THE ROLE AND ACTION OF ENVIRONMENTAL AGENTS IN THE PATHOGENESIS OF LUNG CANCER

I. Air Pollutants

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COMPREHENSIVE epidemiological studies have demonstrated a world-wide increase in the frequency of and absolute mortality from lung cancer.[5, 32] These investigations leave little question that a portion of the observed increase is both real and, at least until very recently, progressive. An analysis of the data in the various studies reveals a number of consistent findings that appear to have significant etiological implications. These common factors include: (1) a greater frequency of lung cancer in men than in women;[18, 39] (2) a greater liability to the development of lung cancer in urban residents than in rural residents;[5, 16, 20, 22] (3) an increased incidence associated with a history of prolonged excessive cigarette smoking;[6, 7, 15, 28] and (4) a temporal pattern of increasing frequency compatible with a possible etiological influence of influenza pandemic of 1917–1920.[9, 42] These considerations indicate the need for studies aimed at: (1) establishing the exact nature of and the significance of the various associations (e.g., spurious, cause and effect, etc.); and (2) elucidating the biological mechanisms responsible for and compatible with the epidemiological characteristics of lung cancer. In all instances in which an association between a disease state and environmental factors has been demonstrated, the isolation and identification of specific etiological agents and the determination of their mode of action have been primarily laboratory undertakings. In the panorama of lung cancer, all available evidence points to a dominant etiological role for exogenous environmental agents.[31]

TABLE 1

QUANTITATIVE ANALYSIS OF ATMOSPHERIC SAMPLES COLLECTED IN LOS ANGELES*

Compound	Sample 1, mg.† Tot.	Sample 1, mg.† Per 1,000,000 cu. ft.	Sample 2, mg.‡ Tot.	Sample 2, mg.‡ Per 1,000,000 cu. ft.
Pyrene	0.28	0.14	0.90	0.32
3,4-Benzpyrene	1.84	0.92	2.35	0.84
1,12-Benzperylene	1.45	1.00	0.72	0.35

*Data are reprinted from *Cancer* 9: 905, 1956.
†Sampled from Aug. 1, 1952, to Oct. 15, 1952 (42 days of actual sampling).
‡Sampled from Oct. 21, 1952, to June 1, 1953 (59 days of actual sampling).

On the valid assumption that bronchogenic cancer develops subsequent to the action of multiple factors, a broad experimental program was undertaken for the purpose of evaluating the relative etiological significance of several of these factors. Atmospheric pollution, cigarette smoking, and viral agents were selected as the primary environmental conditions to be investigated since they were preeminently compatible with the epidemiological pattern of lung cancer. Two cardinal facts were recognized at the onset. First, there are unequivocal instances of lung cancer in which one or several of these environmental agents appear to have played no role (e.g., nonsmokers, residents in rural areas or in agricultural economies, etc.) and second, when the factors under study have been present, the etiological contribution of each presumably has varied from case to case. Nevertheless, one may properly postulate the existence of a pathogenetic milieu compatible with the activity of several or all of the suspected environmental factors operating, at different

