobacco additives.

compared to the control, probably a selective reduction in nicotine occurred, and reconstituted sheets did contribute to the selective modification of the nicotine content of smoke.

By calculating the percentage of the nicotine in the burned tobacco which appears in the mainstream smoke (*i.e.*, in the particulate matter), one may obtain additional insight. In some respects, this is a more informative figure than "percent nicotine in 'tars,'" since the latter quantity can be substantially reduced through increases in the "tar" production of the burned material as well as by actual reduction in nicotine. The percentage of nicotine in the tobacco in the mainstream smoke for Samples A, B, D–H, J, and K is calculated in table 3. The calculation is:

$$\text{Percent of consumed nicotine in mainstream smoke} = \frac{\text{mg nicotine/cigarette in mainstream smoke}}{\left\{\text{Cigarette weight (mg)}\right\}\left\{\frac{47\text{ mm}}{70\text{ mm}}\right\}\left\{\begin{matrix}\%\text{ nicotine}\\ \text{in tobacco}\end{matrix}\right\}} \times 10^4$$

The comparisons are not valid unless they are made at the same pressure drop, since the numerator in the above equation will vary with pressure drop, but the denominator (nicotine content of the portion of the cigarette which is smoked) is unaffected. Accordingly, the results are sensitive to pressure-drop differentials (table 3). When control Sample K cigarettes, with a pressure drop of 1.3 inches, were smoked, 13.6% of the consumed nicotine was in the mainstream smoke. However, when Sample K cigarettes, with a pressure drop of 2.0 inches were smoked, 7.2% of the consumed nicotine was in the mainstream smoke.

A reduction in the quantity of consumed nicotine in mainstream smoke occurred with Sample H, as compared to control Sample K with the same pressure drop of 2.0 inches water. Thus, the nicotine content of smoke must have been selectively reduced.

Modification of the Burn Rate-"Tar" Relationship

Those natural-tobacco cigarettes with faster static burn rates invariably produce lower amounts of "tar," because faster burn rates are generally associated with more complete combustion. However, as shown in table 4, a reconstituted-tobacco-sheet cigarette (Sample J) produced significantly more "tar" than the control, although it burned significantly faster than the control.

TABLE 4.—Modification of the burn rate-"tar" relationship

	Sample K	Sample J
Tobacco type	Natural-control	Reconstituted
Average cigarette weight (g)	1.07	0.99
Average pressure drop—inches water	1.1	1.0
Average puffs/cigarette	11.3	7.3
Static burn rate (mg/sec)	0.99	1.44
Dry tar:		
Per cigarette (mg)	26	33
Per g tobacco consumed (mg)	36	49

Other Smoke Analyses

Table 5 tabulates the results of analyses of the "tars" and the gaseous phase from Samples A–H, J, and K. The carbon monoxide data for the smoke of the reconstituted sheet cigarettes varied from values substantially lower than those of the controls to values somewhat higher than those of the controls. The acrolein values for the smoke from reconstituted sheet cigarettes were greater than those of their natural-tobacco-smoke control. Before attempting to draw conclusions from these data, one should consider that the results for carbon monoxide and acrolein were obtained on "average" weight and "average" pressure drop cigarettes from each experimental and control group. Accordingly, although the results may be considered average for each sample type, no mechanistic conclusions can be drawn because there was no attempt to determine or hold constant variables which may profoundly affect the results obtained. For example, although a variable pressure drop between samples will not result in a variable filtering action for the gaseous phase as it did for the particulate phase, most likely it has an effect on the completeness of combustion, which would be reflected in the carbon monoxide and acrolein levels. To determine whether basic differences in the gaseous phase from these samples exist, a study must be undertaken to determine the factors influencing the gas-phase results.

TABLE 5.—Chemical analysis of smoke*

Smoke constituent	Sample									
	A	B	C	D	E	F	G	H	J	K
	Particulate phase									
Benzo[*a*]-pyrene† (μg)	0. 020	0. 019	0. 033	0. 029	0. 047	0. 034	0. 019	—	—	—
Phenol (μg)	† 125	†112	†101	†134	† 218	150	99	10	82	153
	Gas phase									
Acrolein (μg)	144	117	117	155	73	—	—	—	—	—
Carbon monoxide (mg)	19	16	11	17	17	11	6	9	13	10

*Expressed as amount per gram of tobacco consumed.
† Values obtained by Sloan-Kettering Institute.

The results for benzo[*a*]pyrene and phenol also represent the yields from "average" cigarettes of each sample type. The values for both benzo[*a*]pyrene and phenol were generally lower for the smoke from reconstituted sheet samples than for the natural-tobacco controls.

Based on the previous discussion of nicotine yield in smoke, the data in table 5 do not reveal whether the reductions in the particulate matter constituents are nonselective, resulting from a gross reduction of the particulate phase (due to pressure-drop differences, etc.), or if they are, in fact, selective.

More meaningful conclusions may be drawn from the data in table 6. In this table, the values for benzo[*a*]pyrene and phenol per gram of tobacco

TABLE 6.—Composition of "tars"

"Tar" constituent	Sample									
	A	B	C	D	E	F	G	H	J	K
Benzo[*a*]-pyrene* (ppm)	0. 6	0. 7	1. 2	0. 9	1. 1	1. 0	0. 7	—	—	—
Phenol (%)	0. 37*	0. 40*	0. 40*	0. 40*	0. 53*	0. 41	0. 40	0. 05	0. 15	0. 39

*Results obtained at Sloan-Kettering Institute.

consumed have been divided by the total "tar" yield, so that the yield of these "tar" constituents is expressed in terms of its percentage or concentration in the "tar." Accordingly, factors affecting the total "tar" and the benzo[*a*]pyrene to the same extent are eliminated, and reductions observed may be classified as selective. It is apparent from the data in table 6 that selective reductions of up to 45% for benzo[*a*]pyrene and up to 87% for phenol were achieved with some of the experimental reconstituted tobacco leaves.

CONCLUSIONS

The utility of reconstituted-tobacco-leaf technology as a tool to obtain quantitative and qualitative modifications in tobacco smoke has been demonstrated. The possibilities, illustrated by the data presented, for gross reductions in smoke condensate as well as selective reductions of specific smoke ingredients indicate that this technology warrants expanded research. Tobacco leaf technology may be a more basic tool for smoke modification than filter technology, since changes effected during the combustion process itself could be more profound than those achieved thereafter.

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Chapter III

POSSIBLE SPECIFIC HARMFUL SUBSTANCES OTHER THAN "TAR" AND NICOTINE AND MEANS OF THEIR REDUCTION

Polynuclear aromatic hydrocarbons are regarded as the main tumor initiators in cigarette-smoke condensate. These types of chemical compounds can be reduced through the addition of nitrates to tobacco. At this time less is known about the makeup of tumor promoters though it is realized they are present in several fractions of tobacco-smoke condensate as well as in certain tobacco extracts. Nitrosamines and radioactive substances are not believed to play a significant role in experimental tobacco carcinogenesis and the role of free radicals has not as yet been fully evaluated.

Gaseous and particulate components adversely affect cilia activity in the experimental setting. Gaseous components are not believed to be carcinogenic nor is there any evidence that they affect the development of coronary artery disease. Carbon monoxide in the amounts present in cigarette smoke is not thought to adversely affect myocardial infarction. Certain types of gaseous components can be selectively removed from tobacco smoke through filtration.

Selective Reduction of the Tumorigenicity of Tobacco Smoke. Experimental Approaches [1]

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LABORATORY investigations throughout the world have proved the carcinogenicity of tobacco-smoke condensate to a variety of animal species and tissues (*1*, *2*). Thus, the chemical nature of carcinogens and possible co-carcinogens in tobacco "tar" [2] had to be determined. Identification of these tumorigenic agents should lead to measures for their reduction, and thus to lowering the tumorigenicity of tobacco smoke. This communication discusses data which have led to and may be extended further toward a reduction of the tumorigenicity of tobacco products.

CARCINOGENS AND TUMOR INITIATORS

Identification

The method of fractionation of tobacco-smoke condensate and results of bioassays on mouse skin are outlined in text-figures 1 and 2. Of the 5 major "tar" fractions, only the neutral portion is carcinogenic in concentrations of 10, 25, and 50% (*3–6*). This was reconfirmed by a recent large-scale study of the Tobacco Research Council (*7*).

On the basis of our early observations we partitioned the neutral portion by column chromatography into 6 fractions, and, in a subsequent experiment, into 4 fractions (*3*, *5*). In both cases, fraction B, about 2% of the whole dry condensate, was the only fraction to produce a significant number of cancers. Subsequently, B was further divided into 3 parts, of which only BI, which contained all polynuclear aromatic hydrocarbons (PAH),

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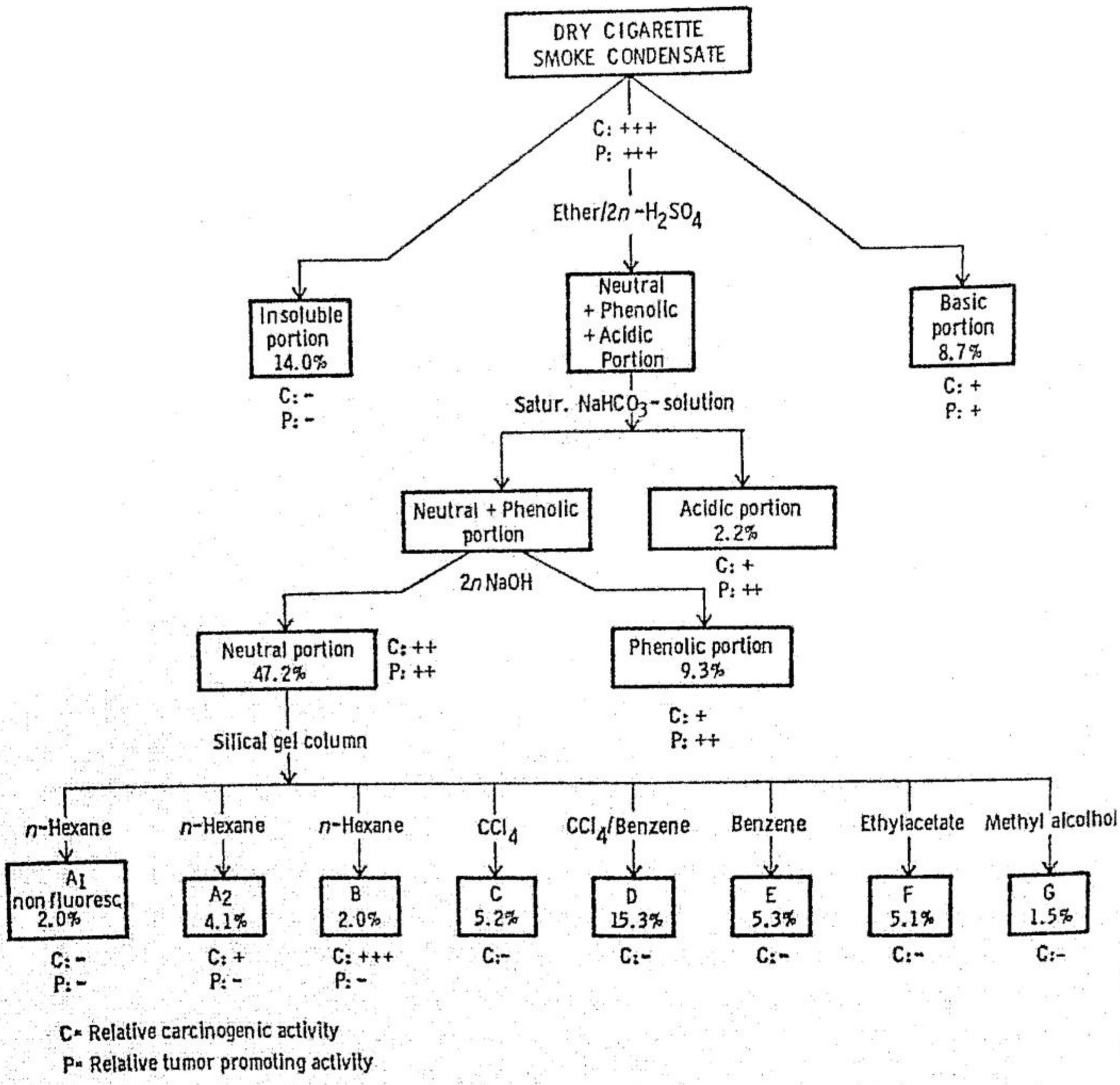
[1] These studies were supported by grant E-231 from the American Cancer Society, Inc., and since 1966, in part by Public Health Service grant CA 08748 from the National Cancer Institute.

[2] The term "tar" is used throughout for convenience, although it is not chemically accurate in the strictest sense.

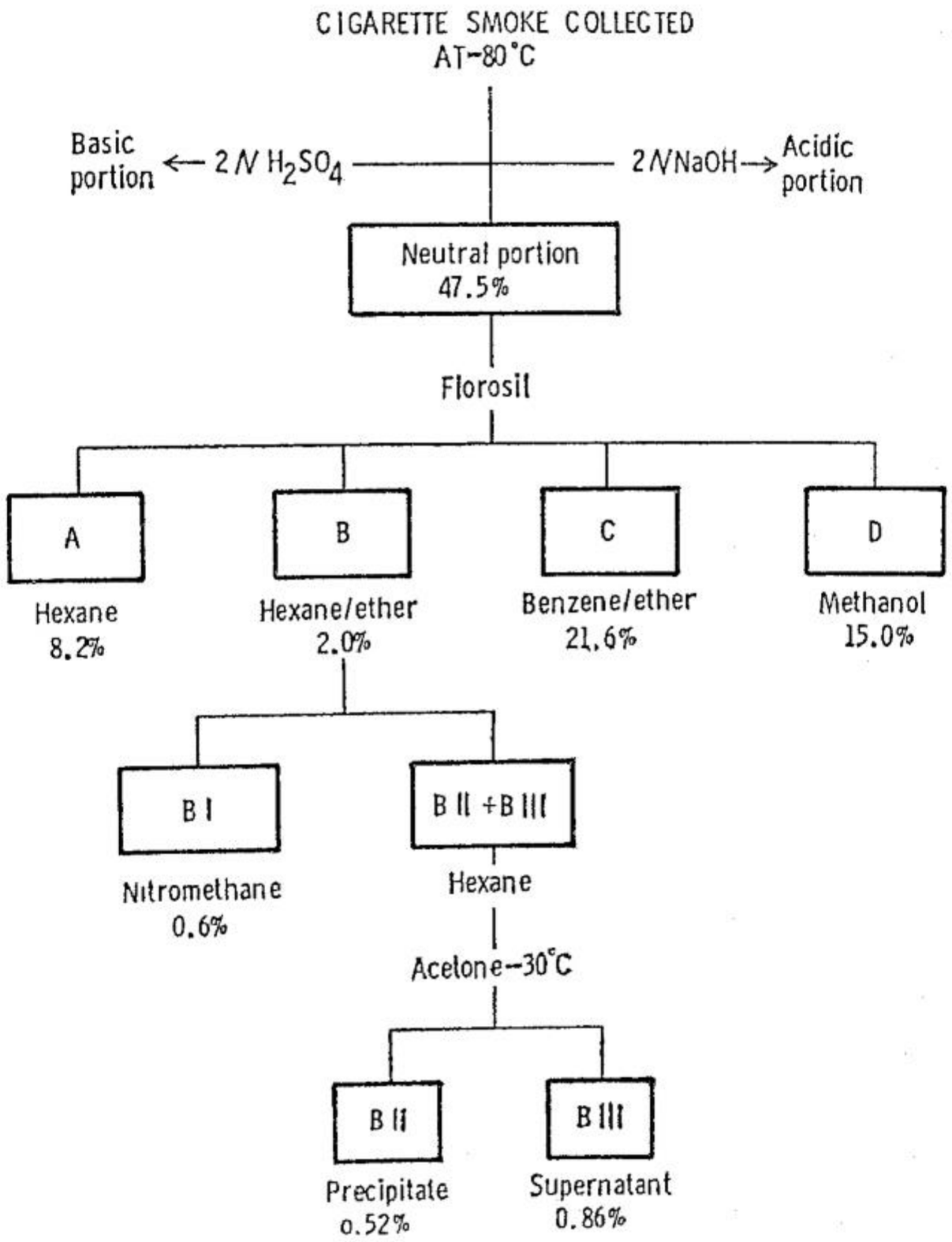


TEXT-FIGURE 1.—Fractionation scheme I for cigarette-smoke condensate and summary results of bioassays on mouse skin (*1*).

proved active in 5 and 10% concentrations. About 0.6% of the dry "tar" was BI.

To assay their possible tumor-initiating activity, the neutral fractions A, BI, BII, BIII, C, and D were applied every other day to mouse skin in 10 doses of 2.5 mg each. The initiated area was subsequently painted with 2.5% croton oil as tumor promoter. Again, only BI was active as a tumor initiator (*5*). To assay the initiating activity of BI, which is probably due to its PAH content, we compared its tumor yield with that obtained on mouse skin initiated with known doses of benzo[*a*]pyrene (BaP). A comparison with 50, 100, and 200 μg BaP as initiator, promoted by 2.5% croton oil, shows that the tumor-initiating potential of BI exceeds the activity expected on the basis of its BaP content (5 μg) by at least 20 times.

Table 1 summarizes the presently identified constituents of BI. This table lists 36 PAH, 10 N-heterocyclic hydrocarbons, 2 insecticides, and some of their pyrolysis products. The remainder of BI is made up of esters, traces of paraffins, olefins, terpenes, quinones, and weakly polaric



TEXT-FIGURE 2.—Fractionation scheme II of cigarette-smoke condensate (*2, 5*).

carbonyl compounds. To our knowledge, the PAH are the major, though not the only, carcinogens in BI, and they appear to be the most important ones in terms of mouse-skin bioassays. Table 2 summarizes the amounts of PAH isolated from BI with known carcinogenic and/or tumor-initiating properties.

A synthetic admixture of 17 aromatic hydrocarbons in the concentrations corresponding to those applied as constituents of BI was inactive as a tumor initiator (text-fig. 3). However, when the PAH admixture was applied together with 10 mg of highly purified skatol as an initiator, a significant induction of tumors was observed. Skatol is not tumorigenic by itself, but has the highest concentration (6.9%) of any component in BI (*12*). Ten mg of an indole-carbazol subfraction of BI, free of PAH, and applied together with the 17 synthetic PAH, have an even greater initiation potential than the 17 hydrocarbons in combination with skatol. These two examples of "augmentation" of the tumor-initiating activity of the PAH indicate the complexity of assaying the tumor-initiating activity of BI and that of the whole smoke condensate. Nevertheless, the data obtained support the working hypothesis that the carcinogenic hydrocarbons play an essential role in the induction of epithelial tumors by tobacco "tar."

TABLE 1.—Chemical constituents of tobacco-smoke condensate fractions BI

	Group of constituents	Number of compounds		Percent of fraction BI
		Non-carcinogen	Carcinogen	
I	Polynuclear aromatic hydrocarbons			
	Naphthalenes	5		2. 07
	Anthracenes, phenanthrenes, fluorene	7		0. 46
	Four-ring hydrocarbons	7	3	0. 21
	Five-ring hydrocarbons	4	5	0. 036
	Six-ring hydrocarbons	2	3	0. 007
II	N-Heterocyclics			2. 8
	Indole + derivatives	6		10. 8
	Carbazole + derivatives	3		0. 66
	9.9-dimethylacridane			0. 12
III	Insecticides			
	2,2-Bis(*p*-chlorophenyl)1,1-dichloroethane (DDD)			0. 54
	2,2-Bis(*p*-chlorophenyl)1-chloroethylene (DDM)			0. 03
	4,4′-Dichlorostilbene			0. 51
	2,2-Bis(*p*-chlorophenyl)1,1,1-trichloroethane (DDT)			0. 13
	2,(*o*-chlorophenyl),2(*p*-chlorophenyl)1,1-dichloroethane(*o*,*p*-DDD)			Trace
	Groups I–III 51 components			18. 4
IV	Esters			
	Di-isooctyl and *n*-alkyl-phthalates			
	Saturated and unsaturated C_{12}–C_{28}			
	Fatty acid esters of solanesol, phytol, and long-chain alcohols			
V	Paraffins C_{15}–C_{33}			Below 1
VI	Olefins			
VII	Terpenes			
VIII	Cyclic and acyclic carbonyl-components			
IX	Quinones			

The concentration of BaP as an indicator for the carcinogenic PAH in a smoke condensate is demonstrated to correlate with the tumorigenicity to mouse skin of a given "tar" (text-fig. 4). One major effort in selectively reducing the carcinogenic activity of tobacco "tar" concerns therefore the specific reduction of carcinogenic PAH.

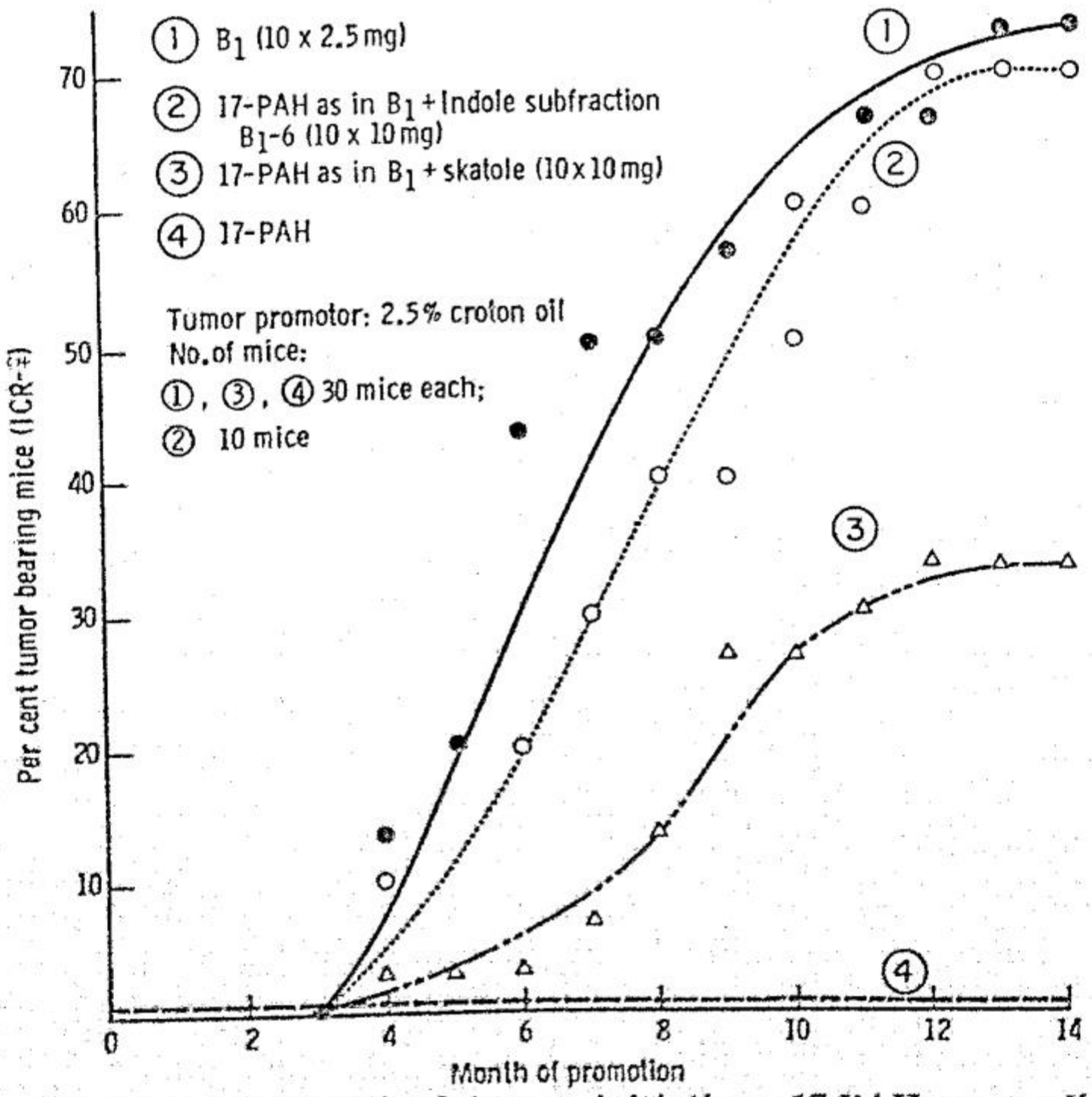
Formation of Polynuclear Aromatic Hydrocarbons

The processing of tobacco and the manufacture of cigarettes result in tobacco with only traces of polycyclic hydrocarbons. The published values for BaP vary from 3–20 parts per billion (*2*). As to be expected, the complete carcinogenic activity of tobacco extracts is also quite low (*2*, *3*).

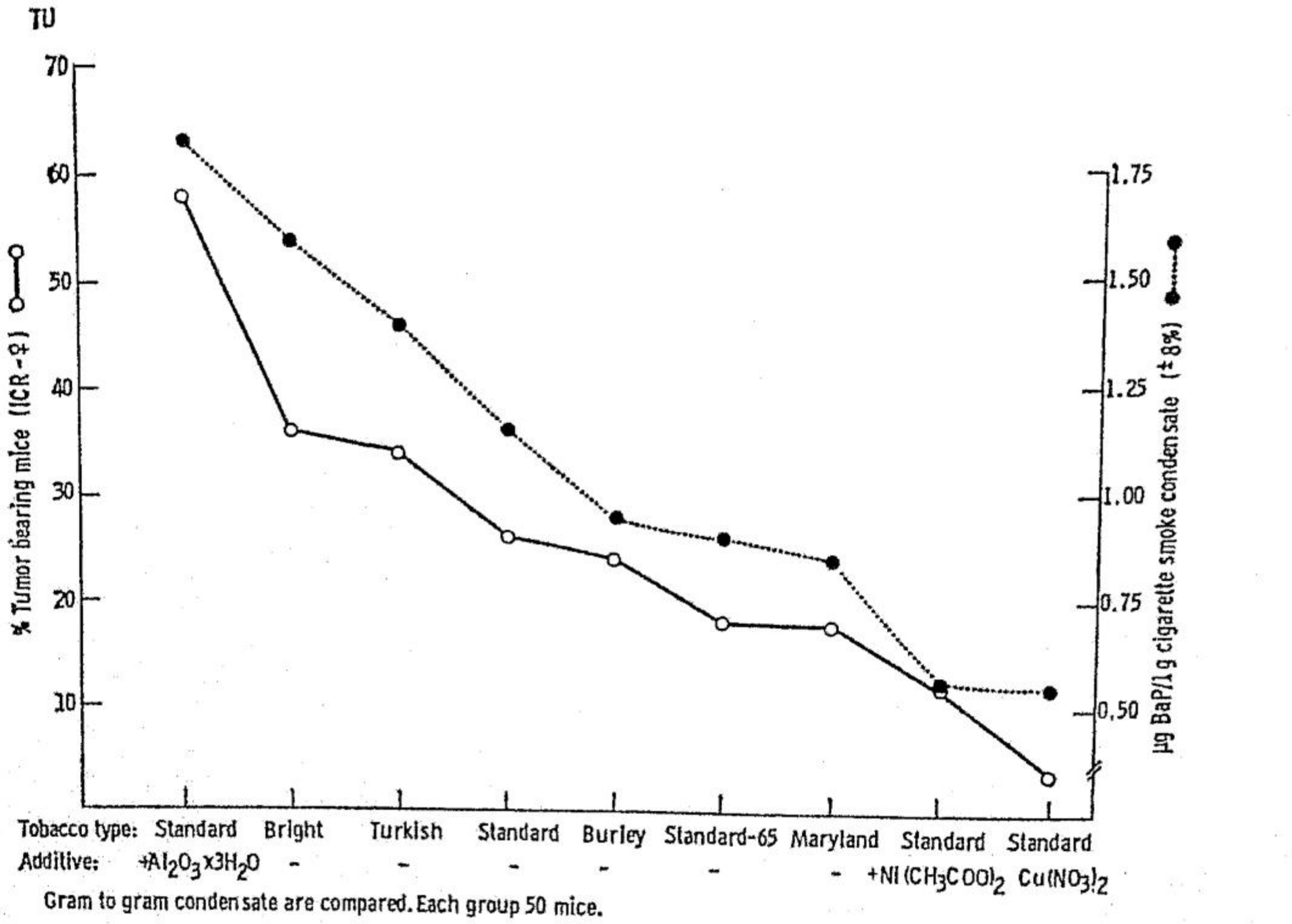
TABLE 2.—Tumor-initiating polynuclear aromatic hydrocarbons in cigarette smoke*

PAH	Isolated from smoke of 100 cigarettes (μg)	Content in fraction BI (%)	Relative carcinogenicity on mouse skin*	Tumor initiator†
Benzo [*a*] pyrene	3. 0–4. 0‡	0. 02‡	+++	+ (*8, 9*)
Dibenz [*a,h*] anthracene	0. 4	0. 004	++	+ (*2*)
Dibenzo [*a,e*] fluoranthene§	Traces	0. 001	++	+ (*8*)
Dibenzo [*a,i*] pyrene	Traces	—	++	+ (*8*)
Benzo [*b*] fluoranthene	0. 3	0. 004	++	+ (*9*)
Benzo [*j*] fluoranthene	0. 6	0. 01	++	?
Indeno [1,2,3,-*cd*] pyrene	0. 4	0. 003	+	+ (*9*)
Benz [*a*] anthracene	0. 3	0. 002	+	+ (*10*)
Benzo [*a*] phenanthrene	Traces	Traces	+	?
Chrysene	6. 0	0. 05	+	+ (*9*)

*See (2).
†Numbers in parentheses are references.
‡Quantitative value.
§Originally considered to be dibenzo (*a*, 1) pyrene (*11*).