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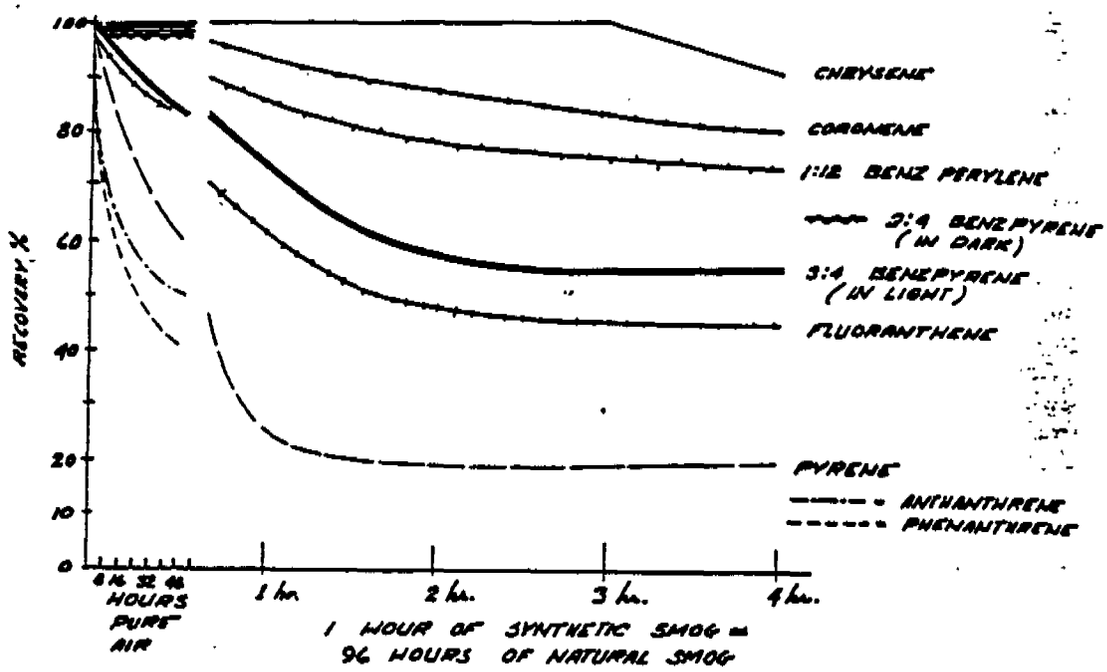


FIG. 1. Recovery of aromatic polycylic hydrocarbons after exposure to washed air and synthetic smog.

intensities, in the development of lung cancer. Representative experimental findings in chemistry, physics, and biology that are related to atmospheric pollution will be presented. Then the data will be utilized in formulating a concept of the pathogenesis of lung cancer that is consistent with the epidemiological characteristics of the disease. The role of cigarette smoking and viral infection will be presented in a subsequent article and will be integrated with the concepts being currently presented. The mechanism whereby these environmental agents working singly or in combination may produce a neoplastic response will be discussed in the final article of this series.

METHOD

Soot, an experimentally and clinically recognized carcinogen, is a major component of smoke emitted into the atmosphere incidental to the use of solid, liquid, and gaseous fuels.[2, 29] A product of the incomplete combustion of most organic materials when they are heated or burned in the absence of an adequate supply of oxygen, soot's carcinogenic properties are presumably due to the presence in it of certain aromatic polycyclic hydrocarbons.[40, 41] Although quantitative data are lacking, the chemistry and method of formation of soot established its continuous presence in the atmosphere for centuries. To ascribe an etiological role to soot and its aromatic polycyclic hydrocarbons present in the air, therefore, requires either the identification of significant new sources for these compounds or the recent introduction into the environment of potentiating or cofactors. An additional reason for considering the atmosphere in the pathogenesis of lung cancer may be the presence in the air of a new class of carcinogenic agents capable of acting in association with or independently of the traditional aromatic polycyclic hydrocarbon carcinogens. We, therefore, established as initial goals the quantitation of aromatic polycyclic hydrocarbons in the atmosphere and the simultaneous determination of their sources. Special interest was shown in compounds recently introduced into our environment. The exhaust products of the internal combustion engine were chosen as the first materials for quantitative studies. Widespread home and industrial use of petroleum products, especially in gasoline and diesel engines, is the hallmark of our industrial society. The entry of these products into our environment is temporally compatible with a possible etiological role.

FINDINGS

CHEMICAL

A broad spectrum of aromatic polycyclic hydrocarbons was found in the atmosphere and in the exhaust products from both gasoline engines and diesel engines.[22, 24, 25] The potent carcinogen 3,4-benzpyrene was identified and quantitated in all of them. It is our belief that 3,4-benzpyrene is best regarded as but one of several possible carcinogenic pollutants in the atmosphere. Rather than assuming it to be the only or even necessarily the most significant carcinogen present, we let its presence serve as both a chemical index of aromatic polycyclic hydrocarbon contamination of the air and a biological index of carcinogenic potency. As anticipated, in an essentially liquid or gaseous fuel–consuming community, such as Los Angeles, 3,4-benzpyrene was identified in concentrations lower than those reported[4] in certain other urban communities (Table 1). A listing of the vehicular exhaust products, as shown in Tables 2 and 3, establishes the presence of significant amounts of benzpyrene and allied hydrocarbons.