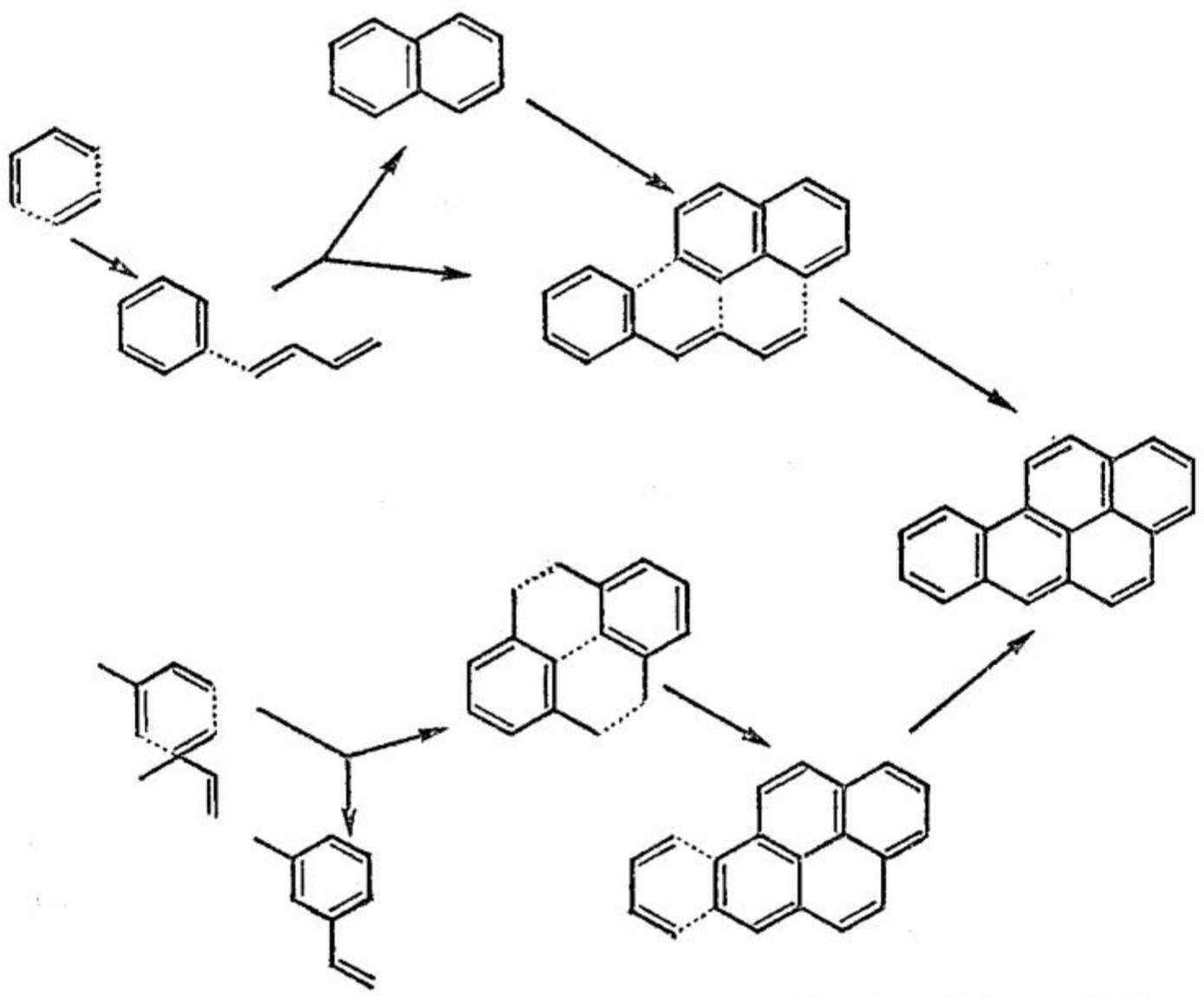
TEXT-FIGURE 3.—"Augmentation" of tumor initiation—17-PAH as applied with 10 initiator doses (in μg): BaP, 5: dibenz[*a,h*]anthracene, 1; benzo[*b*]fluoranthene, 1; benzo[*j*]fluoranthene, 2.5: benz[*a*]anthracene, 0.5; indeno[1,2,3-*cd*]pyrene, 0.75; chrysene, 1.25; benzo[*e*]pyrene, 0.4; benzo[*ghi*]pyrene, 2.2; perylene, 1.0; fluoranthene, 41; benzo [*mno*]fluoranthene, 0.4: benzo[*k*]fluoranthene, 1.2; pyrene, 41; 1-methylpyrene, 5: 3-methylpyrene, 5; 4-methylpyrene, 5; 11*H*-benzo[*b*]fluorene, 3.



TEXT-FIGURE 4.—Tumor response on mouse skin and BaP in cigarette mainstream smoke condensate.

The PAH in tobacco are for the most part pyrolyzed; only a minor amount appears unchanged in the smoke. We estimate that between 2 and 4% of PAH in the smoke may be attributed to material that persists during the combustion. Bentley and Burgan estimated this figure to be about 8% (*13*). Thus, most carcinogenic PAH are formed during the burning of tobacco.

The burning cone of a cigarette creates a reducing atmosphere (*14*) and has been reported to have peak temperatures between 700 and 900° C (*2*). According to Badger, these temperatures represent a decisive environmental factor for the pyrosynthesis of PAH from organic matter in an inert or reducing atmosphere (*15*, *16*). All organic constituents that are not removed from the hot zone and burning cone by distillation and/or sublimation will therefore have a potential for forming PAH. The PAH pyrosynthesis apparently occurs in at least two distinct steps. First, the organic material is partially fragmentated by chemical decomposition (pyrolysis) and then a recombination (pyrosynthesis) occurs in which small molecules recouple to yield new compounds. Some of these are aromatic hydrocarbons. Text-figure 5 shows some of the most likely pathways for the pyrosynthesis of carcinogenic BaP. Logical reasoning and knowledge from other studies established that individual aromatic hydrocarbons, such as BaP, are not the only pyrosynthesized products, but that a large spectrum of aromatic compounds is formed in the process. In tobacco smoke, at least 24 aromatic hydrocarbon ring systems are known to be



TEXT-FIGURE 5.—Pathways for the pyrosynthesis of benzo[*a*]pyrene.

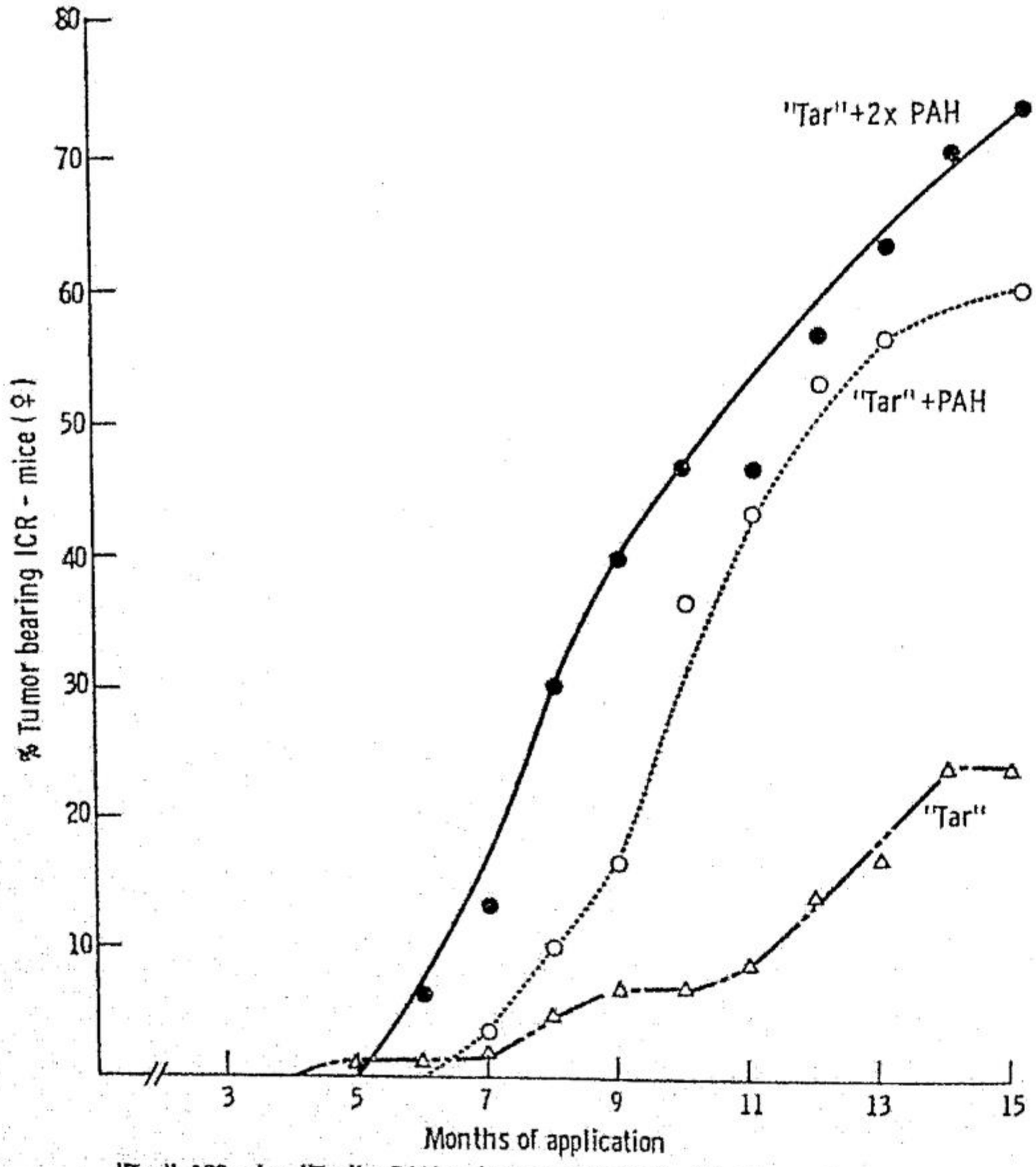
formed. Therefore, we need to stress, as before, that BaP values serve merely as indicators for the pyrolytically formed carcinogens.

In view of this, one is surprised to find in the recent literature that "BaP is an unimportant factor in the carcinogenicity of tobacco "tar" since an addition of BaP alone to the "tar" did not result in an increase of the activity of the material" (*17*, *18*). As a case in point, if one artificially doubles or triples the concentration, not only of BaP but of 17 identified PAH in the tobacco "tar," tumorigenicity increases significantly (text-fig. 6).

Reduction of PAH in Tobacco Smoke

Considering the two-stage pyroformation of carcinogenic hydrocarbons in tobacco smoke, at least three processes may cause an inhibition of the pyrosynthesis of these agents: 1) change in the temperature profile of a burning tobacco product, 2) reduction of the precursors for the pyrosynthesis of PAH, and 3) inhibition of the actual pyrosynthesis of aromatic hydrocarbons by chemical interference.

Change of temperature profile.—Although Muth suggested in 1956 that reducing or increasing the burning characteristics of tobacco would lead to a change in the tumorigenicity of the resulting tobacco "tar" (*2*), no scientific papers on the subject have appeared. The possible usefulness of such an approach can be deduced from the following data. Lam demonstrated a correlation of the pyrolysis temperature of tobacco paraffins and PAH yield (*19*): Wynder *et al.* showed a correlation between the pyrolysis temperature of tobacco hexane extract and the carcinogenicity of the resulting tars (*20*); and Ingram demonstrated *in vitro* a temperature dependence of the radical formation from organic matter (*21*). Since



"Tar" 100 mice, "Tar" + PAH and "Tar" + 2 x PAH - 30 mice each.

TEXT-FIGURE 6.—Cigarette-smoke condensate plus PAH.

organic radicals are considered actual precursors of PAH, this latter suggestion is certainly a very important aspect.

The U.S. patent literature contains at least a dozen claims of reducing the burning temperature of tobacco by additives. We concur with Stills and Stedman who regard these claims as unsubstantiated by experimental data (*22*). However, these authors confirmed a claimed reduction of the burning temperature by addition of alumina trihydrate to tobacco (*23*). In our laboratory, alumina trihydrate, though most likely of another form and added by a rather different process, effected no decrease, but a significant increase of BaP. The tumorigenicity of the resulting "tar" was likewise increased [(*4*); *see* text-fig. 4].

Reduction of precursors of PAH.—Considerable attention has been given to the reduction of the tumorigenicity of tobacco products by selective extraction. In our experience, the extraction of tobacco most often leads to a product of reduced combustibility and/or changed water-retaining power. While such secondary effects may be overcome, another consideration concerning tobacco extraction becomes more significant. Each selective extraction of tobacco should lead to a quantitative increase in cellulose, hemicellulose, and lignin, the essential matter of the cell walls of raw tobacco. Owing to their low volatilities, these polysaccharides are

relatively good precursors of PAH and phenols on incomplete combustion (*2, 24, 25*).

An earlier detailed study on the selective reduction of the tumorigenicity of tobacco-smoke condensate dealt with hexane and hot hexane extractions of tobacco (*20*). These extracts consisted mainly of waxes of the leaf cuticles that are composed essentially of paraffins, esters, and carbonyl compounds. Although the extracts amounted to only 4.5 and 5.4% of the whole tobacco weight, the "tar" reduction for both 70- and 85-mm cigarettes averaged 30%. This finding was to be expected, since significant amounts of the waxes in unextracted tobacco distill off during the smoking process. However, the yield of BaP and the tumorigenicity of the "tars" were within the experimental deviations (*20*).

A study by Mouron *et al.* (*26*) dealt with extracts of tobacco with halogenated hydrocarbons, benzene, alcohol, and petroleum ether. Methylene chloride (a) and carbon tetrachloride (b) removed matter 1.9–2.0 and 2.5–2.8% of organic matter, respectively. Cigarettes made of these extracted tobaccos were reported to have mainstream constituents reduced in the following amounts: "tar," 27% by (a) and 26% (b), aliphatic hydrocarbons 79 and 85%, anthracene 11 and 12%, pyrene 16 and 26%, and BaP 79 and 85%. Nicod (*27*) tested "tars" from methylene chloride-extracted Bright and Turkish tobaccos on mouse skin (*27*) and observed no significant reduction of tumorigenicity (*see 28*). Contrary to this finding stands the claim in a patent that this extraction method results in a reduction in carcinogens and of tumorigenicity (*29*).

The "Tobacco Smoke Committee" of Coresta then studied the extraction methods with chlorinated hydrocarbons in detail and concluded that a significant "tar" reduction can indeed be achieved by these means, although a reduction of carcinogens does not occur (*30–32*). Thus tobacco extraction has as yet failed to bring about a significant specific reduction of the tumorigenicity of tobacco-smoke condensate.

Inhibition of the actual pyrosyntheses of PAH.—The first applications of additives to tobacco or cigarette paper were mainly exploratory, since these studies did not immediately suggest mechanisms to alter the concentration of PAH in cigarette smoke (*3, 33, 34, 36–40*). Table 3 summarizes the reports on tobacco additives. Most of the data are based on irreproducible methods, as was shown by deSouza and Scherbak (*35*) in a well-designed experiment using reproducible analytical methods to prove the ineffectiveness of glycerol in respect to BaP in the smoke. The usefulness of ammonium sulfamate as additive to tobacco and cigarette paper has been debated for several years. A summary of the reported effects of this salt as additive to cigarette paper is shown in table 4. The differences of the reported data were ascribed in part to the "aging effect" by partial sublimation and/or diffusion of the ammonium sulfamate from the paper into the tobacco. This concept, however, has been disproved by the quantitative studies summarized in the last column of table 4.

TABLE 3.—Reduction of BaP in cigarette mainstream smoke by tobacco additives*

Authors	Tobacco additive		BaP in smoke (μg)†		Reduction (%)
	Name	Conc. (%)	Per 100 g tobacco‡	Per 100 cigarettes	
Bentley and Burgan (1960b)	KNO_3	2	1.95 (4.5)		57
		4	1.8 (5.5)		67
	Copper nitrate	5	1.3 (8.0)		84§
		5	1.6 (6.2)		74§
		2.5	1.3 (6.2)		79§
		1	2.2 (6.2)		64§
	$NaNO_2$	5	1.7 (6.2)		73
	Glycerol	3	2.1 (5.5)		62
	Ethylene glycol	3	2.4 (5.5)		52
	Ammonium sulfamate	4	0.4 (1.4)		71‖
			0.3 (0.6)		50‖
			0.16 (1.01)		84‖
Wynder and Hoffmann (1961a)	$Cu(NO_3)_2 \cdot 5H_2O$	5		2.0 ± 0.5 (3.8 ± 8.3)¶	45**
Wynder and Hoffmann (1963a)	$Cu(NO_3)_2 \cdot 5H_2O$	5		1.5 ± 0.1 (3.9 ± 0.3)¶	62††
	$Ni(CH_3COO)_2$	3.1		2.5 ± 0.2 (3.9 ± 0.3)¶	36††
Hoffmann and Wynder (1967)	$NaNO_3$	8.3		0.95 ± 0.12 (2.9 ± 0.23)¶	67††

*Toxic effects of the decomposition products of the additives are disregarded.
†Figures in parentheses are values for control cigarettes.
‡Isolated amounts.
§ Pyriki *et al.* (1965) report a 40% decrease, in agreement with Wynder and Hoffmann (1961a, 1963a).
‖Since the experimental variation for the control cigarette for BaP is 40%, the actual reduction may be only 30%. Pyriki *et al.* (1965) report a 65% increase in BaP with 4% ammonium sulfamate as a tobacco additive.
¶BaP determined by isotope dilution method.
**The cigarettes were smoked with 3 puffs per minute. Tumorigenicity of "tar" on mouse skin reduced.
††Tumorigenicities of "tars" on mouse skin reduced.

From the data in table 3, one may deduce that the use of nitrates offers means of reducing PAH in the smoke and at the same time a reduction of tumorigenicity of the resulting "tars." In this respect it should be recalled that the smoke of Bright and Turkish tobaccos delivers significantly more BaP and the corresponding condensates exhibit significantly higher tumorigenicities than the smoke and "tars" from Burley and Maryland tobaccos (text-fig. 4). Since the latter contain up to 5.5% nitrate, while Bright and Turkish leaves have relatively low nitrate concentrations with less than 0.5% (*41–43*), we may correlate the nitrate content of tobaccos and the tumorigenicity of corresponding condensates.

In recent years we have investigated this correlation in chemical and biological experiments. The prevailing nitrates in tobacco are alkali nitrates which decompose at temperatures above 400° C into nitrite and oxygen [I]. These nitrites are instable at the decomposition temperature of the corresponding alkali nitrates and break down further into alkali oxide, oxygen, and nitrogen oxide [II]. Nitrogen dioxide is formed by oxidation of nitrogen oxide [III]. Neurath calculated a reaction time of

TABLE 4.—Reported effect of ammonium sulfamate in cigarette paper on BaP concentrations in smoke

Authors	Ammonium sulfamate in cig. paper (%)	BaP* (μg)	Reductions (%)	Remarks
Alvord and Cardon (*36*)	4. 25	B 7.0 (17.5) B 7.75 (16.9)	60 54	Comparison based on isolated amount
Bentley and Burgan (*33*)	4. 0	A 1.5–1.6 (1.6)	—	Isolated amounts
Lindsey *et al.* (*37*)	4. 25	Not given	60	Isolated amounts
Candeli *et al.* (*38*)	4. 25	Not given	60	Isolated amounts
Cuzin *et al.* (*39*)	4. 25	B 1.0 (1.2)	—	Isolated amounts
Pyriki *et al.* (*40*)	5. 7	B 2.1 (2.0)	—	Isolated amounts; pyrene 7.5 (11.5); methylpyrene 9.0 (10.0); anthracene 10.8 (10.6)
Hoffmann and Rathkamp†	15. 7			Quantitative values; smoked after paper treatment:
		B 3.85 (2.35)	—	15 minutes
		2.95 (2.35)	—	1 day
		3.0 (2.35)	—	3 days
		2.95 (2.35)	—	1 week
		2.75 (2.35)	—	2 weeks
		2.95 (2.35)	—	4½ weeks
		2.8 (2.35)	—	6 weeks
				Quantitative phenol values were in mg: 23.5, 18.5, 16.8, 14.0, 12.9, and control, 11.0

*A = values for 100 g of tobacco. B = for 100 cigarettes. Values in parentheses = values of control cigarettes.
†Unpublished data of Hoffmann, D., and Rathkamp, G.

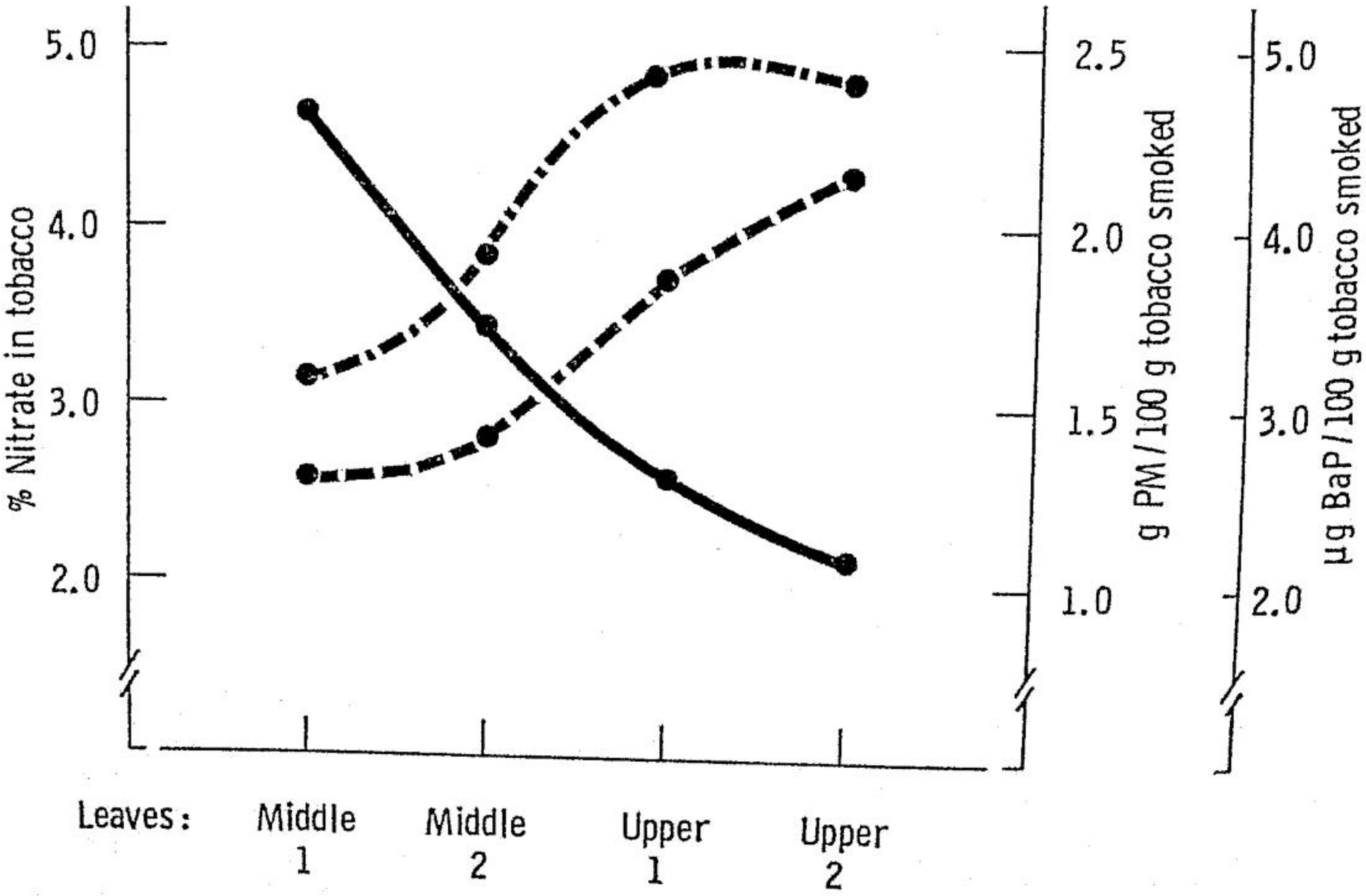
about 500 seconds for the oxidation of half the NO in tobacco smoke to NO_2 (*44*).

$$4\ NaNO_3 \xrightarrow{380^\circ\ C} 4\ NaNO_2 + 2\ O_2 \qquad [I]$$

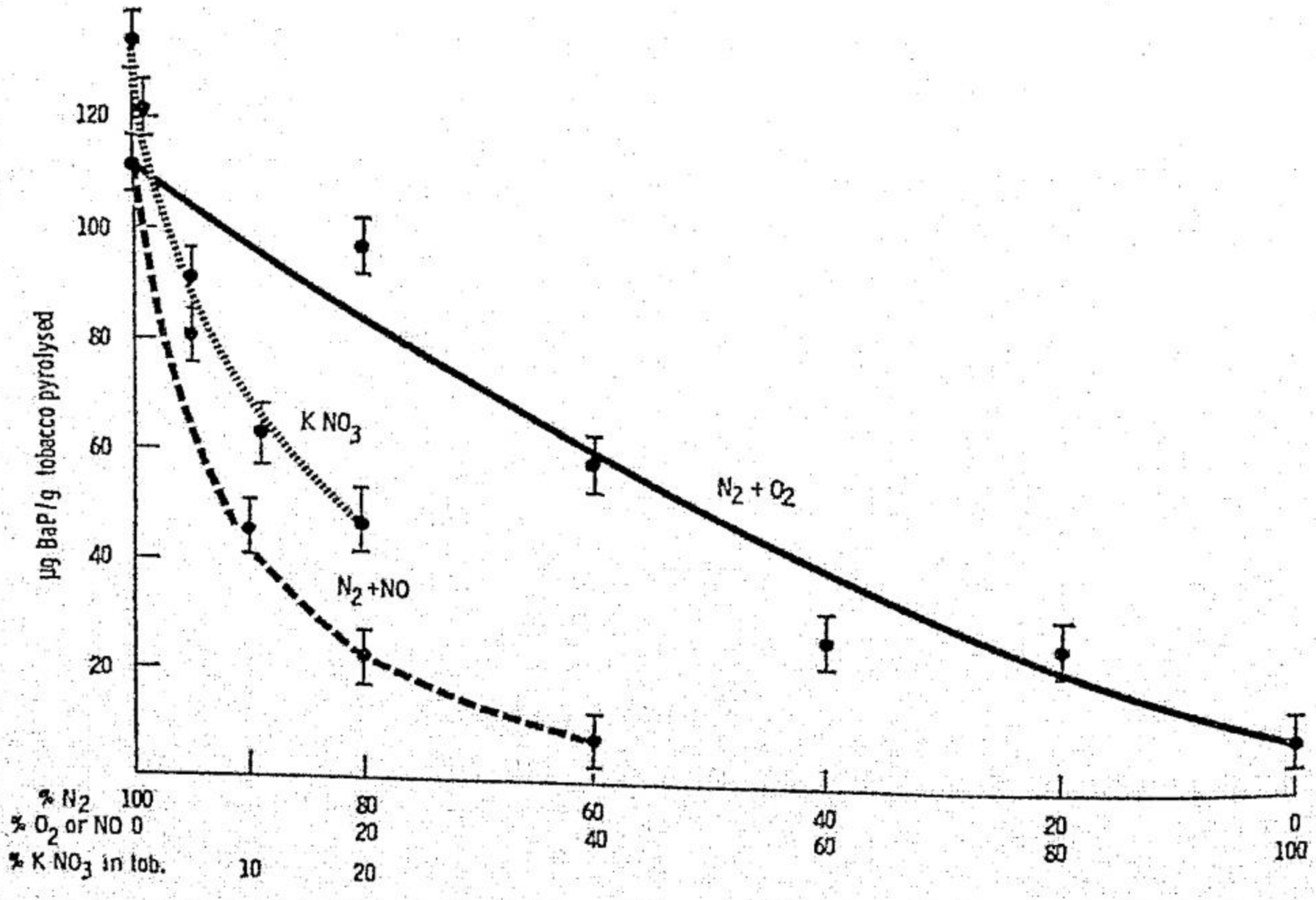
$$4\ NaNO_2 \xrightarrow{300^\circ\ C} 2\ Na_2O + 4\ NO \qquad [II]$$

$$4\ NO + 2\ O_2 \longrightarrow 4\ NO_2 \qquad [III]$$

Since, according to Eqs. [I] and [II], oxygen is formed in the hot zones of nitrate-containing tobacco, one may assume that the burning of tobacco with high nitrate is more complete than that of nitrate-free tobacco. That this indeed occurs was demonstrated by two experiments in which 4 cigarettes were made of Burley leaves which differed only in nitrate content. An increase in nitrate resulted in a higher static burning rate and, consequently, a lower number of puffs and a lower "tar" yield per puff. However, a comparison of the reduction by nitrate of the smoke constituents TPM and BaP revealed a significantly higher, and therefore selective, reduction of BaP (text-fig. 7). Nicotine appears to be also selectively



TEXT-FIGURE 7.—Burley tobacco smoke analysis. PM = particulate matter; nitrate in tobacco = •———• ; g PM/100 g tobacco smoked = •-·-·-• ; µg BaP/100 g tobacco smoked = •– – – – –•.



TEXT-FIGURE 8.—Pyrolysis of tobacco.

reduced. However, in this regard our studies have not sufficiently advanced to permit a definite conclusion.

Comparative pyrolysis (at 880 ± 10°C) of tobacco in nitrogen-oxygen admixtures and admixtures of nitrogen with nitrogen oxide (text-fig. 8)

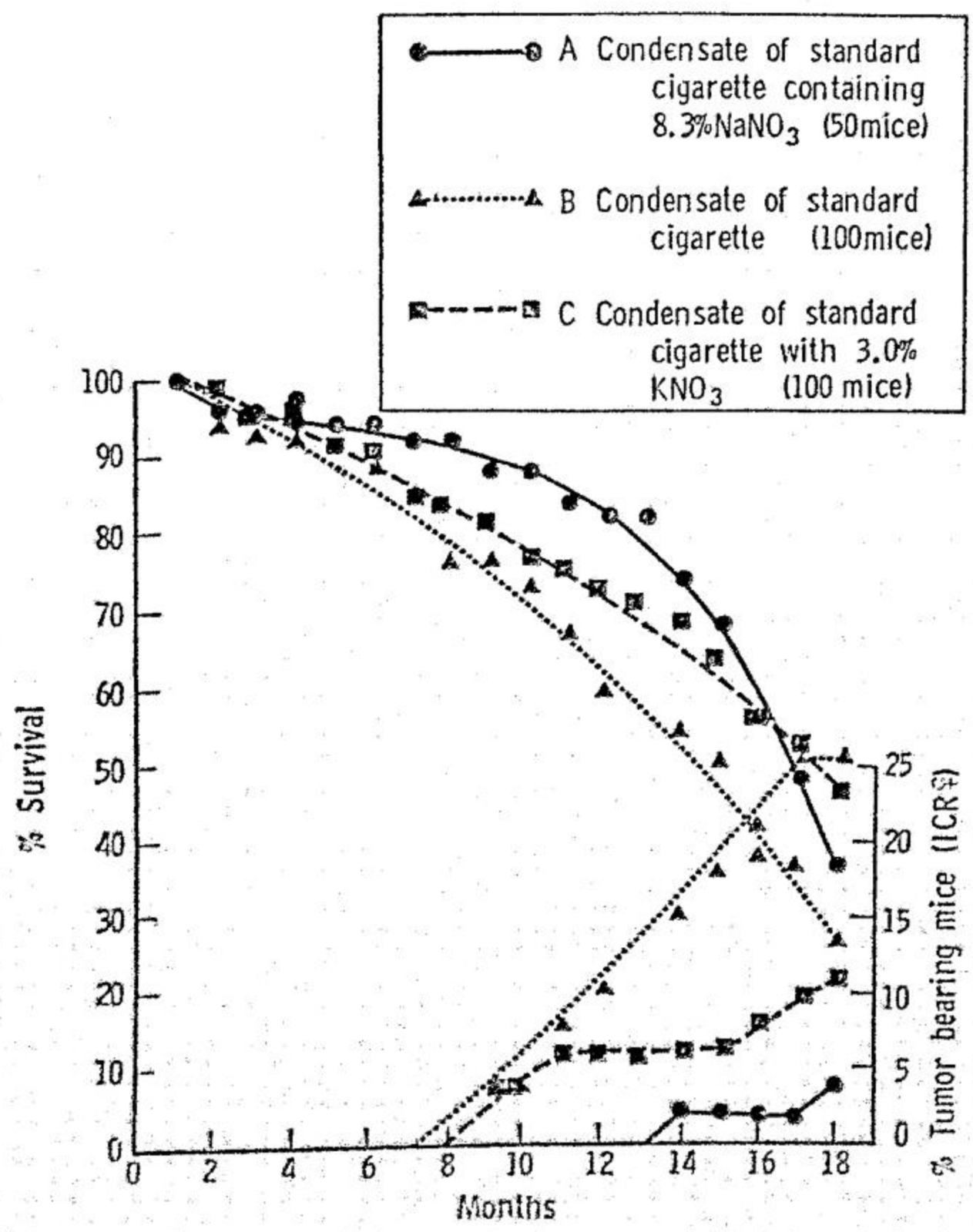
proved the latter to be superior for PAH reduction. This is indicated by BaP values obtained from the pyrolysis products, though the reduction is clearly not limited to BaP (table 5).

TABLE 5.—Relative PAH-yield in pyrolysis*

Tobacco	BaP	BaA	Pyrene	Chrysene	Anthanthrene
Standard	85	39	46	41	39
Standard + 8.5% KNO_3	45	20	21	22	22

*μg from 1.0 g tobacco.
BaP and BaA are quantitative values; pyrene, chrysene, and anthanthrene are isolated amounts.

Since we find with increasing nitrate content of tobaccos a significant selective PAH reduction in their smoke condensates, we expect this to be reflected in lower tumorigenicity of such "tars" when tested on an equal dose basis. Text-figure 9 compares the survival rates of animals in the tumorigenicity tests as well as tumorigenicity of three "tars." "Tar" B derives from a U.S. blended cigarette 85 mm long, "tar" A from cigarettes



TEXT-FIGURE 9.—Survival rate and tumor response of Swiss ICR ♀ mice to cigarette-smoke condensates.

manufactured with the same tobacco blend with addition of 8.3% sodium nitrate, and "tar" C from cigarettes made of the same blend with 3% potassium nitrate. The corresponding analytical data are given in table 6 and demonstrate for the nitrate cigarettes a higher burning rate, lower number of puffs, lower yields of TPM, volatile phenols, and BaP. Here we see again an indication for a selective nicotine reduction.

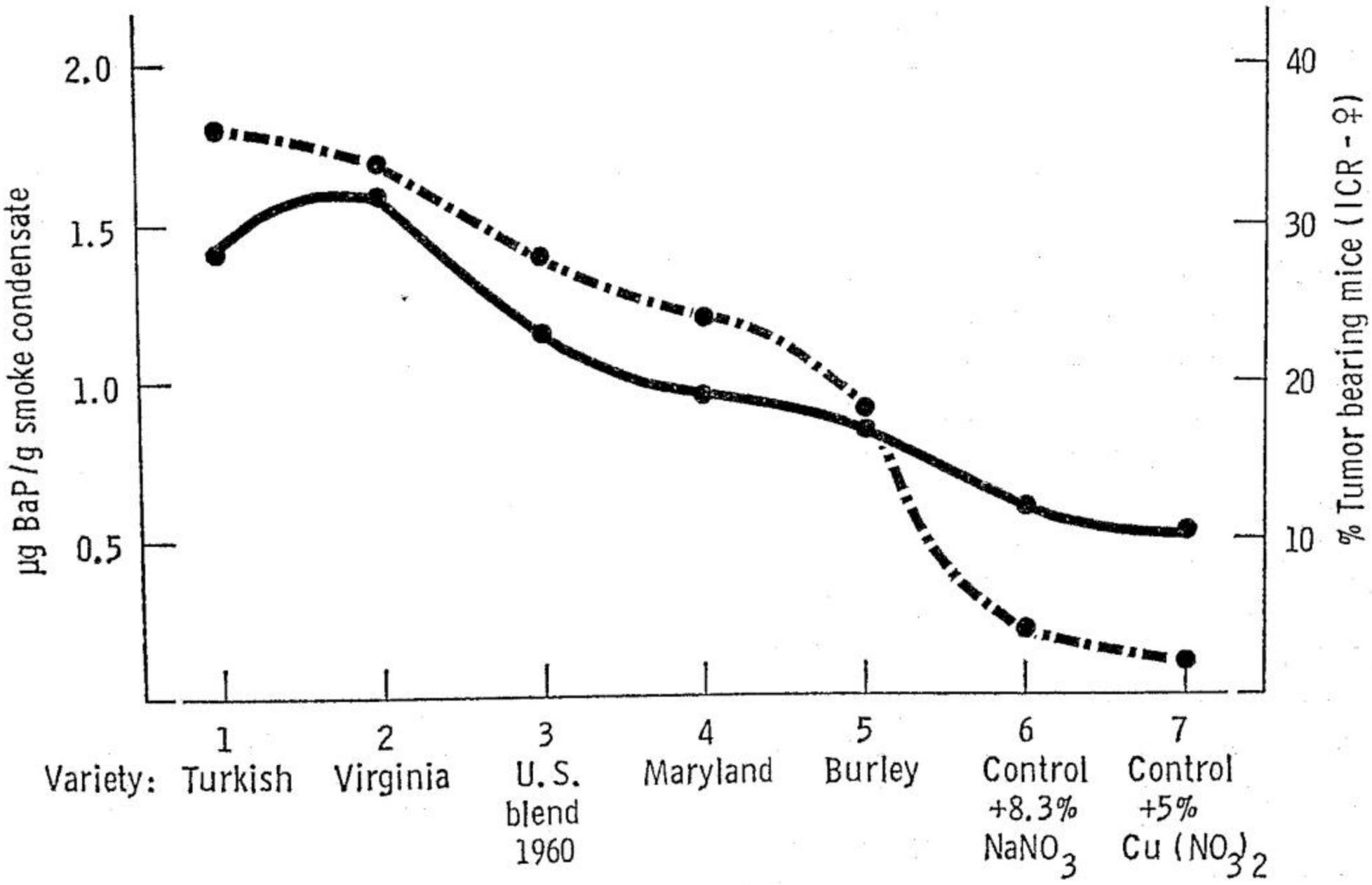
TABLE 6.—Analyses of cigarette smoke

Per cigarette	Standard + 8.3% $NaNO_3$	Standard
Cigarette weight (mg)	1200	1090
Number of puffs	8.8	10
Static burning rate	1.19	1.03
Dry PM (mg)	19.0	27.8
Nicotine (mg)	0.91	1.88
Phenol (μg)	60.4	96.0
BaP $\mu g \times 10^{-2}$	0.95	2.92

In another experiment, 2.5% potassium nitrate was added to Bright tobacco and again a reduction in tumorigenicity was found, though it proved statistically insignificant ($P>0.05$). This experiment will be repeated with increased additions of potassium nitrate.

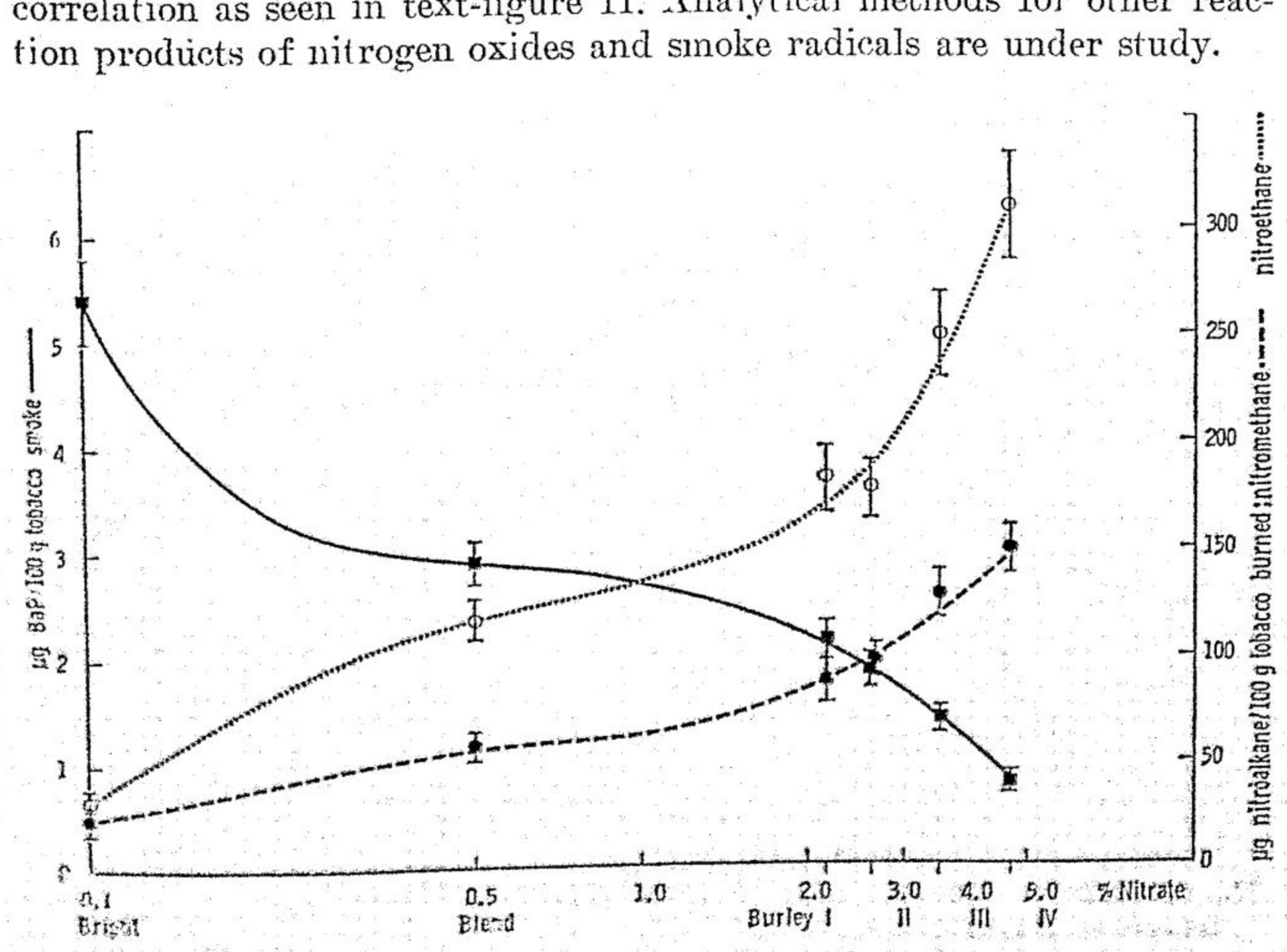
The nitrate experiments completed to date permit the conclusion that an increased nitrate content of tobacco leads to a more complete tobacco combustion, a step reflected by an increased burning rate of higher nitrate tobaccos, a lower yield of TPM per puff, and a reduced phenol and nicotine yield. The latter compound is probably also selectively reduced. The pyrosynthesis of PAH is inhibited as proved by selectively reduced yields, especially of the carcinogen BaP. Significant reduction of the tumorigenicity of such "tars" also substantiates this finding (text-fig. 10). The addition of nitrate to various tobacco fines during tobacco sheet preparation is currently being investigated, since it represents a method with most promising academic aspects as well as practical implications.

As discussed before, the pyrosynthesis of PAH in tobacco smoke occurs by the conglomeration of pyrolytically formed smaller C,H-units, including radicals, to ring hydrocarbons with thermodynamic stability and low energy content. Theoretically, it appears likely that scavengers in the burning cone of tobacco may inhibit the actual pyrosynthesis. Tobacco pyrolysis in nitrogen at 880°C was studied with addition of 5% elementary iodine (*45*). This additive gave a significant reduction for BaP of more than 40% in comparison to the control pyrolysis without iodine. During the tobacco pyrolysis, iodine is vaporized and is known to act in this state as a scavenging agent (*46*). Thus the PAH reduction with iodine further incriminates C,H-radicals as precursors of PAH in tobacco smoke. A similar scavenging activity can be assumed for nitrogen oxides (*47*). The qualitative and quantitative identification of nitro hydrocarbons in cigarette smoke and the dependence of their concentration in the smoke on the nitrate content of tobacco support this concept. The experimental details



TEXT-FIGURE 10.— Benzo[*a*]pyrene and tumorigenicity of cigarette-smoke condensates.

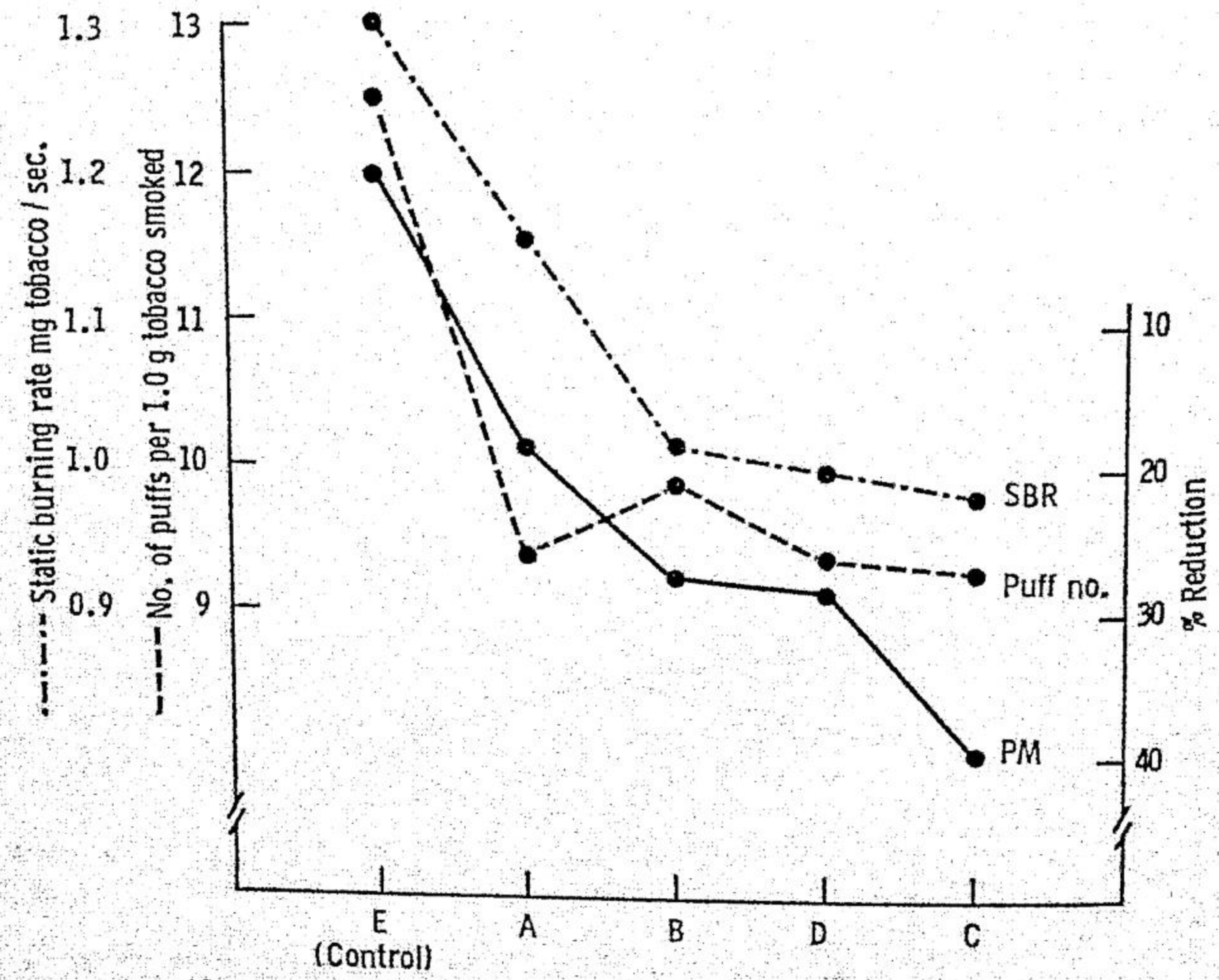
for the analysis of nitro hydrocarbons will be presented elsewhere; however, quantitative nitromethane and nitroethane data suffice to outline this correlation as seen in text-figure 11. Analytical methods for other reaction products of nitrogen oxides and smoke radicals are under study.



TEXT-FIGURE 11.—Benzo[*a*]pyrene, nitromethane, and nitroethane in the mainstream smoke of 100 g cigarette tobacco.

TOWARD A LESS HARMFUL CIGARETTE

Since the addition of nitrate or selection of high nitrate tobacco results in a faster burning rate, a lower number of puffs per given amount of tobacco, low TPM, phenol, nicotine, and PAH yields, and low tumorigenicity of "tars," we consider this approach encouraging. On the other hand, we have to regard the possibility that the smoke of nitrate-rich tobaccos may have altered chemical composition that may enhance the irritating effect of tobacco smoke on the respiratory epithelium. It must also be considered that tumorigenic agents such as *N*-nitrosamines and nitroalkenes may be formed or are present in the smoke in increased concentrations. Pending the development of adequate techniques (*2*, *44*, *48*), the presence in smoke of these nitroso compounds should be verified, especially, since in the case of Burley we deal with a type of tobacco that is rich in nitrate and has been cultivated and smoked for about 100 years (*49*). Nickel acetate, though not of practical use, is so far the only other additive for which sufficient experimental data are at hand to demonstrate a significant reduction of carcinogenic hydrocarbons and tumorigenicity of tobacco "tars" (*50*). However, our group and others are currently testing other additives. Based on the knowledge gained from earlier studies with tobacco additives, we can today eliminate the empirical selection of additives and concentrate on studying those agents that appear theoretically promising.

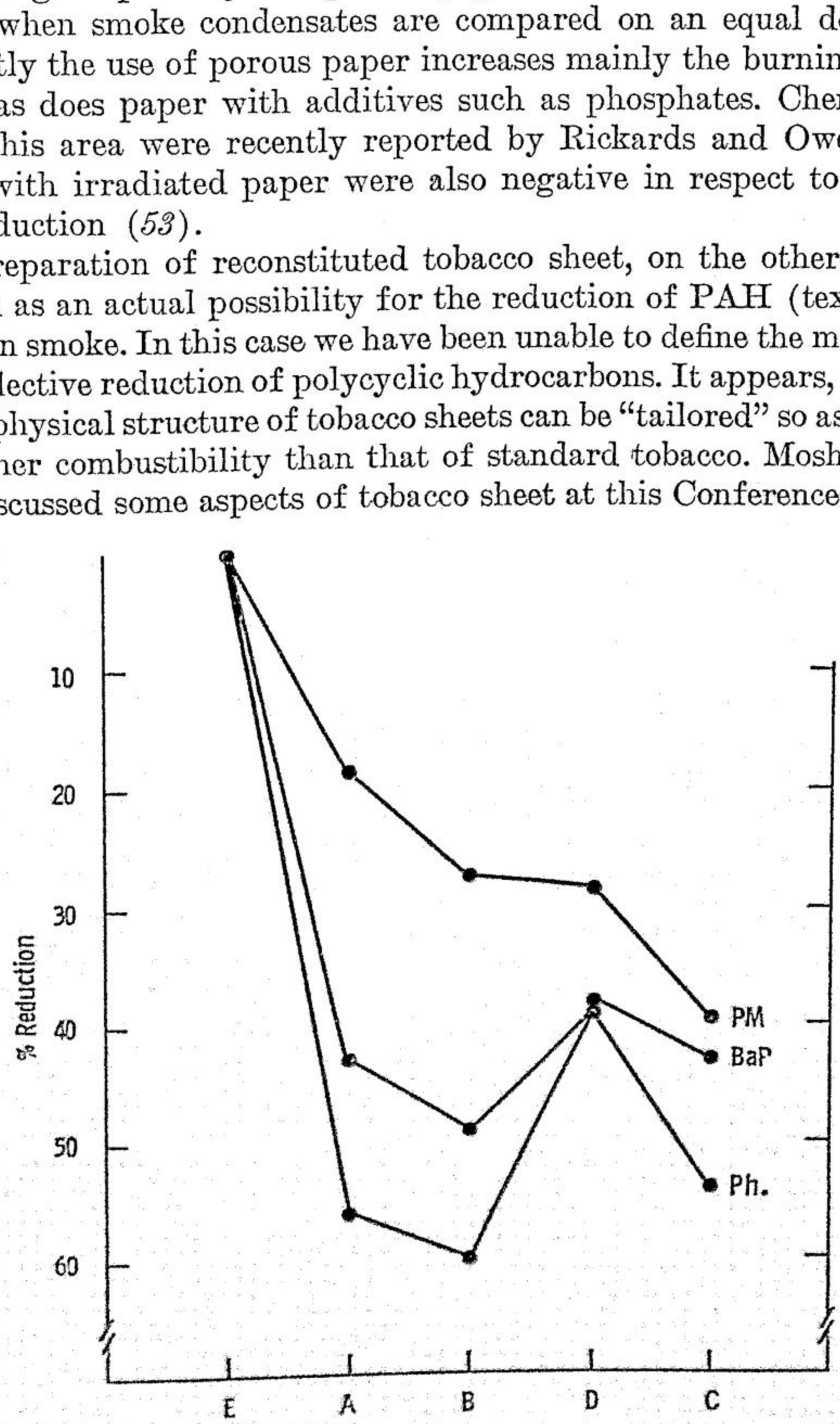


TEXT-FIGURE 12.—Burning characteristics of experimental cigarettes made from tobacco sheets. SBR = static burning rate; PM = particulate matter; E = control cigarettes made from natural tobacco which was used for the preparation of the cigarette sheets of cigarettes A–D.