Comparison of the analytical findings from vehicular exhausts with those from atmospheric studies revealed a reversal of the ratio of benzpyrene to pyrene. Benzpyrene was consistently emitted from vehicular exhausts in smaller quantities than was pyrene. Atmospheric analyses showed benzpyrene to be present in larger amounts than pyrene. Two alternative explanations for this finding suggested themselves. First, a significant emission source of benzpyrene existed of which we were unaware, or second, marked variations in the stability of the various aromatic polycyclic hydrocarbons existed. A search for proof of the first possibility proved unsuccessful. An investigation of the stability and survival of aromatic polycyclic hydrocarbons under varying atmospheric conditions was then undertaken. Our data readily disclosed that the reversal in the benzpyrene to pyrene ratio was a reflection of differences in hydrocarbon stability.[11] As can be seen in Fig. 1, pyrene proved to be the most labile of this group of hydrocarbons while 3,4-benzpyrene, even under the most adverse atmospheric conditions, proved to be notably more stable.

TABLE 2

AROMATIC HYDROCARBONS ESTIMATED IN 1-MINUTE SAMPLES OF GASOLINE EXHAUST, USING VARIOUS ENGINE REVOLUTION SPEEDS

Speed, r.p.m.	At 0 load, μg./minute of: Pyrene	Compound X	Benzpyrene	Benzperylene	Anthanthrene
500	225	289	120	235	153
1,000	439	325	61	177	102
1,500	507	266	33	60	36
2,000	374	142	40	73	27
2,500	346	127	25	70	31
3,000	121	25	13	85	14
3,500	48	5	10	39	15

TABLE 3

AROMATIC HYDROCARBONS ESTIMATED IN 1-MINUTE SAMPLES OF DIESEL EXHAUST, USING VARIOUS LOADS AND ENGINE REVOLUTION SPEEDS

Load*	μG./minute of: Pyrene	Compound X	Benzpyrene	Benzperylene	Anthanthrene
At 1,000 r.p.m.					
0	137	22	146	22	0.
¼	267	76	465	42	43.
½	536	175	772	124	223.
¾	1,800	640	1,320	610	472.
1	2,500	639	876	1,265	469.
At 1,200 r.p.m.					
0	208	0	9	79	4.3
¼	257	0	47	40	24.
½	448	278	437	171	197.
¾	888	488	432	930	320.
1	1,912	614	1,706	976	944.
At 1,400 r.p.m.					
0	188	0	80	0	20.
¼	177	56	78	0	16.
½	220	76	1,372	368	69.
¾	734	337	982	1,071	577.
1	822	346	1,687	944	666.

*All with compression release.

PHYSICAL

Having established the presence of 3,4-benzpyrene and allied hydrocarbons in common and consistent sources of air pollution and having demonstrated their survival in the atmosphere, we then became concerned with the relation of these hydrocarbons to the soot particles on which they are adsorbed. Physical factors play a significant role in the deposition of particulate matter in the respiratory tract. The biological potential of carcinogen-laden soot is, therefore, significantly related to the size of the soot particles present in the atmosphere, which in turn determines their epithelial deposition capability. The fundamental biological importance of particle size resides in two independent factors. First, a major por-