Other approaches for the reduction of PAH in tobacco smoke.—A selective filtration of carcinogenic hydrocarbons from cigarette smoke was claimed in one scientific communication (*51*). Testing one of the supposedly effective filter additives, mineral oil, we were unable to confirm the reported selective reduction for BaP (*2*). Theoretically and experimentally, such selective removal from the particulate matter of tobacco smoke appears most unlikely (*2*).

The change in porosity of cigarette paper does not decrease the tumorigenicity when smoke condensates are compared on an equal dose basis. Apparently the use of porous paper increases mainly the burning rate of tobacco, as does paper with additives such as phosphates. Chemical details of this area were recently reported by Rickards and Owens (*52*). Studies with irradiated paper were also negative in respect to selective PAH reduction (*53*).

The preparation of reconstituted tobacco sheet, on the other hand, is indicated as an actual possibility for the reduction of PAH (text-figs. 12 and 13) in smoke. In this case we have been unable to define the mechanism of this selective reduction of polycyclic hydrocarbons. It appears, however, that the physical structure of tobacco sheets can be "tailored" so as to result in a higher combustibility than that of standard tobacco. Moshy has already discussed some aspects of tobacco sheet at this Conference (*54*).



TEXT-FIGURE 13.—Selective reduction of several cigarette-smoke constituents; PM = particulate matter; BaP = benzo[*a*]pyrene; Ph. = phenol.

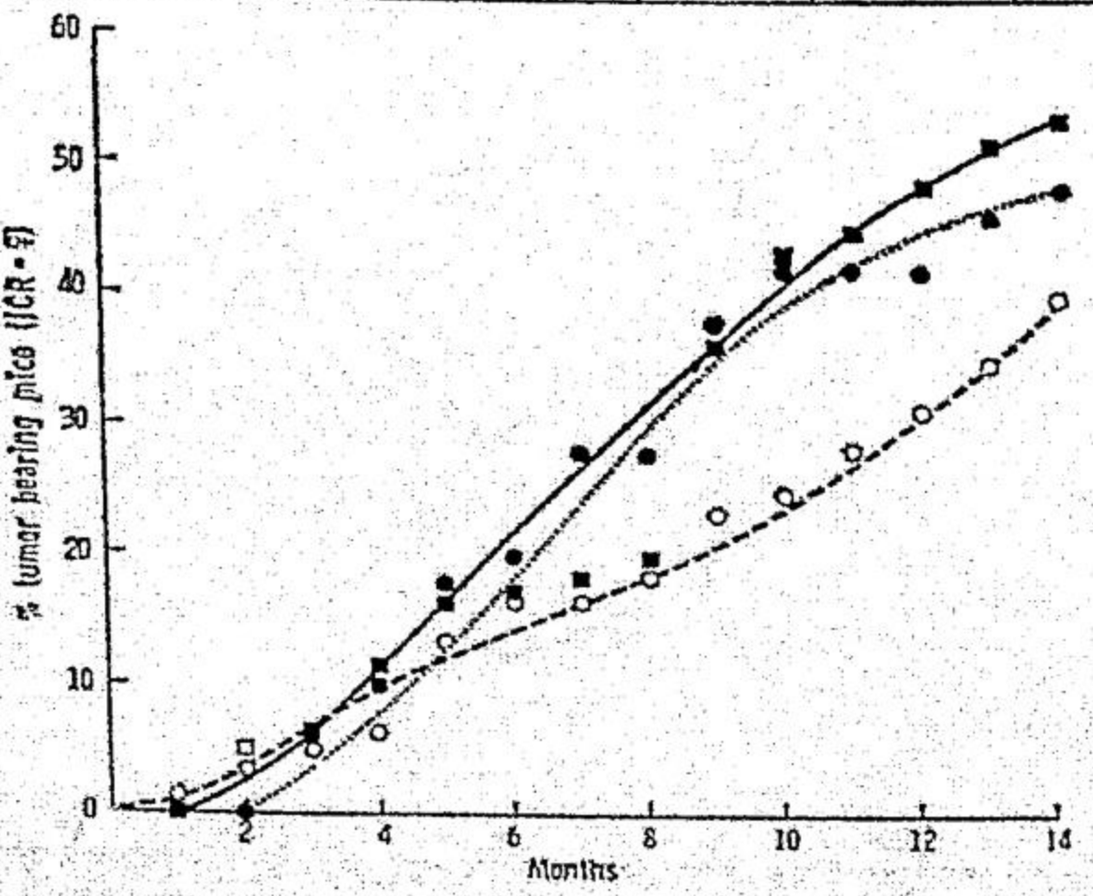
TOWARD A LESS HARMFUL CIGARETTE

The importance of the material structure in respect to completeness of tobacco combustion is apparent from studies with sheet preparations. The tobacco geneticist might possibly breed tobacco strains with leaves of increased combustibility. The tobacco growing practices which lead to some TPM and nicotine reduction in smoke have been discussed by Tso (*55*). Other attempts to reduce the tumorigenicity of smoke by growing specific tobaccos are currently evaluated by the U.S. Department of Agriculture (*56*). This important research area may well include the reduction of tumor-promoting agents or their precursors in the tobacco leaf.

Reduction of Tumor-Promoting Activity of Tobacco Smoke

The preceding discussion related mainly to the selective reduction of PAH in tobacco smoke. It could be shown that a significant selective reduction of these agents, the only known tumor initiators in the smoke, was paralleled by an overall reduction of the tumorigenicity of the particulate matter of the smoke without reducing significantly the tumor-promoting activity of these "tars" (*see* text-fig. 14). One may reason that a significant reduction of the tumor-promoting agents in tobacco smoke may also lead to an overall reduction of the tumorigenicity of the "tar." Despite the fact that a selective filtration of the tumor-promoting volatile phenols (*2*, *57*, *58*) occurred, such reduction did not suffice to reduce significantly the tumorigenicity of the "tars" from cigarettes with such filters (*2*). Further

"Tar"	Mice started	After 14 months promotor application			
		Papilloma bearing mice	Total number papillomas	Carcinoma bearing mice	Survivors
■—■ Standard	60	26	55	9	8
●—● Cigarettes + 5% $Cu(NO_3)_2 5H_2O$	50	24	59	8	14
○—○ Cigarettes + 8,3% $NaNO_3$	60	21	24	6	24



TEXT-FIGURE 14.—Tumor-promoting activity of cigarette-smoke condensates. Tumor initiator: 300 μg DMBA, single application. Tumor promoter: 50% smoke condensate, thrice weekly for 12 months.

studies on the selective reduction of other tumor-promoting agents represent an important challenge.

In our search for tobacco-specific as well as general types of tumor promoters in tobacco extract and tobacco smoke condensate we consider chemical and biological evidence. In order to enrich tumor promoters in certain fractions we employ countercurrent distributions. In biological tests we determine the influence of curing and secondary treatment of tobacco on the tumor-promoting activity of its extracts and smoke condensates.

SUMMARY

Tobacco smoke contains at least two types of tumorigenic agents, tumor initiators and tumor promoters. The polynuclear aromatic hydrocarbons (PAH) were identified as the only known group of tumor initiators in tobacco smoke. A significant reduction of these agents in the smoke is paralleled by a significant reduction of the tumorigenicity of the particulate matter, when assayed on mouse skin. Several methods that appear theoretically feasible for the reduction of PAH include: breeding of specific tobacco strains, modification of the combustion temperature, extraction of tobacco, changing of cigarette paper, addition of alkali nitrates or selection of tobaccos rich in alkali nitrates, and the manufacture of reconstituted tobacco sheets. So far only the last two methods have brought encouraging results.

Pyroformation of PAH occurs in the hot zones of burning tobacco in at least two distinctive, successive steps: formation of small fragments from the nonvolatile tobacco constituents followed by a recombination of the pyrolytically formed reactive fragments to ring hydrocarbons with thermodynamic stability and low energy content. Inorganic compounds already present in genuine tobacco, or added to tobacco, may thermically decompose, yield volatile scavengers, and interfere with the actual pyrosynthesis.

Of all agents so far tested, alkali nitrates resulted in the most significant inhibition of PAH formation and thereby most effectively reduced the overall tumorigenicity of the particulate matter. Encouraging data were obtained by selection of nitrate-rich tobaccos as well as by the addition of alkali nitrates to tobacco. Extensive chemical-analytical data and mouse-skin tests of several tobacco-smoke condensates are the main basis for this finding.

An inhibition mechanism for the PAH pyrosynthesis is discussed and supported by the analytical finding of an interdependence of the yield of nitrohydrocarbons in the smoke with the nitrate content of the tobacco.

A second potential for reducing the carcinogenic hydrocarbons in tobacco smoke and the tumorigenicity of the resulting "tars" was demonstrated by the use of reconstituted tobacco sheets with and without the addition of nitrates.

The importance of tumor promoters in experimental tobacco carcinogenesis is apparent and much remains to be learned about them. Concerted efforts toward their elucidation are therefore imperative.

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Initiators and Promoters in Tobacco Carcinogenesis[1]

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MANY studies have been carried out (*1*) and many others are under way (*2*) in an attempt to define the tumorigenic agents in tobacco smoke and from this knowledge to develop a less harmful cigarette.

Since it has become evident that the carcinogenic hydrocarbons are most probably not alone responsible for the total tumorigenic activity of cigarette "tars" on mouse skin (*3–5*), further studies on carcinogens and tumor-initiating and tumor-promoting agents are clearly indicated.

One aspect of such studies is the isolation and chemical identification of the tumor-promoting agents from tobacco leaf and from tobacco-smoke condensate which are being examined in several laboratories (*5–7*). Our own recent studies in this area will be reported elsewhere. Several related aspects of initiators and promoting agents will be dealt with in this report.

1) *Initiating agents.*—Earlier work in this laboratory (*7*) has shown that a "borderline" carcinogen such as chrysene (present in cigarette "tars") is an effective initiating agent in initiation-promotion experiments on mouse skin with the use of a mixture of phorbol esters (croton resin) from croton oil as promoting agent. The present report describes the initiating activity of other borderline or noncarcinogenic hydrocarbons, some of which occur in cigarette "tars."

2) *Synthetic promoting agents.*—Through work in several laboratories the structures of the tumor-promoting principles of croton oil have been established (*8–10*). With this knowledge, it is now possible to examine similar compounds, with lipophilic-hydrophilic structures, for their tumor-promoting activity. Boutwell and Bosch (*11*) have described the tumor-

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[2] We are indebted to Dr. Hans L. Falk of the National Cancer Institute for fruitful discussions and encouragement during this work.

promoting activity of several phenolic compounds. The bioassay of a series of known or suspected constituents of tobacco leaf or smoke condensate of these two types is described.

3) *Cigarette tobacco additives.*—Many chemicals are introduced into cigarette tobacco, *e.g.*, in the growing stage as pesticides, during storage as fungicides, and during processing as flavoring agents and humectants. Relatively little is known about the biological properties of many of these chemicals. This report describes a study on the thermal degradation of menthol, a known flavoring agent in some cigarettes.

4) *Chemical modification of tobacco.*—Bock *et al.* (*6*) have described the tumor-promoting activity of a barium hydroxide extract of tobacco leaf. It is expected that some chemical changes, such as air oxidation and condensation reactions, occur during the extraction procedure (*12*). These changes are under investigation in this laboratory. The present report describes the bioassay results of a barium hydroxide extract and an acetone-benzene extract prepared according to the procedure of Bock *et al.*

MATERIALS AND METHODS

Animals.—The mice used were females of an ICR/Ha Swiss strain obtained from Millerton Research Farms, Millerton, New York. They were vaccinated against ectromelia and tested at age 8 weeks. Mice were housed on sterile wood chips in metal cages, 10 to a cage, fed Purina Laboratory Chow and water *ad libitum*, and weighed regularly. The animal rooms were maintained at 22–24°C.

Bioassay procedure.—The backs of the mice were clipped 2 days before the initial treatment and as needed for the duration of the experiment. The initiating agent was applied by micropipet in a single dose in 0.1 ml of solvent. This primary treatment was followed 2 weeks later by 3 times weekly applications of promoters. Promoter solutions were applied to the clipped dorsal skin with a micropipet, delivering 0.1 ml of solution per application. In all cases, short-term toxicity was evaluated; in some instances a decrease of the preselected dose of 25 mg in 0.1 ml solvent per application of promoter had to be used because of toxicity.

Because of the solubility properties of some of the compounds, the solvent of choice, acetone, could not always be used and other solvents were employed. Animals were observed regularly and tumors were recorded; tumors greater than 1 mm in diameter were counted and charted monthly. The data are based on these monthly chartings. Animals bearing tumors that appeared grossly to be carcinomas were killed approximately 2 months after the tumors were classified as cancers. All animals were autopsied at death, and specimens from tumors and any abnormal tissues were excised and confirmed histologically. Included in the experimental protocol were control groups that received initiator or promoter only, solvent only, and a group given no treatment.

Aromatic hydrocarbons.—Commercial grade aromatic hydrocarbons were purified by column chromatography and/or recrystallization, and their purity was verified by thin-layer chromatography, melting points, ultraviolet absorption spectra, and fluorescence spectra. 6-Methylanthanthrene was kindly supplied by Dr. N. P. Buu-Hoï, Radium Institute, Paris, France.

Laurate esters.—The synthesis, characterization, and purification of the laurate esters of pyrogallol, catechol, resorcinol, and hydroquinone were described in another report from this laboratory (*13*).

Phenols and coumarins.—Quercitrin, mp 185° [reported 183–185° (*14*)], quercetin, mp 314° [reported 305°, decomp. (*15*)], and morin, mp 298–300° [reported 300° (*16*)], were obtained from K. & K. Laboratories (Plainview, N.Y.) and purified by recrystallization from ethyl alcohol-water. Rutin, mp 197° [reported 180–200° (*17*)], and umbelliferone, mp 233–237° [reported 238° (*18*)] from the same supplier, were purified by crystallization from water. Hesperidin, also from K. & K. Laboratories, was purified by dissolving in alkali at *p*H 10 and then acidifying to *p*H 6.5, mp 253–255° [reported 251–252° (*19*)]. The following reagent grade chemicals (Aldrich Chemical Company, Milwaukee, Wisc.) were used as such: phenol, 2- and 3-hydroxyacetophenone, *p*-anisaldehyde, and caffeic acid.

Solvents.—Reagent grade acetone and spectroscopic grade benzene were used for mouse skin applications. These solvents were routinely checked for purity from one batch to another by spectrofluorimetric analysis. Dimethylsulfoxide was pure material prepared expressly for biological testing (Crown Zellerbach Corp., Camus, Wash.).

Croton resin.—This material is a mixture of the phorbol esters from croton oil. The procedure for the preparation of this material from the seeds of *Croton tiglium* L. was described in an earlier report (*8*).

Pyrolysis of dl-menthol.—The pyrolysis tube consisted of a central section of vycor glass, 6 inches long, with an internal diameter of ~1 inch. One side of the vycor tube was connected to a standard pyrex inner joint and the other side to a standard outer joint. The vycor section was wrapped with nichrome wire and the wire connected to a rheostat. The tube was clamped at a 30° angle to the horizontal, and the molten menthol, ~1.0 g, was run into the heated tube for 5 minutes at the standard upper end. An adapter connected to the lower end of the tube led to a round-bottom flask immersed in an ice bath. The temperature of the tube was determined with a pyrometer. Menthol was passed through the tube at temperatures from 200–700°C. For some experiments, the tube was packed with glass beads. After the sample had been added, the tube, cooled to room temperature, was washed with acetone. Portions of the acetone solution were assayed by gas chromatography with the use of a column packed with di-*n*-propyl tetrachlorophthalate on chromosorb at 125°, with a flame ionization detector.

RESULTS

Bioassays.—The results of the initiation-promotion experiments are summarized in tables 1–3. Coronene, dibenz[*a*,*c*]anthracene, and 6-methylanthanthrene all had tumor-initiating activity with croton resin promotion. Benzo[*e*]pyrene and anthanthrene did not have initiating activity at the doses used. The most effective of the initiators was 6-methylanthanthrene at a dose of 0.2 mg per application; in a group of 20 mice, 6 animals bore a total of 14 papillomas; 4 of the tumor-bearing mice developed squamous carcinomas. The times to first tumors were 78 days for benign and 223 days for malignant tumors from the beginning of the experiment.

All the groups treated with the initiator alone were without tumors and there were also no tumors in the control group treated with acetone alone, and in the no-treatment group. However, 1 of the 2 control groups treated with croton resin alone did have an unusually high tumor incidence. In our experience with 258 mice in 12 different control experiments run at different times, 20 animals with papillomas were observed, *i.e.*, a 7.8% tumor incidence. Of the 17 compounds tested as promoting agents (table 2), only phenol showed promoting activity. Of 20 animals, 4 bore papillomas and 1 bore a squamous carcinoma. The first papilloma appeared after 167 days of testing and the squamous carcinoma appeared after 355 days. Animals treated with quercitrin and morin as promoting agents each bore 1 papilloma.

The tumor-promoting activity of the materials prepared by the procedure of Bock *et al.* is summarized in table 3.

Menthol was not degraded thermally from 200–500°C by the procedure used. When the tube was packed with glass beads and menthol run through between 500 and 700°C, more than 98% of the compound still remained unchanged.

DISCUSSION

The most notable findings described in this report are that noncarcinogenic hydrocarbons, such as coronene or dibenz[*a*,*c*]anthracene, or borderline carcinogens, such as 6-methylanthanthrene, exhibit tumor-initiating activity in 2-stage tumor induction experiments on mouse skin. These findings have both theoretical and practical implications of considerable interest. Dibenz[*a*,*c*]anthracene is usually cited as the classical noncarcinogenic hydrocarbon which does not have a K-region. Nevertheless, it is a tumor-initiating agent. 6-Methylanthanthrene has been tested for carcinogenic activity by subcutaneous injection into 14 mice (*20*). One animal bore a fibrosarcoma after 353 days. It therefore has weak activity but is a potent initiating agent. Coronene is also generally considered to be a noncarcinogenic hydrocarbon, yet it has some tumor-initiating activity.

TABLE 1.—Aromatic hydrocarbon initiators. Mouse skin application (20 ♀ ICR/Ha Swiss mice/group except where noted)

Treatment		Cumulative No. of mice with:		Total No. of:		Days to first:		Weeks on test
Primary (single dose)	Secondary dose* per animal	Papilloma	Cancer	Papilloma	Cancer	Papilloma	Cancer	
Benzo[*e*]pyrene, 1.0 mg in 0.1 ml acetone	Croton resin, 25 μg in 0.1 ml acetone	2	0	2	0	103	—	64
Benzo[*e*]pyrene, 1.0 mg in 0.1 ml acetone	None	0	0	0	0	—	—	64
Coronene, 0.5 mg in† 0.5 ml benzene	Croton resin, 25 μg in 0.1 ml acetone	6	0	8	0	192	—	64
Coronene, 0.5 mg in† 0.5 ml benzene	None	0	0	0	0	—	—	64
Anthanthrene†, ‡, §, 1.0 mg in 0.4 ml benzene	Croton resin, 25 μg in 0.1 ml acetone	2	0	2	0	341	—	65
Anthanthrene†, ‡, §, 1.0 mg in 0.4 ml benzene	None	0	0	0	0	—	—	65
Dibenz[*a*, *c*] anthracene, 1.0 mg in 0.1 ml benzene	Croton resin, 25 μg in 0.1 ml acetone	5	2	11	2	65	310	65
Dibenz[*a*, *c*]anthracene, 1.0 mg in 0.1 ml benzene	None	0	0	0	0	—	—	65
6-Methylanthanthrene‖, 0.2 mg in 0.1 ml acetone	Croton resin, 25 μg in 0.1 ml acetone	6	4	14	4	78	223	64
6-Methylanthanthrene‖, 0.2 mg in 0.1 ml acetone	None	0	0	0	0	—	—	64
None	Croton resin, 25 μg in 0.1 ml acetone	5	1	5	1	150	231	66
None	Croton resin, 25 μg in 0.1 ml acetone	1	0	1	—	293	—	60
None	Acetone, 0.1 ml	0	0	0	0	—	—	54
None	None	0	0	0	0	—	—	59

*Three times weekly beginning 14 days after primary treatment.
†Applied as consecutive 0.1 ml doses.
‡Dibenzo[*def*, *mno*]chrysene according to *Chemical Abstracts*' nomenclature.
§Thirteen mice in original test group.
‖Sixteen mice in original test group.

TABLE 2.—Phenols, coumarins, and related tobacco leaf and smoke constituents tested for promoting activity on mouse skin*

Promoting agent	Dose in 0.1 ml solvent†
Phenol	3 mg/Ac‡
Caffeic acid	10 mg/DMSO‡
2-Hydroxyacetophenone	10 mg/Ac‡
3-Hydroxyacetophenone	2 mg/DMSO‡
p-Anisaldehyde	10 mg/Ac‡
Rutin	25 mg/DMSO
Quercetin	25 mg/DMSO
Hesperidin	25 mg/DMSO
Quercitrin	25 mg/DMSO
Umbelliferone	25 mg/DMSO
Morin	25 mg/DMSO
Pyrogallol-1-laurate	3 mg/Ac
Pyrogallol-1,2-dilaurate	10 mg/Ac
Pyrogallol-trilaurate	13 mg/Ac
Catechol monolaurate	6 mg/Ac
Resorcinol monolaurate	6 mg/Ac
Hydroquinone monolaurate	6 mg/Ac

*Twenty ICR/Ha Swiss mice/group.

†Applied 3 times weekly for one year. Abbreviations: Ac, acetone; DMSO, dimethylsulfoxide.

‡Single 150 μg dose of 7,12-dimethylbenz[*a*]anthracene in 0.1 ml of acetone 14 days before promoting treatment; all other groups were pretreated 14 days before promoting treatment with a single dose of 100 μg benzo[*a*]pyrene in 0.1 ml acetone.

TABLE 3.—Promoting activity of extracts of tabacco leaf on mouse skin (20 ♀ ICR/Ha mice/group; results at 1 year)

Treatment		Cumulative No. of mice with:		Days to first:	
Primary (single dose)*	Secondary dose per animal (3 × weekly)	Papilloma	Cancer	Papilloma	Cancer
DMBA	Barium hydroxide extract, 83 mg/0.1 ml water	6	1	176	265
None	Barium hydroxide extract, 83 mg/0.1 ml water	0	0	—	—
DMBA	Acetone-benzene extract, 50 mg/0.1 ml acetone	2	0	238	—
None	Acetone-benzene extract, 50 mg/0.1 ml acetone	0	0	—	—
DMBA	Acetone	2	1	247	373
DMBA	Croton resin, 25 μg/0.1 ml acetone	19	8	51	231
None	Croton resin, 25 μg/0.1 ml acetone	1	0	293	—
None†	None	0	0	—	—
None	Acetone, 0.1 ml	0	0	—	—

*Single 150 μg dose of 7,12-dimethylbenz[*a*]anthracene in 0.1 ml acetone applied 14 days before promoting treatment.

†Sixty mice in this group.

From the point of view of tobacco carcinogenesis, these findings suggest that noncarcinogenic compounds, such as the hydrocarbons mentioned and including chrysene described earlier (*7*), may be initiating agents. This is particularly important since it is clear that tobacco leaf extracts and smoke condensate contain tumor-promoting agents (*5–7*). This raises the question about such initiating effects that may be caused by other noncarcinogenic tobacco "tar" hydrocarbons.

Boutwell and Bosch (*11*) showed that phenol and several substituted phenols are tumor-promoting agents and this has been confirmed for phenol in our work. Nevertheless, a variety of substituted phenols (table 2) has been devoid of promoting activity. These findings, although largely negative, suggest that though phenols probably play some role as promoting agents in tobacco carcinogenesis, other unknown and/or untested constituents of tobacco or smoke are promoting agents. This aspect is being explored further. Our experiments indicate that without a catalyst, brief heating of menthol up to 700°C does not yield any appreciable amount of degradation products. It is known that menthol can be converted to a variety of products with a catalyst such as aluminum oxide. Pines and Pillai (*21*) showed that the number and kind of pyrolysis products of menthol, mainly isomeric menthenes, are determined by the nature of the catalyst present.

SUMMARY

The aromatic hydrocarbons, coronene (noncarcinogenic), 6-methylanthanthrene (weak carcinogen), and dibenz[*a*,*c*]anthracene (noncarcinogenic), show initiating activity, *i.e.*, single applications of these hydrocarbons on mouse skin followed by repeated application of croton resin result in tumors. These findings imply that noncarcinogenic tobacco "tar" hydrocarbons may be tumor-initiating agents. In a series of 17 phenols, coumarins, and related compounds, only the parent compound, phenol, had promoting activity. A barium hydroxide extract of tobacco leaf had tumor-promoting activity. The thermal degradation of menthol was examined. The relevance of these findings to tobacco carcinogenesis is discussed.

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Nitrosamines in Tobacco Smoke

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IT was first suggested by Druckrey and Preussmann (*1*) that conditions exist in the burning of tobacco which could produce nitrosamines. They pointed out that tobacco is known to contain nitrates and a number of amines which are burned in tobacco in an atmosphere with a limited amount of oxygen; thus the production of compounds such as nitrosamines is favored.

The extreme carcinogenic activity of nitrosamine compounds has been shown by numerous investigators and was reviewed by Magee and Schoental (*2*). One of the more interesting features of these alkyl nitroso compounds is their capacity to induce a great variety of tumors at different sites. It was also pointed out that despite their rapid decomposition in the body some of them can induce cancers by single doses. Nitrosamines can be converted to strong alkylating agents such as diazomethane by microsomal enzyme systems and these compounds can alkylate vital parts of the cell such as DNA and RNA. Establishing the presence of nitrosamines in cigarettes has been made difficult by the lack of procedures for measuring these compounds in a very complex mixture.

The probable presence of nitrosamines in cigarette smoke condensate has been shown by Serfontein and Hurter (*3*) and Neurath *et al.* (*4–6*). Serfontein and Hurter's work indicated that at least 3 nitrosamines might be present in cigarette smoke; however, they could not quantitate the compounds present although they estimated that there might be 1–5 μg of *N*-nitrosopiperidine formed per cigarette under the given conditions. Neurath (*6*) has shown from model experiments that the conditions for formation of nitrosamines in tobacco smoke require a secondary amine plus an equimolar mixture of NO and NO_2, producing two moles of the corresponding nitrosamine. Thus in a model experiment with a secondary amine, gas mixtures containing NO only or NO_2 only did not produce the cor-

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responding nitrosamines. Only when an equimolar mixture of NO and NO_2 was present were there measurable quantities produced. Neurath pointed out that this reaction should be carried out with the exclusion of oxygen. The reaction is also time-dependent and temperature-sensitive.

It has been reported by Norman and Keith (*7*) that of the nitrogen oxides present in tobacco smoke only NO is found under normal smoking conditions. Very little, if any, NO_2 was found. The absence of NO_2 in the cigarette smoke could stem from a number of reasons; however, one of these is that NO_2 is a highly reactive substance in the sense that it is readily absorbed. For example, the moisture and organic reactive materials in the smoke stream and tobacco column may readily absorb and react to eliminate NO_2. Neurath has shown that under normal smoking conditions very little of the nitrosamines could be detected; however, when smoke condensate was collected in a cold trap, thus allowing more time for interaction, some nitrosamines were found. These compounds increased with time in the cold trap. Neurath feels that these are artifacts that represent primarily oxidation of NO to NO_2 in the cold trap thereby generating a required compound for the production of nitrosamines.

Serfontein and Smit (*8*) presented evidence for the occurrence of nitrosamines in unburned tobacco. These authors analyzed unburned Burley tobacco for the presence of nitrosamines and were able to find a zone of compounds which appeared to be nitrosamines based on a colorimetric spray; they suggest that possibly nitrosamines in tobacco smoke originate, at least in part, from material of the tobacco plant. Neurath *et al.* (*4*) indicated that addition of *N*-nitrosodibutylamine to a cigarette could be identified in the cigarette smoke condensate in a 10–15% yield. They suggested that much of the loss was via side stream smoke or by combustion processes. The recovery of *N*-nitrosodimethylamine was considerably poorer than for the corresponding dibutyl compound.

This paper evaluates the reports of the presence of *N*-nitrosamines in cigarette smoke and presents some of the work that has been carried out in our laboratories. For the purpose of this evaluation the problem has been divided into 4 subgroups: 1) collection of smoke condensate, 2) cleanup of the condensate, 3) detection and identification of nitrosamines, and 4) a biological test of the significance of these nitrosamines.

Collection of Smoke Condensate

The method used for collection of smoke condensate for examination for nitrosamines is of vital importance as Neurath (*4–6*) has pointed out quite clearly. He concluded that the use of cold traps, liquid trapping, and electrostatic precipitation should be avoided since these procedures can produce artifacts. In cold traps, NO and NO_2 are collected and oxygen is allowed to flow over the trapped solution, converting some of the NO to NO_2, which eventually produces nitrosamines. Liquid trapping gives essentially the same result, and electrostatic precipitation can produce

ozone which converts NO to NO_2. In Neurath's trapping procedure he used a horizontal glass tube with cotton wadding as a more realistic means of trapping smoke condensate followed by the use of a pentane trap. The cotton wadding occasionally showed some nitrosamine compounds, primarily from those cigarettes which were enriched with nitrates; however, the pentane trap consistently produced nitrosamine compounds which Neurath concluded was an artifact. He also showed that if the residence time, *i.e.*, the time from which the smoke exited from the cigarette to the time trapped, is increased production of nitrosamine compounds increases. This indicates that the reaction also proceeds by a gaseous route. Neurath also concluded that trapping should be done at 37°C.

Serfontein and Hurter (*3*) condensed smoke in 2 traps containing dichloromethane, cooled in dry ice and acetone at freezing mixtures. It must be concluded that the results obtained in Serfontein's laboratory on nitrosamines represent artifacts at least with respect to smoking conditions of man. A method of smoking cigarettes and trapping of the condensate has been developed in our laboratories and is being presented elsewhere (*9*). It utilizes a jet impaction principle as shown in figure 1. The method satisfies most of Neurath's recommendations in that the smoke is condensed at temperatures near 37°C and no solvent is used. The smoke travel time can be varied to meet his recommended 5-second residence time. This procedure also has an advantage in the number of cigarettes that can be smoked per day by two apparatus operated by one technician as shown in figure 1. A thousand cigarettes can be easily smoked in one day. Smoking conditions can be reproduced with accuracy so that very uniform doses (for example, 45.3 mg mean dose with a standard deviation of 2.1 mg) of tobacco smoke can be delivered by direct condensation onto test animals. Or, if indirect application is preferred, the condensate can be easily recovered from vials and either worked up or applied after a chosen aging period. The quantity of smoke condensate prepared daily is important since the condensate should be worked up as soon as possible after collection as artifacts can be produced when the extract stands for a long time. Since it has also been shown that nitrosamines if present in tobacco smoke appear in rather small quantities, then large amounts of smoke condensate must be prepared for their identification.

Cleanup of Smoke Condensate

Smoking of approximately 1,000 cigarettes will produce as much as 50 g of smoke condensate. Under the best conditions, there may be as much as 100–1000 μg of nitrosamines in 50 g of smoke condensate. It is obvious that some method of purifying the nitrosamine fraction is necessary for detection and identification. The standard procedure used is to take up the smoke condensate in an organic solvent such as pentane or dichloromethane and wash this organic extract with sodium hydroxide and hydrochloric acid solutions to remove acid and basic components, leaving a neutral frac-

tion. These 2 treatments remove considerable quantities of substances; however, the neutral fraction still contains too many interfering compounds for use in most detection methods. Further cleanup of the sample has been accomplished generally in 2 ways—one utilized by Neurath *et al.* (*4–6*) converts the nitrosamines to hydrazines by reduction, then the resultant basic compound can be removed from the organic extract with an acid wash, leaving much of the impurities behind in the aqueous solution. The hydrazines are then removed from the acid wash by extraction. Serfontein and Hurter (*3*) have cleaned up the sample by distilling the neutral fraction and collecting the fraction which distills between 130 and 240°C. In order to obtain a suitable concentration in the final extract, it must be concentrated by evaporation which can lead to problems of loss of the more volatile components such as dimethyl- and diethylnitrosamine. The selection of a very volatile solvent in the final evaporation process is essential and dichloromethane is an excellent choice for nitrosamines. A microextraction procedure or column cleanup method should be very useful.

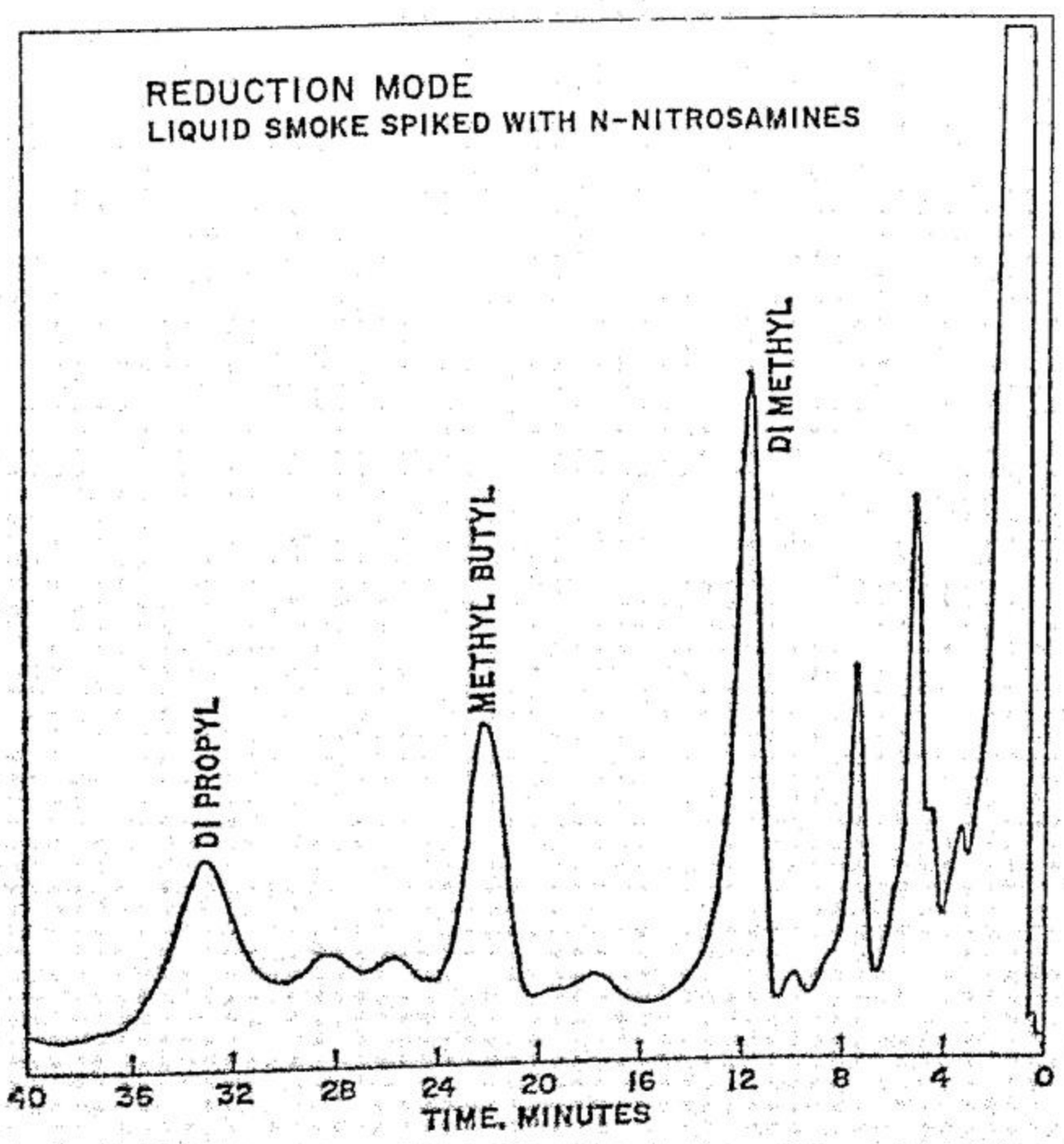
Detection and Identification of Nitrosamines

To detect and identify nitrosamine compounds in cigarette-smoke condensate it is necessary to have a high resolution procedure such as thin-layer or gas-liquid chromatography coupled with some means of selectively detecting these compounds on the plate or in the gas stream. Once the compounds have been tentatively established as being present in tobacco smoke then the individual components can be precisely identified by other means—for example, by preparative procedures followed by infrared and Nuclear Magnetic Resonance (NMR) techniques.

Preussmann *et al.* (*10*) and Serfontein and Hurter (*11*) have developed methods for the selective detection of nitrosamine compounds on thin-layer chromatograms at the microgram level. Methods utilizing gas-liquid chromatography have been reported by Serfontein and Hurter (*3*) who used a hydrogen flame detector. This type of detector will detect any organic compound which comes off the column and is not selective to nitrosamine compounds. There are a number of interfering substances and it is unlikely that the very small quantities of nitrosamine compounds could be seen by this technique. The coincidence of retention time with that of a nitrosamine standard is not a satisfactory procedure when there are so many peaks present. In our laboratories we have utilized a selective detector approach for identifying nitrosamines with gas chromatographic procedures. The initial work utilized a nitrogen detector system which eliminates many of the interferences found in smoke yet retains high sensitivity, down to a few nanograms of individual components. It was shown, however, that even with a selective nitrogen detector there were numerous interfering peaks during gas chromatographic examination of a neutral extract from smoke. Recently a further development (*12*) has

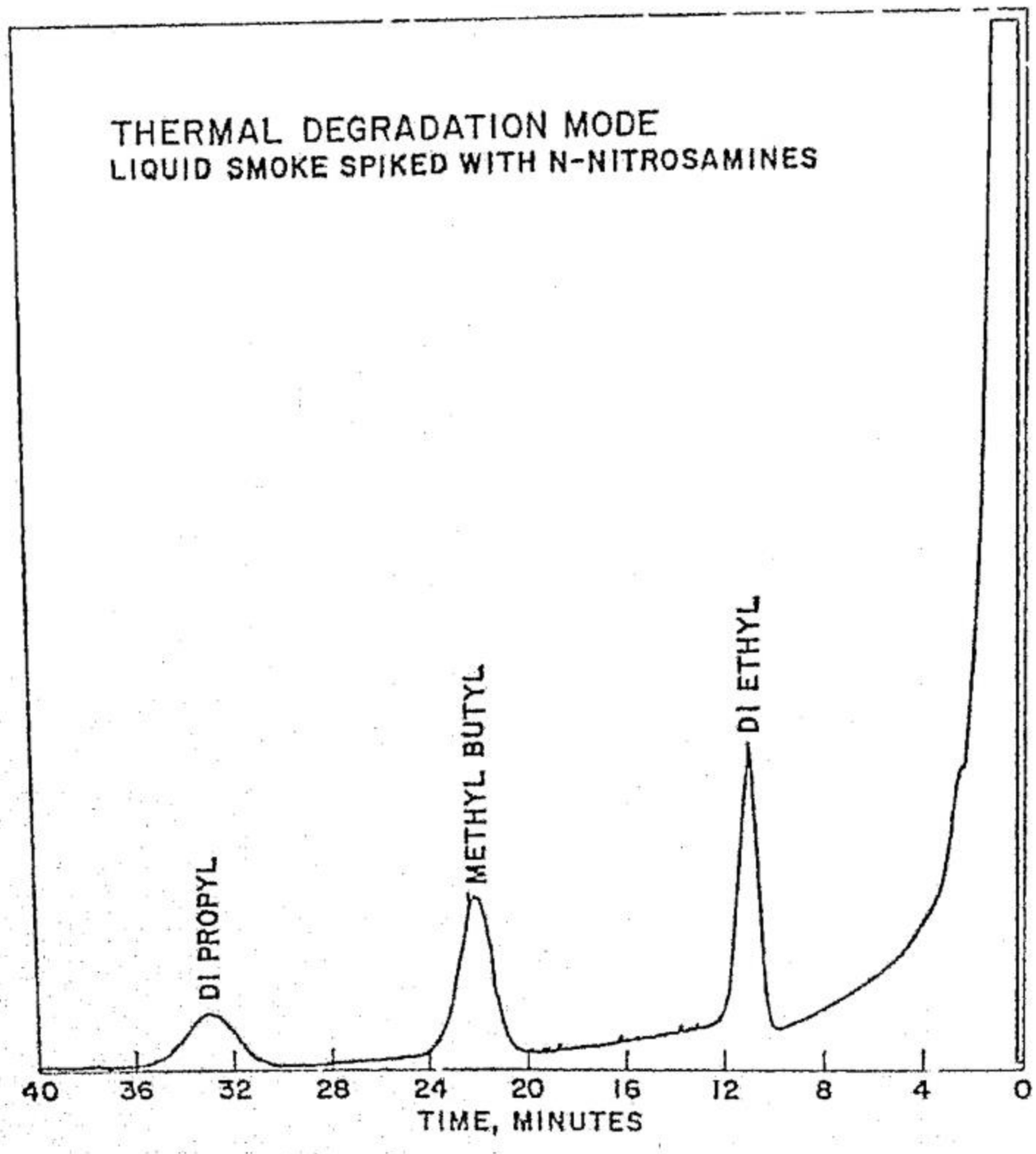
produced a detector system which is selective for amine compounds including nitrosamines. This detector system eliminates many classes of nitrogen compounds in addition to standard hydrocarbons, chlorine- and phosphorus-containing compounds. Examination of neutral smoke condensates with this detection system revealed no detectable peaks.

It is known that nitrosamine compounds are present in other products such as wheat flour, smoked fish, and a product known as "liquid smoke." In the latter 2, concentrations may be at dangerous levels. An illustration of the use of this selective detector system using liquid smoke is shown in text-figures 1 and 2. Text-figure 1 is a gas chromatogram of a neutral extract from liquid smoke with the use of the nitrogen detector. This sample has been spiked with 3 nitrosamine standards. The same extract chromatographed with the selective amine detector is shown in text-figure 2. Note that most of the interfering peaks are not seen here and the nitrosamine standards are clearly shown. Even with this selective and highly sensitive procedure, additional cleanup of the smoke-condensate sample is necessary for routine analysis of condensates for nitrosamines. Gas chromatographic columns generally can take up to 10 μ liter and in some cases slightly larger quantities of an extract. The neutral fraction from cigarette smoke cannot be evaporated past a certain point because of the accumulation of solid material. Thus the final sample is too dilute and injection of quantities of 100–1000 μ liter of sample into a gas chromatographic column is prohibitive.



TEXT-FIGURE 1.—Chromatogram of liquid smoke using nitrogen detector.

TOWARD A LESS HARMFUL CIGARETTE



TEXT-FIGURE 2.—Chromatogram of liquid smoke using amine detector.

Biological Test of the Significance of Nitrosamines in Cigarette Smoke

After a carcinogen or potential carcinogen has been identified as being present in tobacco smoke under conditions which simulate inhalation of smoke by man, then it is necessary to establish whether the compound produces a biological effect, *i.e.*, a tumor or some other harmful effect at the concentrations present in smoke. There are 2 general procedures for identifying the effect of these individual components. One is to add small quantities of the individual components or mixtures of the components to a biological system such as the back of a mouse or to inject it intramuscularly periodically. This procedure has some objections because there is a co-carcinogen effect in smoke which is difficult to duplicate in a model experiment. Another method is to remove the class of compounds from a smoke condensate without altering the other components of the smoke and then test the biological activity versus a condensate which has not been treated. The latter procedure has objections because it is very difficult to demonstrate that the remaining cigarette smoke has not been altered.

From the available literature it would appear that if nitrosamines are present in tobacco smoke under conditions similar to those prevailing in cigarettes smoked by humans, then they are present in concentrations no greater than 0.01 μg per cigarette. These are for cigarettes which are not enriched with nitrates. This quantity would be increased somewhat in nitrate-enriched cigarettes. It is questionable whether this small amount of material in a cigarette can produce a significant biological effect, and it

would be very difficult to observe the effects produced in a model experiment where a compound is added to a biological system such as the back of a mouse or injected intramuscularly over a period of time. Such an experiment can, however, be carried out and might be done by preparing the small amount of nitrosamine on small particles and depositing these either on the skin or directly into the lung of an animal. It has been shown that the addition of solid particles enhances the toxicity of sorbed individual components by creating a concentrating effect in a given area (*13*). This also tends to keep the compound from being physically removed by the biological system or eliminated through metabolic reactions.

The ideal method would be to produce an effect by inhalation of the nitrosamines, possibly via an aerosol, directly into animal lungs; however, this is extremely difficult. There have been many attempts to develop procedures whereby animals will smoke cigarettes as man does, including experiments with dogs where tracheotomies were performed so that cigarette smoke could be pulled directly into the lungs. The biggest problem is that the animals develop emphysema and other lung disorders and die without developing lung cancer. It may be that the total concentration introduced into the lung in these experiments for a brief time is too large and should be reduced and experiments carried out over a longer period.

CONCLUSIONS

To establish the presence of nitrosamines in inhaled cigarette smoke and to predict the possible hazards from their presence, it is necessary to create experimental conditions simulating as closely as possible the conditions that exist when a man smokes normally. If these conditions do not prevail, then artifacts may occur as it is suspected they do when cold or liquid trapping is used. The smoking of standard cigarettes and condensate collection under conditions which nearly match the recommendations of Neurath *et al.* (*4–6*) do not produce sufficient quantities of nitrosamines to be measured with present analytical procedures. Neurath and co-workers have shown that for the formation of nitrosamines equimolar mixtures of NO and NO_2 must be present and other reports indicate that either no NO_2 or very little is present in cigarette smoke. It is possible then that little or no nitrosamines are present in cigarette smoke under normal conditions of smoking. Because of the importance of these compounds and the known high incidence of lung cancer, the establishment of their presence in smoke is of importance in the health of man. Additional studies are needed utilizing improved methods of collection and cleanup of the sample. Detection procedures need to be made more selective and, if possible, more sensitive. Even if the nitrosamines are identified and unequivocally established as being present in cigarette smoke at a certain level then it must be investigated whether they contribute to the total toxic activity of the tobacco smoke.

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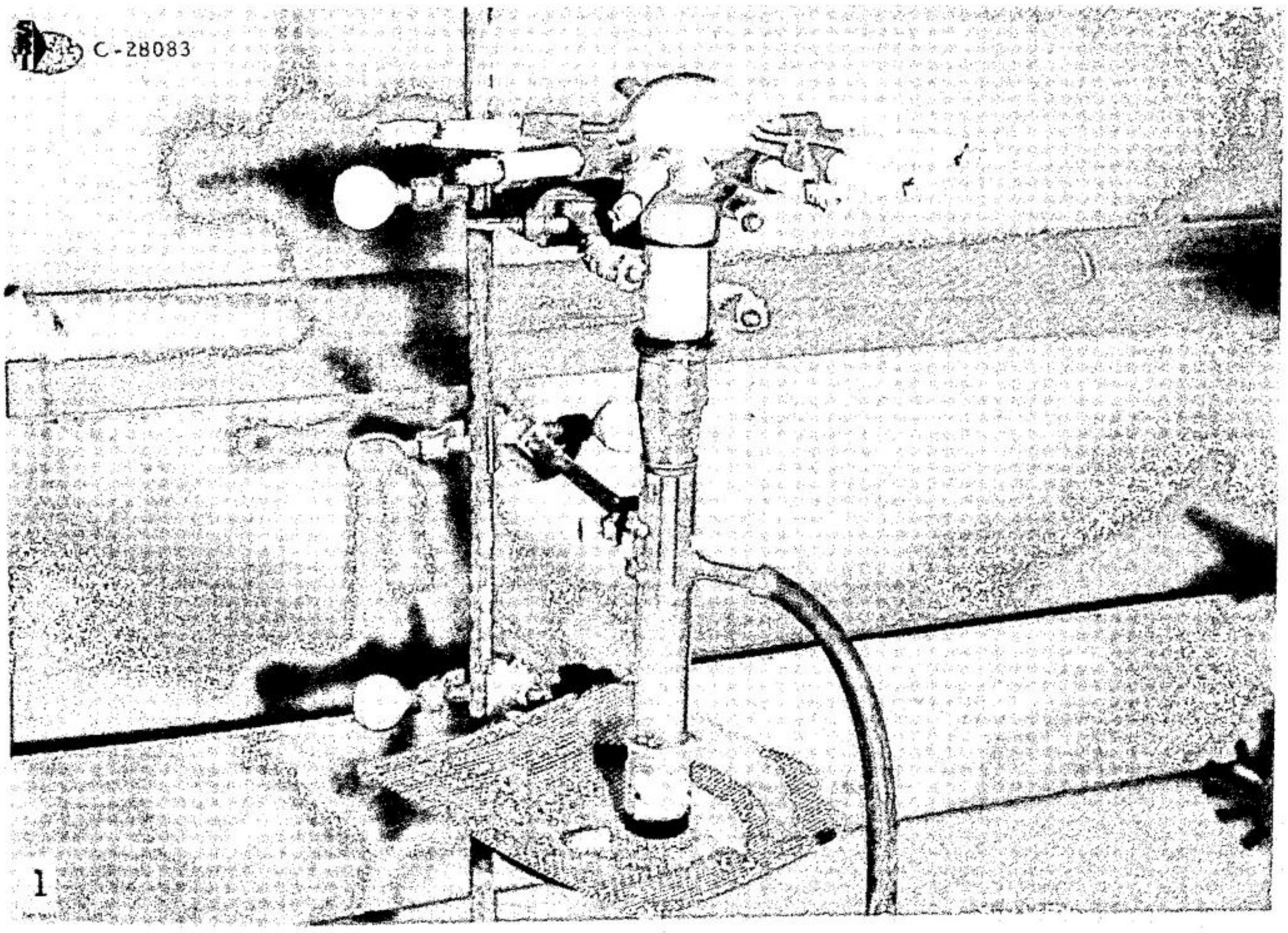
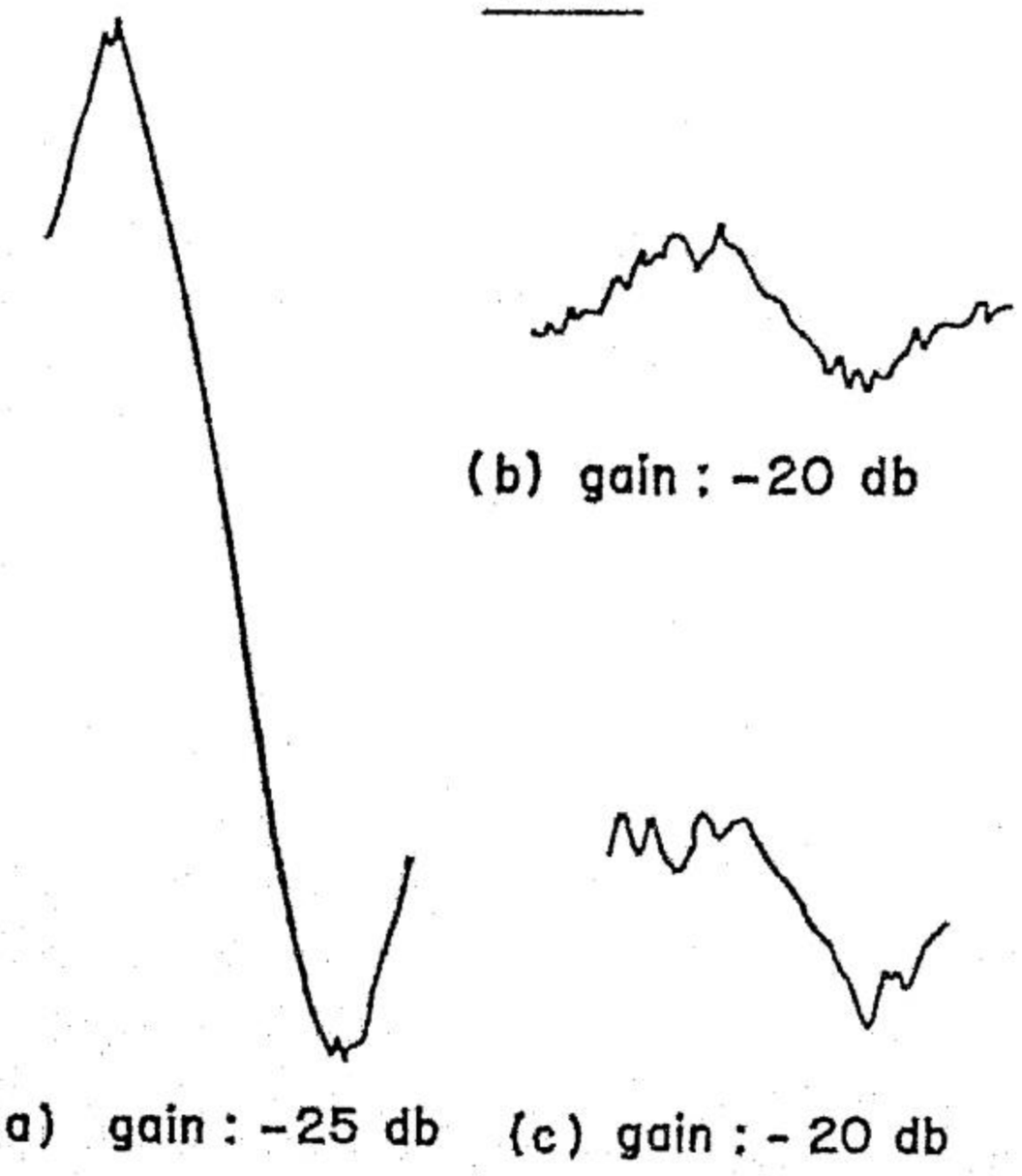


FIGURE 1. Collection of smoke by jet impaction.