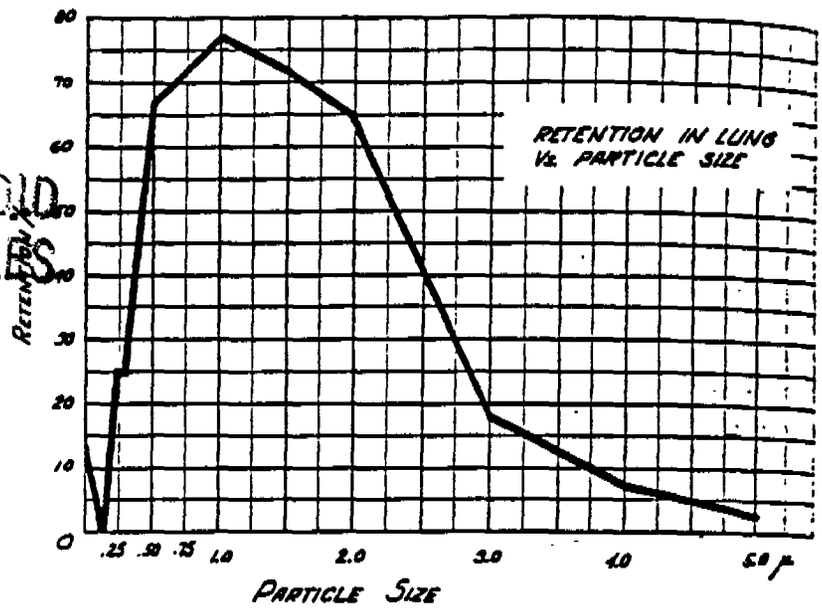


FIG. 2. Rate of retention of particulate matter in lung in relation to particle size. (After Dautrebande et al.[4])

tion of inhaled soot particles (larger in size than 2 μ) are arrested in the mucous membranes of the nose, accessory nasal sinuses, pharynx, and oral cavity. At the other extreme, a lesser but still significant segment of exceedingly small soot particles (less than 125 Angstrom units in size) remain suspended in tidal air, except for that small amount of ultra, ultra microscopic particles that are precipitated by brownian movement. The retention of particulate matter in the lung in relation to particle size is shown in Fig. 2. Dautrebande et al.[4] correlated the location of particulate deposition with specific sites in the tracheobronchial tree (Fig. 3). We have been able to confirm the pattern of pulmonary inhalation deposition and retention through the use of soots of specific particle size.

The elution of carcinogens by appropriate organic solvents or plasma proteins is also critically related to the size of the particle on which the carcinogens are adsorbed. Aromatic polycyclic hydrocarbons cannot be very readily eluted from soots of very small particle size. Further, particles with an average diameter of less than 400 A will actually remove aromatic polycylic hydrocarbons from their immediate environment because of their high surface adsorption. Particles beyond the 400-A average size range will release adsorbed aromatic polycylic hydrocarbons in the presence of appropriate solvents. As particle size increases, these compounds are released more rapidly and in larger amounts.

Studies of the atmosphere and of the exhaust products from vehicular engines show a predominance of soot particles within the 0.1- to 1.-μ diameter range. As will be demonstrated, this range is consistent with the biological action of various particles on the basis of both particle retention in the lung, as shown in Fig. 3, and elution of carcinogenic hydrocarbons, as shown in Table 4.

BIOLOGICAL

Respiratory Epithelial Studies. Experimental. After inhalation, a period of contact with the respiratory tract epithelium is necessary before the carcinogen can be liberated from its soot particle and enter the cell. Ordinarily the necessary prolonged interval for this carcinogenic stimulus to be in contact with the respiratory epithelium is precluded by the physiologic defenses of the tracheobronchial tree. The principal and most potent factors responsible for the prevention of prolonged residence of particles on the lining cells of the tracheobronchial tree are the mucous stream covering the epithelial cells and the activity of ciliated cells that by their action constantly move the mucus cephalad. Particles settling out on this stream are, as a result, immediately set in motion. The normal rate of movement of these settled particles is sufficiently rapid to make elution and local carcinogenic stimulus in the tracheobronchial tree unlikely to occur. The initial