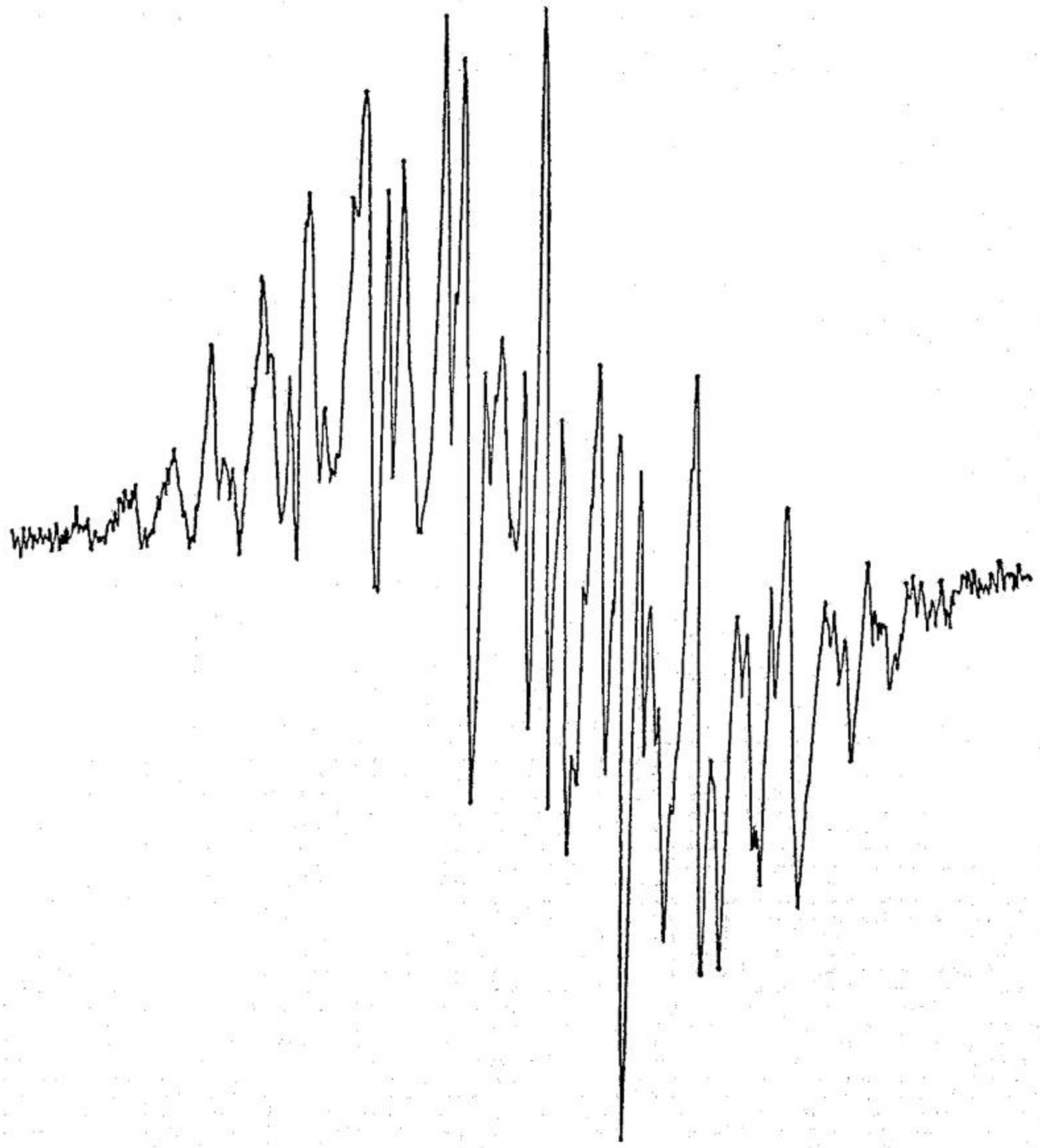
techniques, or, alternatively, that a powerful co-carcinogenic effect occurs. Additional species formed could be meta-stable free radicals or related species. Electron spin resonance (esr) spectra provide a direct measure of unpaired electron concentrations and give some indication of the identity of the free radicals present. The esr spectra of smoke condensates were therefore investigated.



TEXT-FIGURE 1.—The spectrum of smoke condensate: *a*) collected at 135°K, *b*) after warming to room temperature, and *c*) recooled to 135°K.

When tobacco smoke was condensed at low temperatures and subsequently allowed to warm to room temperature, the esr signal intensity obtained was reduced relative to the signal intensity of the "cold spectrum." Subsequent recooling of the smoke condensate to 135°K did not give the more intense spectrum (text-fig. 1). Hence, at least some of the free radicals formed during smoking possess only a limited stability under these conditions [*cf. also* (*1*)]. The shape and the location of the spectrum rule out some possible species, but do not permit identification of the contributing radicals.

To obtain further information about the smoke condensate and its ability to form free radicals, the condensate was dissolved in various acid media, such as concentrated sulfuric acid, and the resulting esr spectrum was studied. Although this gives rise to different paramagnetic species, *i.e.*, cation radicals, such experiments yield information about free radicals obtainable from the complex hydrocarbon mixtures investigated. So far, unambiguous identification of the radicals formed from smoke condensate in acid media has not been possible, although the esr spectra have been determined under a variety of different conditions. A typical spectrum is shown in text-figure 2, which, incidentally, is similar for a number of acid



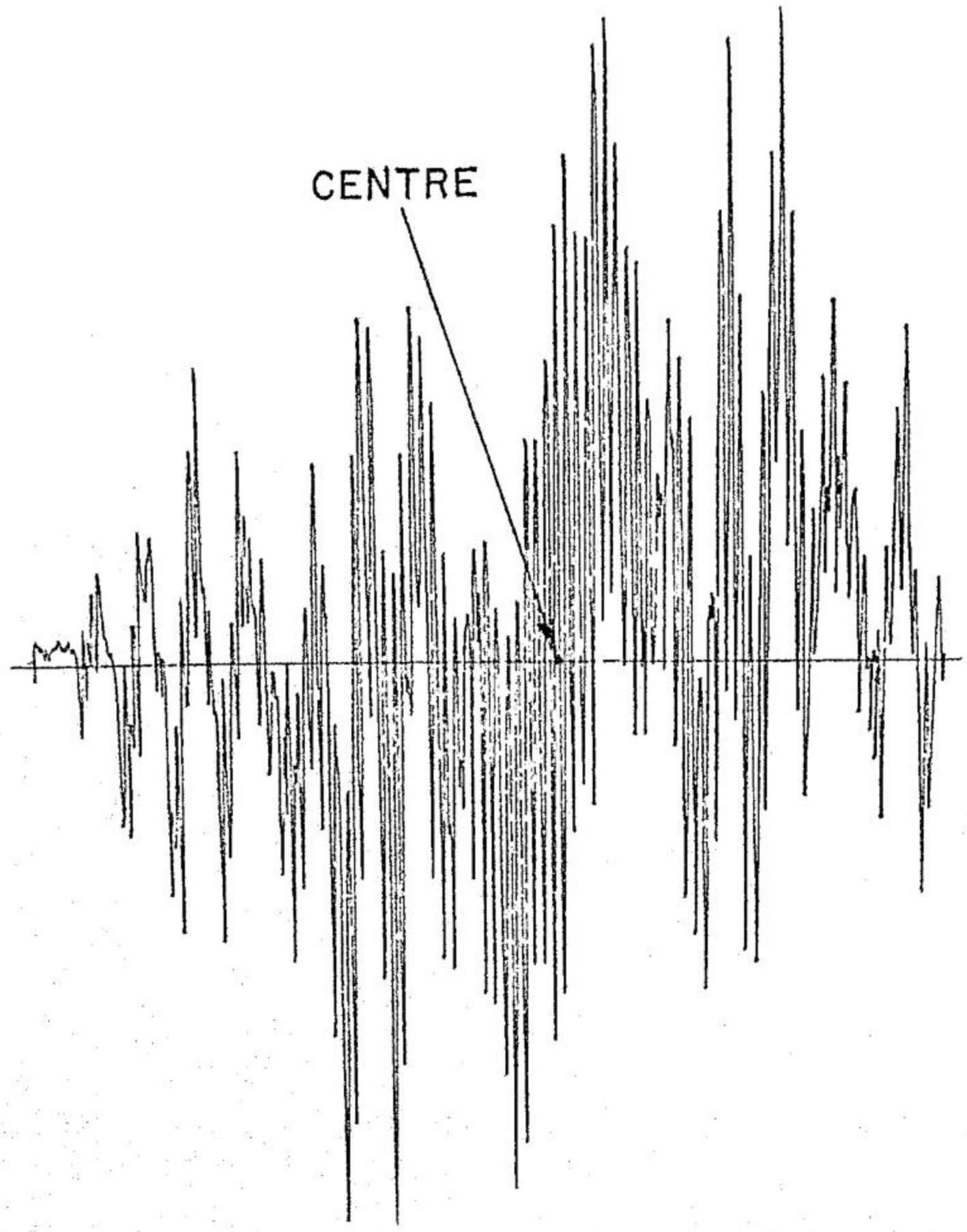
TEXT-FIGURE 2.—The spectrum of tobacco-smoke condensate dissolved in methanesulfonic acid-nitrobenzene.

media, and this and other evidence [*cf.* (*4*)] indicate the presence of only a limited number of radical species. Comparison of this spectrum (especially g-value and spectral width) with a series of reference compounds suggests that the radical cation species of smoke condensates are derived from polynuclear hydrocarbons.

RADICALS FORMED ON HEATING OF POLYNUCLEAR HYDROCARBONS

To investigate how free radicals might be formed and stabilized under actual smoking conditions, a number of polynuclear hydrocarbons—including several known carcinogens—were heated individually in air. For a large number of hydrocarbons, including azulenes and benzo[*a*]pyrene (*5*), reasonably stable free radicals (assumed to be of type RO· where R is the hydrocarbon moiety) were formed. Since RO· radicals are formed from at least some tobacco-smoke constituents on heating, this suggests one mechanism whereby radical species could be formed on smoking. These spectra were remarkably well resolved, which aids in their identification.

A typical spectrum is shown in text-figure 3. The relative stabilities and reactivities of these radicals are being investigated.

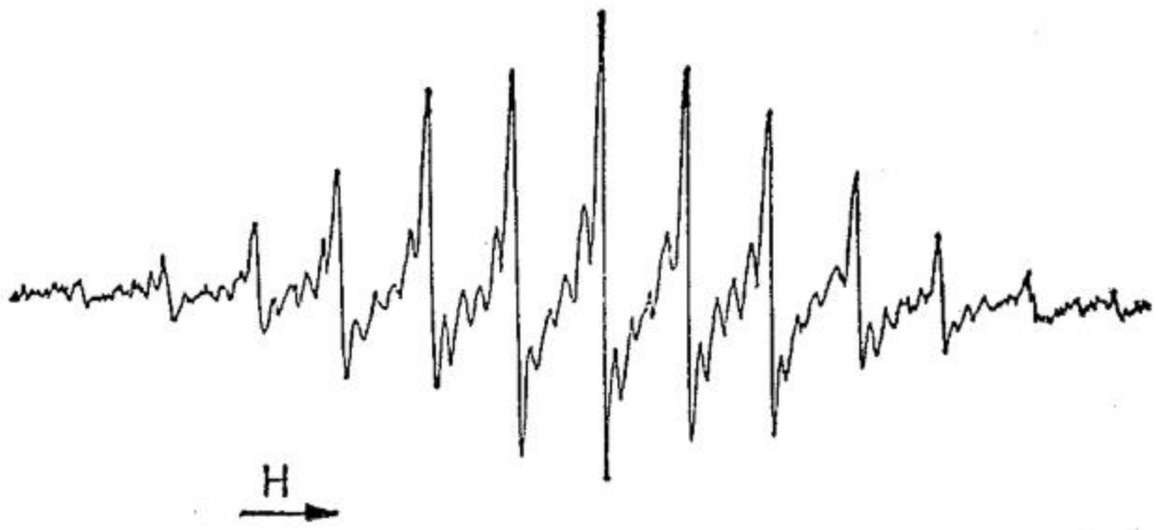


TEXT-FIGURE 3.—Electron spin resonance spectrum of benzo[*a*]pyrene, heated in air.

STABILIZATION OF UNPAIRED ELECTRONS

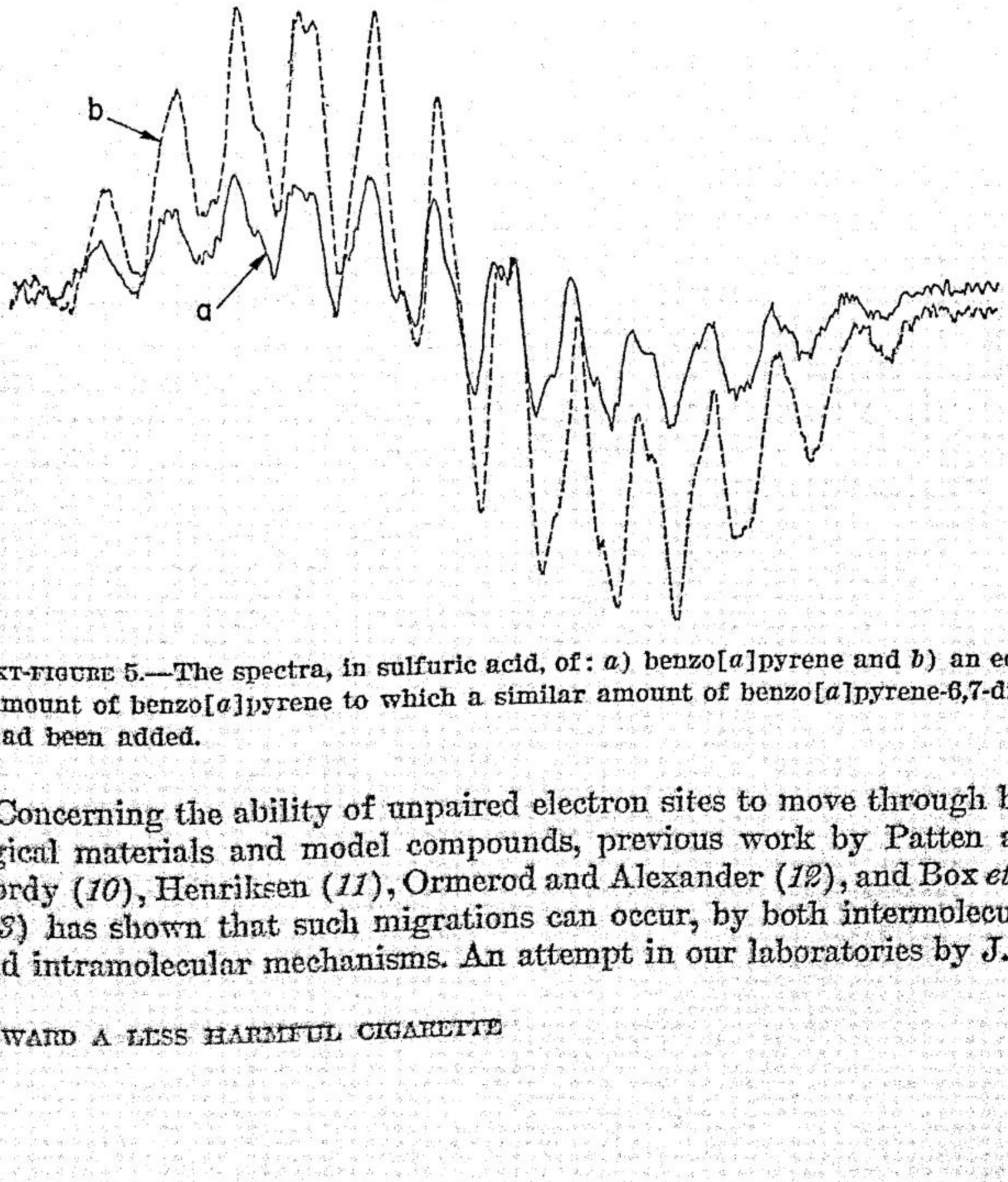
It was shown that some cation radicals may be stabilized by dimer formation. That is, aggregation of hydrocarbon molecules may occur so that the unpaired electron, temporarily at least, is distributed over more than one molecule, resulting in stabilization. Such dimer formation in acid media has previously been described by other workers for napthalene and two other simple polynuclear hydrocarbons (*6*, *7*). Text-figure 4 shows our spectrum of the coronene dimer cation (*8*); other systems, including pyrene (*9*), are being investigated. Since stabilization by dimer formation occurs for certain hydrocarbons in some environments and since smoking, as a form of pyrolysis, would be expected to give rise to various polymerization and aggregation processes, such processes may help to stabilize some of the radicals detected in smoke condensates.

Studies on model compounds also showed that spectra can be drastically altered by the presence of other compounds. This is illustrated in text-



TEXT-FIGURE 4.—Coronene dimer cation in sulfur dioxide-boron trifluoride.

figure 5 which shows that the signal intensity of benzo[*a*]pyrene in sulfuric acid is increased considerably by the addition of its quinone which on its own, in acid, does not afford a detectable signal. Preliminary experiments in sulfuric acid also indicate that tobacco-smoke condensate affords a different spectrum than that obtained from a synthetic mixture of polynuclear hydrocarbons that have been reported isolated from smoke condensate (*4*). These data therefore suggest that radicals are modified within the condensate, given conditions in which migration of unpaired electron sites can occur. These and other data [*cf.* (*4*)] also indicate that the unpaired electron density is confined to a limited number of sites.



TEXT-FIGURE 5.—The spectra, in sulfuric acid, of: *a*) benzo[*a*]pyrene and *b*) an equal amount of benzo[*a*]pyrene to which a similar amount of benzo[*a*]pyrene-6,7-dione had been added.

Concerning the ability of unpaired electron sites to move through biological materials and model compounds, previous work by Patten and Gordy (*10*), Henriksen (*11*), Ormerod and Alexander (*12*), and Box *et al.* (*13*) has shown that such migrations can occur, by both intermolecular and intramolecular mechanisms. An attempt in our laboratories by J. V.

Ramsbottom to investigate these phenomena quantitatively, by irradiating crystals of sulfur-containing amino acids with ultraviolet light and studying the decay rate of the free radicals formed, indicates that some such migrations can occur with surprisingly low activation energies [<5 kcals/mole (*14*)]. Although these results were obtained on relatively simple systems, they suggest that unpaired electron sites may move through biological macromolecules, with greater ease than might have been expected, to reach energetically favorable locations.

CONCLUSIONS

The preliminary results so far obtained are consistent with the hypothesis that a variety of free radicals is formed during smoking. The study of model compounds suggests that different radicals are formed depending on the molecular environment (that is, state of aggregation, amounts of water and air present, etc.) and, consequently, that the type of radical formed may also depend on smoking conditions. Moreover, some of these free radicals may be expected to be able to reach sensitive sites within biological materials to bring about chemical changes.

No direct evidence exists concerning the relation of these changes to carcinogenicity, but free radicals would be expected to cause specific chemical reactions, presumably harmful to biological material. Moreover, exposure to radiation—which causes free radical formation—is known to give rise to cancer and other harmful effects, such as general life shortening. More speculatively, although the passages leading to the lung can remove foreign bodies from the respiratory tract efficiently, radical species may be able to react with tissue before they can be removed in this way. To investigate this possibility, we are condensing tobacco smoke onto lung tissue under different conditions. Although at present there is no direct experimental evidence for a connection between certain free radicals and tobacco-smoke carcinogenesis, if such a relationship exists, this may be relevant to any assumed relationship between "tar" or nicotine concentration and tobacco-smoke carcinogenesis.

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Role of Radioactive Substances in Effects of Smoking

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THE hazard to health posed by cigarette smoking has brought about an accelerated effort in recent years to identify tobacco constituents which might play a contributory role in effects which correlate with smoking. Since radiation has a well-documented contributory role in carcinogenesis, it is not surprising that considerable attention has focused on radioactive substances in tobacco and in the smoke from cigarettes. The pertinent literature on this subject has been reviewed recently (*1*).

The predominant β-emitter in tobacco is K^{40} (*2*, *3*). Although other elements might contribute to the total β-activity in tobacco smoke, only Sr^{90} has been reported to be present in appreciable concentrations relative to K^{40} content (*4*). Of greater interest has been the tobacco content of materials which emit shorter range, more densely ionizing α-particles. Elements of the radium chain have been found in variable quantities in different types of tobacco (*5–7*). Divergent interpretation is exemplified by the calculations of Turner and Radley (*5*) and Marsden and Collins (*8*). Although part of the disagreement arises from differences in analytical results and procedures, a major difficulty lies in uncertainties about the precise patterns of deposition and clearance of the components of interest.

Considerable attention has recently been directed to Po^{210}. The level of this element in cigarette smoke reported by Radford and Hunt (*9*) has been confirmed spectroscopically (*10*). Attempts to arrive at dose-response interpretation have been hampered by the lack of adequate information about deposition and clearance kinetics and/or an apparent reluctance or inability to apply data from animal studies with Po^{210} and other α-emitters. Further emphasis on the need for the use of such information is given by the work of Little and coworkers (*11*, *12*), Holtzman (*13*), and others on the distribution of Po^{210} in tissues of smokers. Among the experimental work on Po^{210} are two volumes which contain a major portion of the work

on this element at the University of Rochester Atomic Energy Project, one appearing in 1950 (*14*) and the other in 1964 (*15*).

ROLE OF RADIOACTIVE SUBSTANCES

1) Do radioactive substances, indeed, play a role in the effects related to smoking and in what terms can the role be defined? 2) To what extent does total radiation dose or dose rate contribute to effects relative to and in context with all other contributions whether in tobacco smoke or not? 3) What is the mechanism by which the contribution is made to the disease process?

These and other similar questions impose certain requirements and restrictions on any further consideration. First, a primary limitation is that the effect under consideration must be identified as precisely as possible. Selection of an endpoint or criterion of effect is critical to establishment of a role for any impinging substance. The direct involvement of a material in a disease process becomes less subject to assessment the more diffuse and general the criteria of effect become. For example, the association of "smoking" and "pulmonary disease," although epidemiologicaly significant, is too imprecise to allow for meaningful assessment of the role of a specific material in fundamental terms.

Second, it is virtually axiomatic that in order to place a causal or contributory label on any material in a specific disease process, there must be a well-defined understanding of the initiation and progression of the disease. Without such an understanding, any relationship between a particular substance and the disease entity is largely suggestive. In short, it is unlikely that a specific "role" can be assigned unless there is prior knowledge of a corresponding niche in the etiology and development of the disease.

Third, with particular regard to radioactive substances, it is essential to take cognizance of all aspects of the pharmacokinetics of the inhaled material, including site of deposition, initial distribution of the deposited material, the redistribution and clearance patterns, and the time of contact with the tissue structures of interest, particularly the respiratory tract. Only by full utilization of such information can one approach questions of the locus of initiation of an effect, the influence on the progress of the disease, and the relation of these events with the temporal and spatial distribution of the radiation dose.

RADIATION AND SMOKING RELATED DISEASES

When effects of smoking are discussed, three diseases are mentioned most often and appear to be most closely linked to smoking: cardiovascular disease, pulmonary emphysema, and neoplastic changes, especially lung carcinoma. Although a detailed review of literature pertinent to the rela-

tions of radiation to these diseases is outside the scope of this paper, general conclusions can be summarized as to the probable magnitude of the role of radioactive substances. Particular reference is made to experimental work with Po^{210}.

Cardiovascular Effects

With respect to cardiovascular problems, the pharmacological effects of nicotine are classical and well documented. Effects of other constituents of tobacco smoke are less well defined but remain as possible contributors to effects on the cardiovascular system. One must also view cardiovascular changes as they might represent sequelae or secondary responses to pulmonary injury. Radioactive materials, at the levels reported in tobacco smoke, produce no known, substantiated, direct effects on the cardiovascular system.

Experimental studies with Po^{210} in rats (*16*) have demonstrated a prematurely occurring, generalized arteriosclerosis, with the extent of temporal advancement related to the size of the single intravenous dose of Po^{210} given (1–20 μc/kg). Of particular interest is the report of arteriolar nephrosclerosis at a single intravenous dose of 10 μc/kg which was more pronounced than at doses of 1, 5, or 20 μc/kg. This finding suggests that the dose or dose rate is crucial in the production of differing types of arteriolar responses. Multiple doses of 1.5 μc/kg/month to a total dose of approximately 10 μc/kg resulted in a considerably less marked arteriolar nephrosclerosis (*17*).

The radiation dose to blood vessels for the lifetime of the rats can arise from the circulating blood which contains a significant fraction of the body burden (*18*) or from tissue concentrations including possible concentration in vessel walls. Examples of this apparent concentration are shown in the rat after inhalation (fig. 1) and in the rabbit after intratracheal insufflation (fig. 2). In addition to general arteriosclerotic changes, some cardiac enlargement (left ventricle) was observed at long periods after a 10 μc/kg single dose. It was suggested that this was secondary to the arteriosclerosis and consequent hypertension (*16*).

These and other changes result from dosages of Po^{210} which are extreme compared to those expected from human smoking, but they serve to illustrate that the possibility of subtle contributions to effects on the vasculature cannot be ignored. Although subject to further experimentation at realistic levels, it is probable that the radioactive contribution is not large, based on current information.

Emphysema

There is probably little dispute that chronic irritation of the lung can be implicated as a possible factor in the etiology of pulmonary emphysema. There would be even more accord that inhalation of cigarette smoke can be a source of chronic irritation to the mucosa of the respiratory tract.

However, there is no unequivocal evidence that the levels of radioactive material identified in cigarette smoke can produce such chronic irritation. On the contrary, there is some evidence that much higher doses are needed to produce a detectable pneumonitis or bronchitis.

For example, a minimal single dose of about 1000 rad of X rays is considered necessary to produce radiation pneumonitis in rat, rabbit, and dog (*19*), and a minimal single dose of over 2000 rad is considered necessary to produce an acute response in human lung (pneumonitis) (*20*). Single inhalation doses of Po^{210} in rats of 0.05–0.1 μc (*21–23*) results in no lung response attributable to the radioactive material. No relation of emphysema was found with single intravenous doses of Po^{210} ranging up to 20 μc/kg (*16*) or with equivalent doses given monthly for about a year (*17*).

Additional data with other radioactive materials in human and animal studies could be cited. It is important to note that, even for minimally detectable responses of the lung, dosage must exceed that found in cigarette smoke by very large factors. A crucial study would be difficult to perform because a nebulous area of borderline judgment would be required. From available data, except possibly for lymphatic reactions (*16*, *17*), it would appear that radioactive substances would play only a small role relative to the rest of the smoke stream in the etiology of emphysema.

Carcinogenesis

Conceptually, carcinoma can be considered to arise from an "initiating" factor that induces a basic change in a cell and "promoting" factors which create or provide a milieu in which growth and development of neoplastic cells can occur. Evidence is ample that radiation can induce cellular changes which carry the potential for tumor development. Citations are not necessary to document this fact whether the mechanism is a true induction, an increased incidence in a population, or the occurrence of a neoplasm at an advanced time compared with the course of spontaneous tumors. Thus, increasing the radiation dose through smoking can be considered to increase the probability of the occurrence of an initiating event. What remains unknown is the degree to which the probability is increased as distinct from all other contributions of other materials in the smoke. Another significant unknown factor is the pattern of dose and dosage resulting from inhalation of these levels of radioactivity and whether the pattern can produce an increase in incidence equivalent to that which might be predicted on theoretical grounds.

PULMONARY CLEARANCE PATTERNS

Consideration of any disease process leads one to the question of dose distribution of the inhaled material. Of particular pertinence is information on the pharmacokinetics and particulate behavior of α-emitters, espe-

cially Po^{210}. Although much of the available information has been gained from studies in animals without the complicating feature of tars and resins as carriers, several points about the patterns of clearance of these materials should be useful.

Among the many reviews dealing with deposition and clearance of particulate material may be cited examples of a few which deal with α-emitting heavy elements (*24–26*). The dose delivered to respiratory tract structures depends on several factors: the locus of deposition, the pathway of clearance, the rapidity of movement, and the relative size of the source (ion, small or large particle) at the onset and during the course of clearance. Several reported observations are relevant in the context of this paper.

In an NaCl vector particle size range of about 0.05μ count median diameter, approximately 50% of the inhaled polonium is deposited in the respiratory tract of rats (*21–22*) and dogs (*27*) which is divided about equally between upper and lower parts of the tract. The biological half time in the upper respiratory tract is about 6–12 hours and approximates 20 days in parenchymal regions. Although a mean dose to the tissue can be calculated based on these data and assumptions of the structural configuration of the respiratory tree, the true dose to specific areas will be different from the average dose, often by decidedly large factors.

Among the observations previously reported for α-emitting radioisotopes, several are worth mentioning to illustrate some of the microdistribution features which must be considered for an adequate assessment of radiation dose in relation to effects. First, it should be pointed out that Po^{210} behaves as a colloid in the body whether administered intravenously or orally (28) or inhaled on an NaCl carrier. With extended periods of time following administration, the colloid is presumably solubilized so that the polonium form is a more diffusible, nonparticulate one. The physical form in which this material exists in tobacco smoke has not been established. Although at elevated temperature polonium is probably adsorbed in widely dispersed form on small smoke particles, it is not known whether it remains bound *in vivo* or whether an effectively particulate mass is formed on deposition, either by recombination of polonium with other materials or coagulation of smoke particles.

Following inhalation of "insoluble" polonium colloid, a fraction of the deposited material is very rapidly distributed systemically. The remaining material, that deposited on large bronchiolar structures, is rapidly cleared by the ciliated epithelium. Particles deposited in alveoli are found in phagocytic cells within 2 hours after deposition. In the movement of phagocytic elements from the alveoli, cells and particles collect at or near the site of the beginning of the ciliated epithelium. Figure 3 illustrates such an accumulation (*23*). Obviously, the dose delivered to this area is higher than would be predicted from calculations based solely on concentration and tacitly or overtly assumed uniform distribution.

At other levels of the tracheobronchiolar tree, similarly inordinate doses result from the ciliary clearance patterns. Bronchiolar branch points receive a higher dose than might be predicted (*23*). This results from the passage of material over a surface at the juncture of bronchioles which is narrower than would be accounted for by customary calculation but also from the change in mucous flow as described by Hilding (*29*) in which material can be caught up in eddy currents. Other related sites of high dosage are the "faults" or nonciliated patches of bronchiolar epithelium.

Another source of dose to the basal layer of the bronchiolar epithelium is not considered in any conventional dose calculations. Transport of material from alveolar regions to regional lymph nodes places sources of radiation doses in small areas of lymphoid tissue in juxtaposition with small bronchioles and in lymphatic vessels near the bronchiolar structures. Lymphatic transport has been demonstrated for polonium (*22, 23*) and the lymphatic contribution to epithelial dose has been well illustrated for PuO_2 particles (*30*).

In addition to the points given above, precise values for the several patterns of mucous movement and velocity of movement are not available. Thus, calculation of the "dose" with which to relate effects is, at best, a rough approximation. Finally, it should be mentioned that other areas of systemic distribution of radioactive components of cigarette smoke might receive some attention. One example is the lymphoid tissue, which is made up of radiosensitive cells, and the vascular deposits illustrated in figures 1 and 2. Other possible sites are the kidney in which most polonium is deposited in the proximal tubules (fig. 4), the biliary system which cycles significant quantities of Po^{210} (fig. 5), and hair follicles (fig. 6) (*27*).

In summary, it cannot be concluded that radioactive materials in cigarette smoke do not contribute to those effects thought to be related to smoking. However, current information suggests that sizable direct contribution cannot be claimed in comparison with the potential contribution of other elements of the smoke. Chronic, subtle contribution is possible, but precise mechanisms are unknown as are the necessary facts for accurate assessment of dose. Finally, other systems might be examined more critically in view of the systemic distribution of the radioactive materials.

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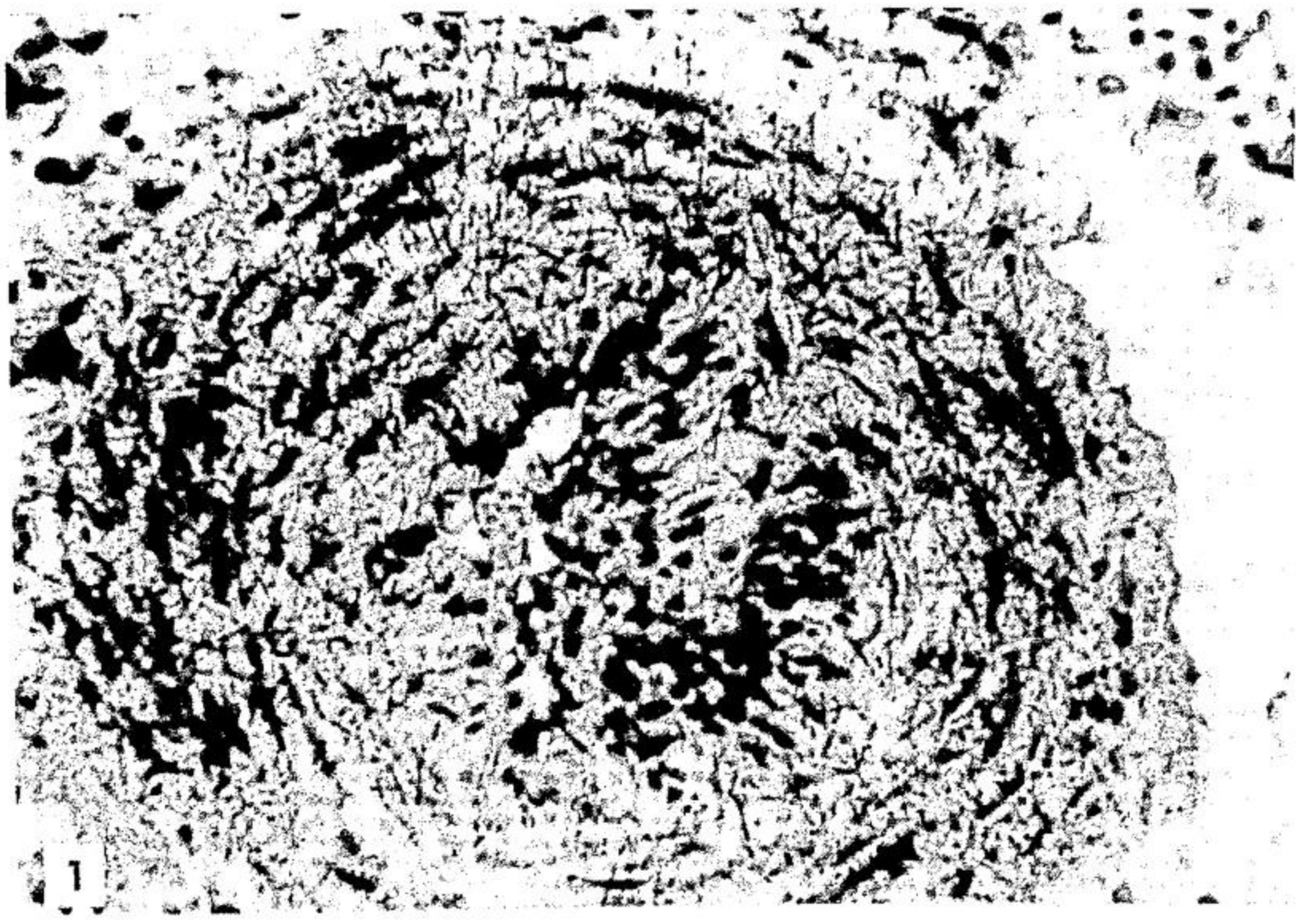

FIGURE 1.—Po^{210} concentration in vessel wall following inhalation in the rat.

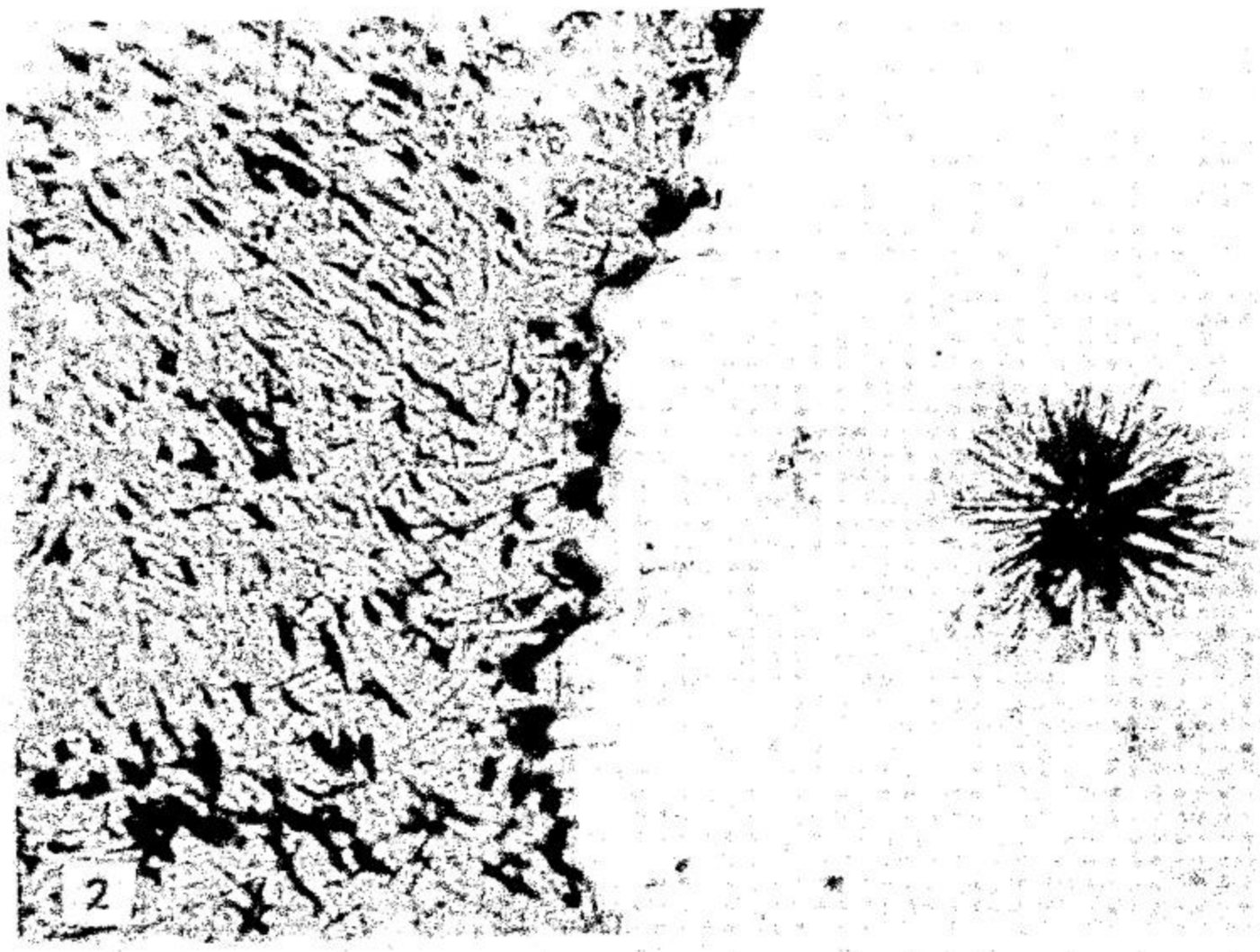

FIGURE 2.—Po^{210} concentration of 'ionic' polonium in vessel wall and colloid in lumen. Intratracheal injection in rabbit.

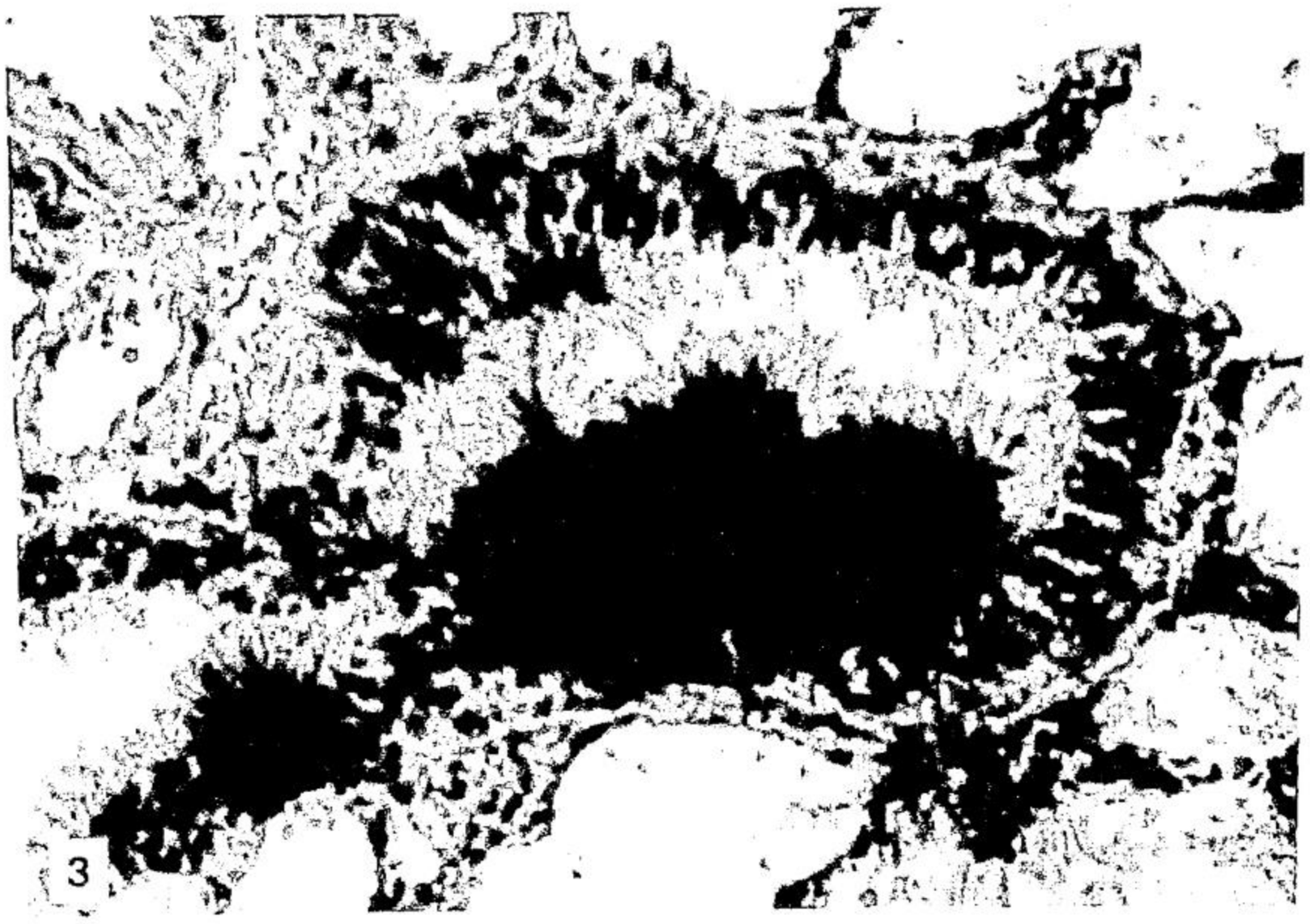

FIGURE 3.—Accumulation of Po^{210} colloid in the bronchiole during early clearance of particles from alveoli. Inhalation in rat.

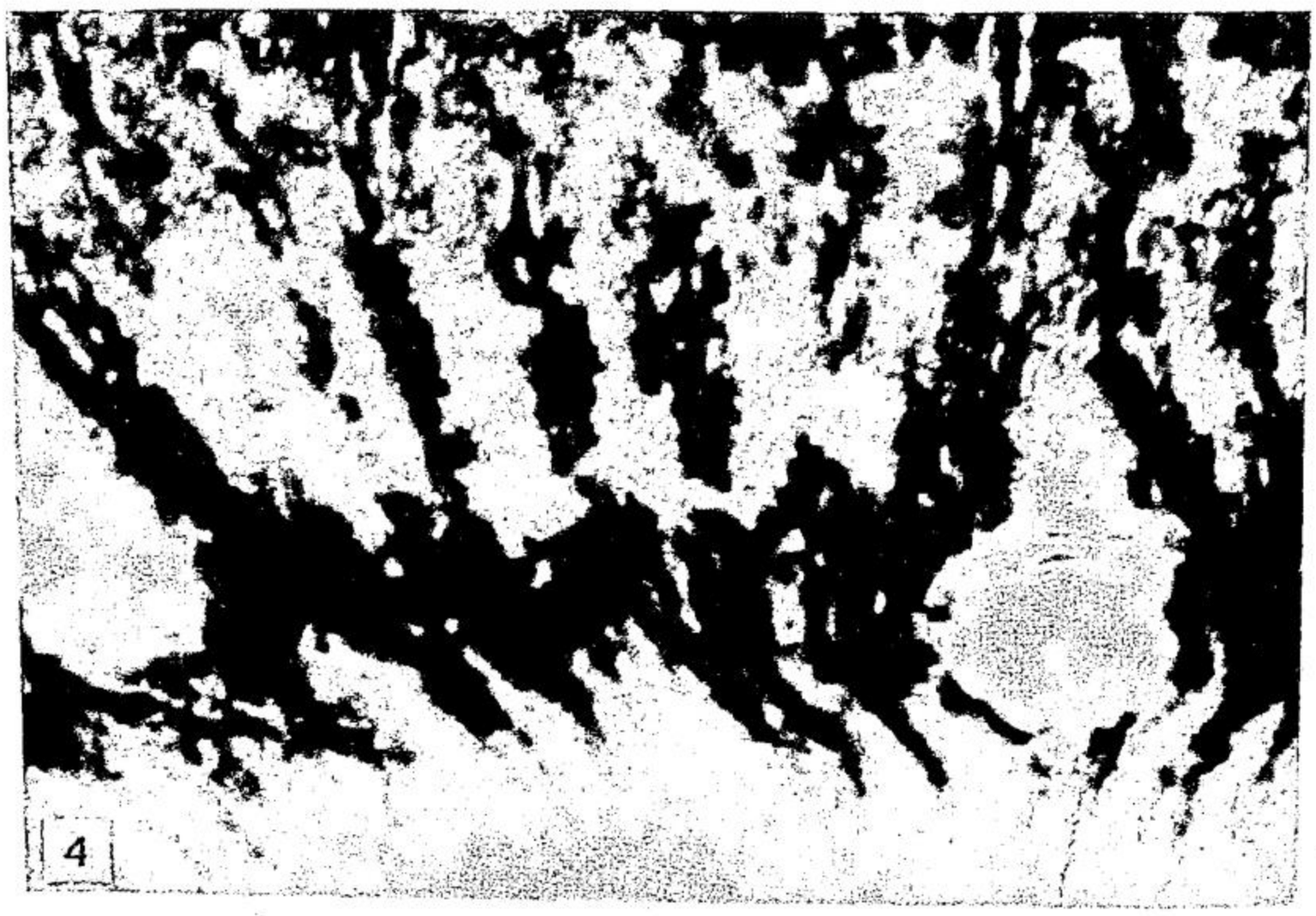

FIGURE 4.—Concentration of Po^{210} (nonparticulate) in the proximal tubules of the rat kidney.

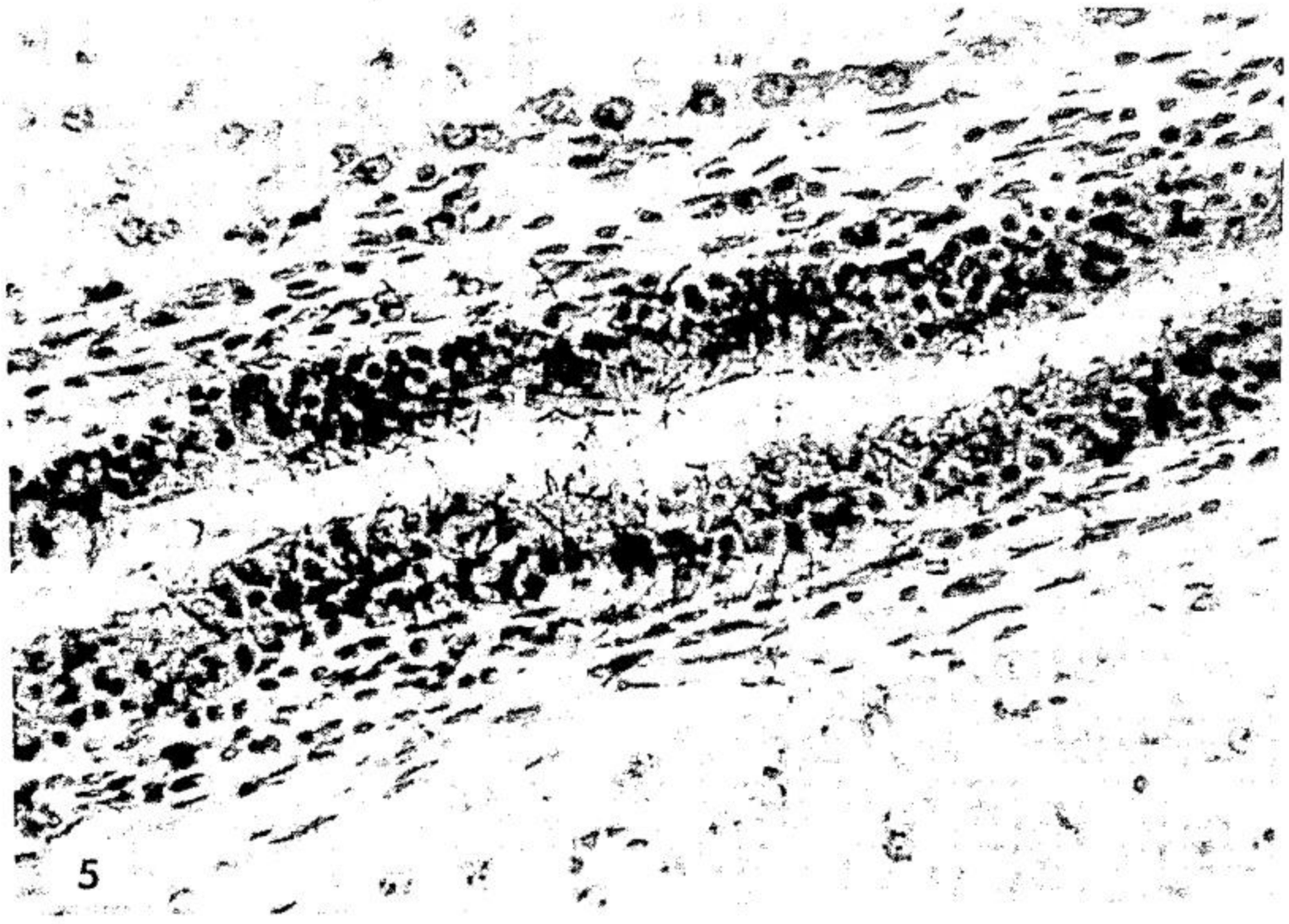

FIGURE 5. Po^{210} in bile duct of rat liver after inhalation.

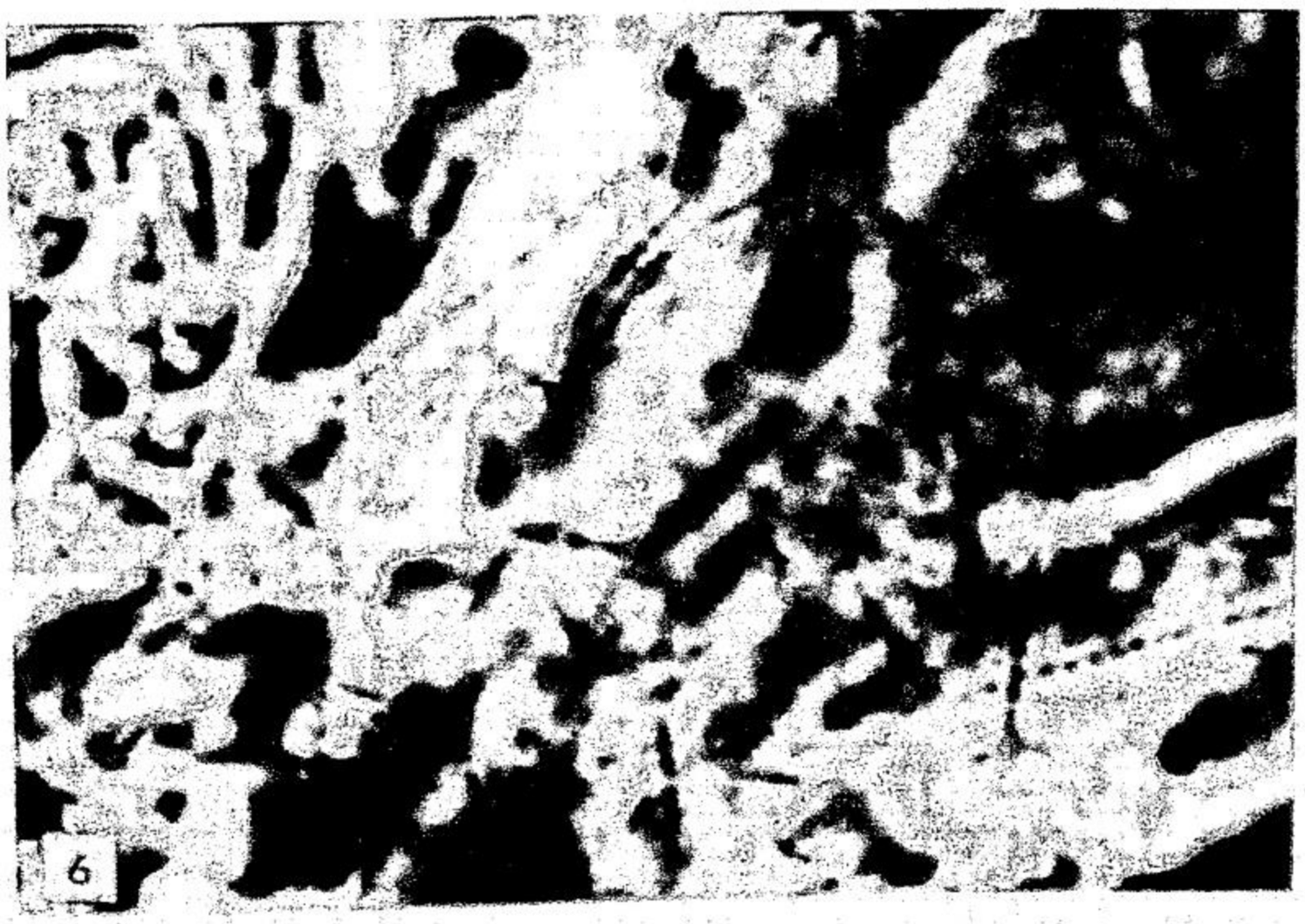

FIGURE 6. Po^{210} in hair follicle of dog after inhalation.

Po^{210} and Pb^{210} in Tobacco

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THE Po^{210} content in various U.S. tobacco types ranges from 0.15 to 1.01 pc/g.[1,2] The natural abundance of Ra^{226} in tobacco soils is between 0.52 and 1.53 pc/g—generally higher in fields of continued cultivation and heavy phosphate fertilization.

Tobacco plants grown in chambers enriched with Rn^{222} in the atmosphere (500 times greater than normal background) only had twice the amount of Po^{210} as the control, indicating that airborne Rn^{222} and its daughters are not the major source of Pb^{210} or Po^{210} in leaf tobacco.[3]

Evidence obtained so far shows that major portions of these radioelements were probably absorbed through the roots. Pb^{210} applied to growing tobacco leaves was not freely translocated with other areas.

Additional studies are under way to find the exact source of these radioelements. Fertilizers, if necessary, can be purified without too much difficulty.

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289-019—69——17

Examination of Aflatoxin in Tobacco and in Cigarette Smoke[1]

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AMONG various microflora identified on tobacco leaf, *Aspergilus flavus* is considered the most important because it produces highly carcinogenic aflatoxins. Studies were made to examine the possible presence of aflatoxin B_1, the most toxic compound among this group, in various tobacco samples including: *a*) good-grade tobacco from flue-cured, Burley, and Maryland types; *b*) three samples of "moldy" tobacco from flue-cured type, and *c*) cigarette-smoke condensate. No aflatoxin B_1 was detected in any of these materials. Pure aflatoxin B_1 added to cigarettes failed to recover in its smoke condensate, indicating this compound was either decomposed or changed through the smoking process.

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Gaseous Components of Tobacco Smoke

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THE GAS PHASE OF CIGARETTE SMOKE

THE gaseous components form what is called the gas phase of the smoke. This term is not clearly defined. In this paper it means the gaseous mixture obtained by elimination of all condensed material from the whole smoke, for instance, by filtration through an efficient aerosol filter such as a Cambridge glass fiber filter. Some authors divide this mixture into gas and vapor phases according to the boiling points of the components below and above room temperature.

RELATION OF GAS PHASE TO WHOLE SMOKE

Table 1 shows the overall composition of cigarette smoke. Among the substances derived from tobacco, excluding air constituents, some 20% are in the gaseous state. More than 90% of this gaseous material is of a very common nature, with carbon monoxide as the only substance of physiological interest. The gaseous components characteristic for tobacco smoke, mainly organic substances, comprise not more than 1.5% of the

TABLE 1.—Overall composition of whole cigarette smoke, weight percent. Figures from (*1*).

Composition	Percent	Derived from:	Percent
Nitrogen	59.0	Air	73.4
Oxygen	13.4		
Argon	1.0		
Carbon dioxide	13.6	Gaseous pyrolysis and evaporation products from tobacco	19.6
Carbon monoxide	3.2		
Water (27°C)	1.2		
Hydrogen	0.1		
Hydrogen cyanide	0.1		
Organic compounds	1.4		
Condensed material (including water)	8.2	Particulate phase	8.2
Total	101.2		101.2

whole smoke. It is still premature to state how many substances form this small part of the smoke. There is good evidence for the existence of approximately 500 components, more than 200 of which are identified or characterized by molecular formulas or molecular weights. There is no doubt that the actual number of components is much greater. However, it is very likely that substances possibly detected in the future will be of minute quantitative importance.

FORMATION OF GAS PHASE

Since volatile constituents of the tobacco leaf are evaporated during the various heating and ventilating steps of cigarette manufacture, most gaseous smoke components are substances not previously present in tobacco, but formed by pyrolysis of nonvolatile leaf constituents. It is not surprising, therefore, that almost every shape of carbon framework, combined with the whole choice of fundamental functional groups, containing oxygen, nitrogen, and sulfur, has been found.

Analysts usually divide the gas phase into three groups: permanent and inorganic gases and vapors, hydrocarbons, and the remaining organic components. Besides different chemical behavior, the three groups differ in volatility, the last group containing the components with highest boiling points. Thus it is impossible to use the same analytical methods for the whole gas phase.

A short survey of the present knowledge about the three groups is given in the following paragraphs, with some examples from the original literature indicated. No complete treatment is attempted.

GASES AND VAPORS OF PRIMARILY INORGANIC NATURE

Profound work has been accomplished on the permanent gases (*2–5*) including accurate quantitative studies and elucidation of the different roles of oxygen and nitrogen entering from the air or bound in tobacco. Work on the latter problem is still under way (*6*). Other authors endeavored to differentiate between main-stream and side-stream smoke (*7*, *8*). Nitric oxide, nitrogen dioxide (*9*, *10*), and nitrous oxide (*11*) have been determined. It seems, however, that formation, behavior, and analysis of the oxides of nitrogen have not yet been treated sufficiently. Among the smoke components of mixed inorganic and organic character, carbon disulfide, methyl nitrite, methyl thionitrite, and methyl isocyanate were investigated (*11–14*). Recently a survey of the sulfur compounds has appeared (*15*).

LOW-MOLECULAR-WEIGHT HYDROCARBONS

Between 1955 and 1960 several authors (*2*, *16*, *17*) found that the most volatile part of the organic gases and vapors essentially was a mixture of saturated and unsaturated hydrocarbons. A few years later a qualitative and quantitative survey of this part of the gas phase was published which is considered an almost complete one (*18*, *19*). Thirty-eight hydrocarbons, from C_1–C_7, were identified and estimated. Saturates and alkenes are present in similar amounts, whereas the level of dienes is approximately 3 times lower. The rough composition does not differ greatly from that observed in the products from thermal cracking of gasoline, except for isoprene which, as a pyrolysis product of terpenoids, is a major gaseous component of tobacco smoke.

ORGANIC VAPORS WITH VARIOUS FUNCTIONAL GROUPS

Analysis of the organic vapors of mixed functionality (aromatics, oxygen, and nitrogen compounds) was started simultaneously with that of hydrocarbons (*20*). But soon it became evident that the number of components was much greater. Thus special gas chromatography equipment with high separation power had to be developed to meet the difficulties (*21*, *22*). The best results were obtained with long, glass capillary columns directly combined with a mass spectrometer. The chromatograms showed some 400 peaks (*22*). Information on 167 neutral components was obtained (96 identifications, 32 molecular structures, 39 molecular weights). There is no doubt that the separation is still insufficient. Thus no prediction on the final number of components is possible. Strongly acidic components, especially the low-molecular-weight fatty acids, present great analytical problems and have not had wide analytical attention (*23*, *24*). The same is true for basic amines (*25*).

Quantitative information on this section of the gas phase, representing the overlap region between the gas and particulate phases, is much poorer than on the more volatile sections: first, because the separation of the numerous components is insufficient, and second, due to the relatively low volatility. Quantitative variations are greater, the higher the boiling points, since high-point boiling substances tend to be condensed or adsorbed on the unburned tobacco or on solid surfaces of the analytical apparatus. The latter problem becomes less important when larger samples are used. This, in turn, decreases the chromatographic separation efficiency. As a compromise, very long, packed, gas chromatographic columns were used (*18*).

HANDLING OF THE GAS PHASE

Sampling, manipulation, and analysis of the gas phase, especially when organic vapors are of interest, are difficult for the following reasons:

Some components, *e.g.*, the unsaturated carbonyl compounds, tend to polymerize or to react with other components.

There is no flask or construction material for apparatus that would not alter the gas phase composition by adsorption; contact with most plastic or rubber material, for instance, is detrimental (*26*).

The high dilution often requires concentration steps leading to artifacts.

Variation in the amount of single components between the gas phases from two "identical" cigarettes may reach the order of ±50% (*18*).

It is difficult to separate the gas phase from the condensed material without influencing the composition (*21*, *27*).

For experiments with the gas phase, it is therefore recommended that variations be measured in the composition or recovery of selected components.

GAS PHASE COMPOSITION AND TOBACCO TYPE

In the early days of analysis by gas chromatography there was much hope that typical differences in the gas phase of different types of tobacco would be detected. Such differences were actually found, but were less frequent and less important than expected. The level of furan and its homologues, for instance, is directly related to the sugar content of tobacco. Similarly, high protein content of the leaf produces a gas phase rich in nitriles. Gaseous components serving as an indicator for a given type of tobacco, however, have not been reported. This is not surprising since, as mentioned above, most gaseous components are pyrolysis products. It is improbable that a leaf constituent typical for a tobacco variety would produce a low-molecular-weight breakdown material not simultaneously yielding from other precursors. Thus moderate quantitative rather than qualitative differences of the gas phase composition are observed.

Unfortunately, there are much greater differences when the same tobacco is smoked under varying circumstances, such as variation in humidity, cut width, and butt length. The greatest differences probably occur between the gas phases from the first and the last puff of a cigarette. All these variations, together with the impossibility of preparing "identical" cigarettes, make it very difficult to identify a tobacco type from its gas phase composition. The particulate phase provides a much better basis for this purpose.

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Relative Role of Aerosol and Volatile Constituents of Cigarette Smoke as Agents Toxic to the Respiratory Tract

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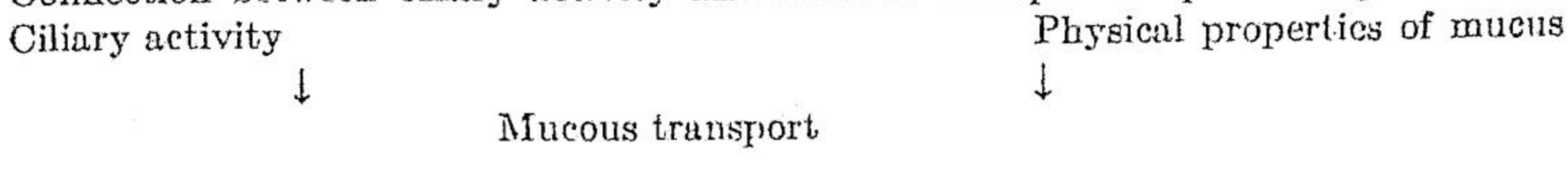
EXPERIMENTS concerned with the toxicity of tobacco smoke on the lung include studies of several different functions of the respiratory tract under a variety of experimental conditions, and thus conclusions concerning effects of various tobacco smoke constitutents can be contradictory.

This paper considers the relative role of aerosol and volatile constituents of cigarette smoke as agents toxic to the respiratory tract. This evaluation is based on the available toxicological information presented in the first part of the paper. To provide a basis for the "Discussion," the conditions present in the human respiratory tract during smoking are reviewed in the next part. The experimental techniques used for the evaluation of the toxicity of aerosol and volatile constituents are then discussed in view of the conditions present in humans. Finally, a conclusion and some suggestions concerning certain requirements on the experimental evaluation of the toxicity of tobacco smoke are presented.

Included in this review are only animal experiments dealing with toxic effects on the respiratory tract or functions thereof either *in vitro* or *in vivo*. The emphasis is on studies which differentiate primarily between the aerosol and volatile constituents of the smoke and in which exposure conditions were comparable to human conditions. Inhalation experiments where tumor-promoting properties of aerosol and volatile constituents are studied have so far not been reported in the literature and this aspect is therefore not discussed here.

The terms "mucous transport, ciliary activity, and pulmonary clearance" are used according to previous definitions (*1*) and are illustrated below.

Connection between ciliary activity and mucous transport in pulmonary clearance



Aerosol constituents are referred to as that part of the whole smoke, retained on an absolute filter (*e.g.*, Cambridge filter).

Volatile constituents are compounds found in the gaseous phase of the smoke which are absorbed if the smoke passes through a filter containing activated charcoal. It must be borne in mind that several compounds are present both in aerosol and in volatile form (*see* pp. 237 and 249). Other differences between aerosol and volatile constituents of tobacco smoke are important in this context. Some aerosol components, *e.g.*, "tar," are present in concentrations several times higher than most volatile components, which are potential irritants to the lung. A nonfilter cigarette delivering 30 mg tar, if smoked under standard conditions, yields about 940 μg of acetaldehyde (2). Other components are listed in table 1. The relative absorption of the various components, which is reported for Cambridge and charcoal filters, might vary somewhat according to the smoking technique and the filter.

TABLE 1.—Example of aerosol and volatile constituents in smoke from a nonfilter cigarette *

Compound	Amount mg/cigarette	Percent removed by Cambridge filter	Percent removed by medium efficiency charcoal filter
Aerosol			
"Tar"	30	~100	~1
Nicotine	1.6	~100	~1
Phenol	0.008	~100	~1
Volatile			
Toluene	0.25	50	53
Acetonitrile	0.31	48	48
Acetone	0.57	12	31
Isoprene	0.56	29	38
Acetaldehyde	0.94	5	20

*Spears and Routh (2).

TOXICOLOGICAL INFORMATION

Whole Tobacco Smoke

That tobacco smoke as such exerts toxicity on various functions of the respiratory tract has been shown in several experiments.

Ciliatoxicity was reported under *in vitro* conditions by Hilding (3) and under *in vivo* conditions by Dalhamn (4) and by several others. An effect on the mucous flow was described for *in vitro* conditions in frogs by Falk *et al.* (5) and *in vivo* on cats by Carson *et al.* (6).

Effects on pulmonary clearance in guinea pigs after chronic exposure to small doses of cigarette smoke were reported by Rylander (*7*). An effect on the functioning of the pulmonary macrophage system was described *in vitro* by Green and Carolin (*8*) and Maxwell *et al.* (*9*). *In vivo* observations were reported by Laurenzi and Guarneri (*10*). Effects on the surfactant property of pulmonary lavage fluid have been reported *in vitro* by Miller and Bondurant (*11*) and *in vivo* by Webb *et al.* (*12*).

The works cited above are but examples of reported findings on the general toxicity of tobacco smoke. Other experimental models with *in vitro* and *in vivo* techniques, using intact experimental animals or isolated preparations of the respiratory tract, have confirmed these findings.

Several chronic inhalation experiments, designed to study the malignant tumor-promoting activity of cigarette smoke, have been reported (*13*, *14*), although no conclusive evidence has yet been presented.

Selective Toxicology of Aerosol and Volatile Constituents

Guillerm *et al.* (*15*) used an *in vivo* preparation by exposing the tracheal mucosa of dogs to smoke under acute exposure conditions. They reported that mechanical filtration of tobacco smoke did not influence the toxic effect of mucous transport. Washing in water, however, reduced the toxicity of the smoke. The authors concluded that the most toxic compounds were in the gaseous phase of the smoke. This conclusion is shared by Kensler and Battista (*16*), who used an *in vitro* system for the measurement of mucous transport. The latter authors denied the aerosol constituents any major role as far as mucous transport and ciliatoxicity are concerned.

Contrary to this conclusion, Dalhamn and Rylander (*17*, *18*), observing the ciliary activity under *in vivo* conditions, reported a correlation and a dose-response relationship between toxicity and aerosol constituents. An explanation of the discrepancy between the above results concerning the relative importance of the aerosol constituents is suggested by Dalhamn and Rylander (*28*), who found that the relative toxicity of the volatile constituents, as compared to the whole smoke, increased with increasing exposure levels.

Falk *et al.* (*5*), using an *in vitro* preparation, concluded that the aerosol constituents were responsible for the effects observed on mucous transport on frog esophagus and cat and rabbit tracheas. Carson *et al.* (*6*) measured the mucous transport in intact tracheas of cats *in vivo*. The findings after acute exposure suggest that both the aerosol and volatile constituents were responsible for the toxicity observed.

An influence on respiratory dynamics of guinea pigs exposed to cigarette smoke was reported by Carson *et al.* (*20*). Less volatile material reduced the effect.

The design of a number of experiments has been very different from human conditions and from those in the above experiments. Thayer and

Kensler (*21*) studied growth of cultured human cells, whereas Walker and Kiefer (*22*) worked with clam cilia. Weiss and Weiss (*23*) used *Paramecium* cultures. Green and Carolin (*8*) studied phagocytic activity of pulmonary macrophages *in vitro*. All the above investigators found toxic effects of volatile constituents of tobacco smoke.

Comments

The experiments reviewed here clearly show that conclusions are contradictory as to which part of the tobacco smoke is most toxic. The available evidence is difficult to weigh, especially in view of the varying exposure conditions for the different experimental models. That differences in results concerning toxicity can be obtained when different experimental models are used has been demonstrated by Dalhamn *et al.* (*24*). Furthermore, data are insufficient on the exposure levels of the agent studied in several reports.

Despite such shortcomings, an attempt will be made to evaluate the relevance of the available information concerning selective toxicology. This evaluation will be based mainly on the exposure conditions present in the human respiratory tract.

EXPOSURE CONDITIONS IN THE HUMAN RESPIRATORY TRACT

Volume and Dilution of Smoke

The volume of a smoke puff varies for different individuals and also for puffs drawn by the same individual. A 35 ml puff has been suggested as a standard volume. Calculating with an average tidal volume of 500–750 ml, one then has to deal with a large dilution of the smoke in the deep parts of the lungs. The dilution in the upper parts of the respiratory tract is less, although the smoke is reduced to about 50% even in its uppermost part.

Selective Absorption

At any given time after the generation, the tobacco smoke aerosol and volatile constituents change, due to interactions of constituents and the environmental conditions. Although a puff of smoke remains in the mouth for only a short time before being inhaled, several compounds are absorbed to a significant degree. Dalhamn *et al.* (*19*) have shown in experiments on humans that, during the smoke's 2-second stay in the mouth, the absorption is greater for water-soluble compounds in the smoke than for water-insoluble compounds. These findings are summarized in table 2.

The smoke entering the trachea thus differs in composition from the smoke coming directly from the cigarette because of a dilution factor and

TABLE 2.—Removal of certain constituents of cigarette smoke during a 2-second stay in the human mouth*

Compound	Water-soluble	Boiling point (°C)	Percent removal
Acetaldehyde	+	21	61
Isoprene	−	34	27
Acetonitrile	+	85	76
Toluene	−	111	33
Aerosol components	−	—	22

*Dalhamn *et al.* (*19*).

its relative higher content of water-insoluble compounds. The selective greater absorption of water-soluble compounds, as observed in the mouth, probably continues as the smoke passes through the trachea into the bronchi.

Duration of Exposure

Aerosol and volatile constituents of tobacco smoke that enter the lungs expose the lung tissue in several different ways. The smoke, passing rapidly through the trachea and the larger bronchi, remains in contact with a certain area of tissue for only a short time, estimated to be about 0.3 seconds (*25*).

Compounds that are absorbed in the mucus of the epithelium under normal conditions are transported rapidly toward the pharynx and swallowed into the gastrointestinal tract. The duration of exposure is therefore also short. If the absorbed compounds are toxic and their concentration is high enough, the mucus or the cilia may be affected. This can be observed initially as an increased mucous flow (*5*, *6*), in which case the duration of exposure to the compound is shorter than normal. If the concentration of the compound is high enough, the ciliary activity and/or the mucous flow may decrease and the exposure duration is then increased (*16*, *26*).

Aerosol and volatile constituents of tobacco smoke, which penetrate into the alveoli during inspiration, are only partly ventilated during expiration. The duration of exposure in the deeper airways is thus longer than in the upper respiratory tract, and favorable conditions for complete absorption of volatile constituents and total deposition of aerosol constituents are present. If the compounds which remain in the deeper parts of the lung influence the pulmonary macrophage system (*9*) or the alveolar fluid-removing mechanism (*12*), the duration of exposure is even longer.

If any of the defense mechanisms discussed above has been impaired in its function by agents other than tobacco smoke, *e.g.*, viral infections (*27*), the duration of exposure to aerosol and volatile compounds of tobacco smoke increases. This might permit compounds, which normally do not cause a toxic effect, to do so, either due to longer contact time with a certain tissue area or to an accumulation of the amount of the compound present.

DISCUSSION

The relative importance of the aerosol and volatile components must, in view of the discussed conditions in the human respiratory tract, be evaluated by experiments that meet the following requirements: 1) realistic dilutions of the smoke as drawn from the cigarettes; 2) selective absorption of volatile, water-soluble compounds from the smoke; 3) realistic exposure duration, *i.e.*, shorter exposure for observations on cilia and mucus than for those on macrophage and alveolar fluid.

1) As Dalhamn and Rylander (*28*) suggested, the relative toxicity of aerosol and volatile constituents may differ at various exposure levels. In view of this, it can be hazardous to draw conclusions concerning human conditions from experiments in which high exposure levels have been applied. Experimental models with a realistic dilution appear preferable, *e.g.*, (*6*, *17*).

The appropriate degree of dilution, however, is difficult to determine. In acute exposure experiments, Dalhamn and Rylander (*18*) administered a 1.0 ml puff of undiluted smoke every minute. Carson *et al.* (*6*) administered 3 breaths of a 35/200 ml smoke/air mixture. In chronic exposures, Bair and Dilley (*29*) allowed dogs to inhale at their own will. One cigarette per day was smoked initially, but this dose was later increased to 20 cigarettes per day. Rylander studied guinea pigs and administered a 35/130 ml smoke/air mixture at 1-minute intervals, with 20 such puffs given twice daily for 3 weeks (*7*).

The relationship between the inhaled smoke volume and the tidal volume of the animals used in these experiments and the corresponding value for humans are shown in table 3. Although these values can be considered only as very approximate, the table indicates that the dilution factor in human smoking is considerable and that several experiments reported use far higher concentrations of smoke.

2) The passage of smoke directly from the cigarette over the area under observation will not fulfill the requirement of selective absorption. In this

TABLE 3.—Relationship between inhaled smoke volume and tidal volume of experimental animals

Experiment	A Concentration of smoke after dilution (percent)	B Smoke mixture administered (ml)	C Undiluted smoke inhaled (ml)	D Tidal volume (ml)	Ratio C/D
Mucous transport, cats (*6*)	18	12.4	2.2	12.4	0.18
Ciliary beat, cats (*18*)	100	1	1	12.4	0.08
Pulmonary clearance, dogs (*29*)	100	35?	35	350	0.10
Pulmonary clearance, guinea pigs (*7*)	27	1.8	0.5	1.8	0.28
Human	100	35	35	750	0.04

respect, exposure models such as those used by Carson *et al.* (*6*), where the smoke is administered through the mouth of the animal, are to be preferred.

The danger of a too large selective absorption of volatile constituents exists when the animals inhale the smoke through their noses, as happens in most experimental models. The absence of an effect of the volatile constituents under these circumstances cannot be applied to human conditions.

Models using smoke that passed through an extensive system of tubes and valves before being administered to the respiratory tract must also be considered less suitable, if realistic exposure conditions are to be achieved. The same applies to models where the smoke is passed into a chamber where it remains for an undetermined time before being inhaled.

Models such as the above must be strongly suspected of selectively removing certain compounds or changing the characteristics of the smoke so much that a realistic exposure agent is no longer present.

Experimental models in which the smoke has been passed through water or salt solutions obviously alter the characteristics of the smoke, so much so that the results have only limited value in the context discussed here. The same objection is applicable when the observed tissue is suspended in a salt solution through which the smoke is passed.

3) As for the requirement of a realistic exposure duration, a continuous exposure, with no chance for the tissue to recover during intervals between smoke puffs, must naturally be avoided. If the animal inhales the smoke during its normal breathing cycle, the exposure duration in the respiratory tract should be reasonably comparable to conditions in humans, and most models suggested for *in vivo* studies have applied an intermittent dosage system with varying exposure duration.

CONCLUSION AND SUGGESTIONS

If special attention is paid to experiments that reasonably meet the requirements discussed in the previous section, available results do not support the hypotheses that either the aerosol or volatile constituents in tobacco smoke should be the more toxic. Whatever the type of filter used in experimental work, a toxic effect has remained in the filtered smoke. From the toxicologists' point of view, the dose-response relationship, as shown for both the aerosol and the volatile smoke components, seems at this time to provide the only basis for a desired reduction in toxicity.

From a hypothetical point of view, the selective absorption in the mouth and the large dilution of the smoke under human conditions could thus indicate a less important role of the volatile constituents. However, this hypothesis has to be tested.

For future work in this area, experimental models designed to approximate human conditions should be used more extensively. Apart from

the technical procedures involved in the production of the smoke, *e.g.*, factors which influence the dilution, the chemical composition of tobacco smoke used should be clearly defined.

SUMMARY

An evaluation of the relative role of aerosol and volatile constituents of cigarette smoke as toxic agents to the respiratory tract is presented. The discussion is based on available experimental results that are commented on in view of the conditions present in the human respiratory tract during smoking of cigarettes. Special attention is paid to the dilution of the smoke, the exposure duration, and the selective absorption of volatile, water-soluble smoke constituents.

Available experimental results do not support the hypothesis that either the aerosol or the volatile compounds are the most toxic. A reduced dosage of either whole smoke, or the aerosol, or only volatile components results in a lower toxicity. As a result, apart from consideration of the exposure conditions discussed, future experiments should be carried out only with tobacco smoke of defined composition.

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Carboxyhemoglobin in Relation to Smoking

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THE main thrust of the Conference and the tenor of discussion in the general sessions and in this workshop have been to review and analyze the various agents in tobacco smoke with regard to their potential threat to the health and well-being of the cigarette smoker. The evidence already presented has dealt largely with those effects of certain components of tobacco smoke as they relate to such problems as myocardial infarction, blood coagulation, and carcinogenesis. What can be done to reduce such hazards as "tar" and nicotine, thus leading to the production of a less harmful cigarette, has been discussed.

This afternoon's workshop seems to me to be a variation on the general theme, being in the nature of a movement written in a minor key. It has dealt with certain components in tobacco smoke, *e.g.*, nicotine, whose deleterious properties have not been experimentally and clinically established, but which are nevertheless under various degrees of suspicion. Therefore, these components must be examined in the process of writing the score for the orchestration of Dr. Wynder's symphony, entitled *Toward a Less Harmful Cigarette.*

Carbon monoxide (CO) is one of these components of tobacco smoke that has long been suspected of being harmful and, hence, has received much study over the years.

The problem of CO as a harmful constituent of tobacco smoke raises two questions:

1. Does the amount of CO in the blood differ between the smoker and nonsmoker?
2. If more CO is present in the blood of the smoker, does it produce either functional or structural pathological changes? Are such changes demonstrable by symptomatic, clinical, or laboratory evidence, and can they therefore be assumed to be detrimental to the health or well-being of the smoker as is true in the case of other components of tobacco smoke?

There is abundant evidence in the literature to answer unequivocally the question of the difference between the CO blood level concentration in the

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