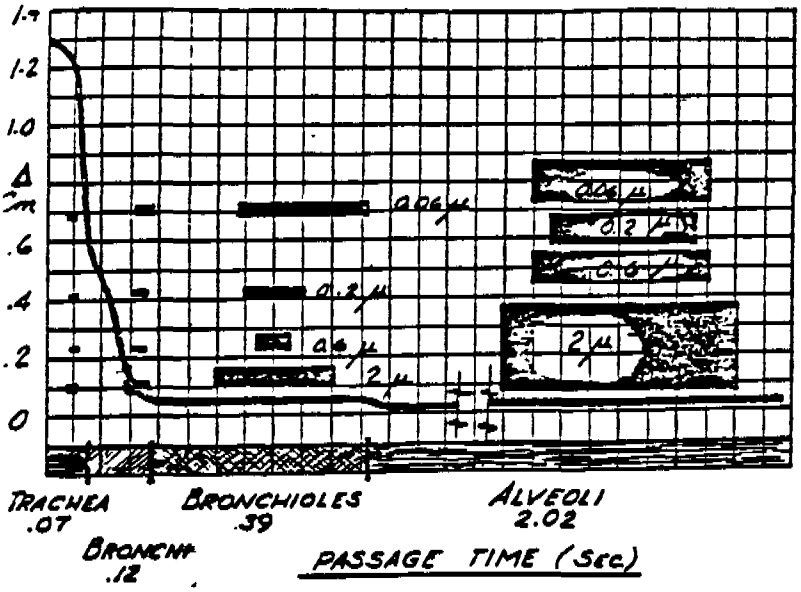


FIG. 3. Relation of particle size to deposition site and time of passage within tracheobronchial tree.

eriod required for elution or removal of the arcinogen from the soot and the added time equired for entry into the cell are fulfilled nly when the ciliary-mucus defense barrier is ed or lost. Carcinogenic particles pen- more deeply into the pulmonary arenchyma to the level of the alveoli are in- ested by the macrophages in the lung. Phago- ytosis is effective in removing these particles, hich then may ultimately be disposed of by heir entry into the systemic lymphatics or by eing advanced to the level of the mucous tream.

Under certain conditions, however, the host efenses can be interfered with, and abnor- nally long residence of particles ensues. Nu- erous irritants of a nonspecific type are pres- nt in polluted atmosphere that, when reathed in sufficient concentration, are cap- ble first of slowing mucous flow and ulti- nately of stopping ciliary activity. These ompounds include both organic and inor- anic acids, the oxidation reaction products f hydrocarbons, the aldehydes, the oxides of itrogen, and the oxides of sulfur. They may e present in the air as gases and aerosols. As rule, the effect of these compounds is tran- ient, and when their concentration drops, ormal activity returns.

The modifying effect of atmospheric irri- ant substances (ozonized gasoline, aldehydes, rganic acids, etc.) on the normal functions of heobronchial tree was studied in two rst, animals in inhalation chambers were exposed to controlled test environments for varying periods. After the animals were removed, the tracheobronchial trees were exteriorized and opened so that ciliary activity and mucous secretion could be observed in the intact animal. The second method of studying the atmosphere's effect consisted of using isolated animal tracheobronchial epithelial strips for in vitro studies. Carbon particles of a size, configuration, and chemical structure identical to those recovered from the atmosphere were used for the determination of ciliary rate. A steromicroscope with scaled ocular lenses was adapted for this purpose. In addition, studies were carried out using the esophageal tract of frogs.

During study by the first method, one initial response of the exposed epithelium was apparent excitation as manifested by an increased rate in the movement of particles. In response to a few specific irritants, however, this primary effect could not be observed. With exposure to these, there followed a period of decreased activity that, if allowed to persist, often resulted in total cessation of particle movement. Prolonged or intense exposure was followed by limited and delayed recovery. Total ciliary paralysis could be maintained for a considerable length of time, after which partial recovery was still possible. Structurally, it was possible to demonstrate a considerable degree of parallelism between alterations in physiological functions and cellular abnormalities. Representative respon-

TABLE 4

RECOVERY OF POLYCYCLIC AROMATIC HYDROCARBONS FROM 500 mμ OF SOOT AND PLASMA AFTER INCUBATION

Compound, μg./100 mg. soot	% recovered after incubation period of:*												
	1½ hr.			16 hr.			96 hr.			192 hr.			Saline
	Soot	Plas.	Tot.	Soot	Plas.	Tot.	Soot	Plas.	Tot.	Soot	Plas.	Tot.	Soot
Pyrene 8.4	9	81	90	34	61	95	9	50	59	6	61	67	100
Fluoranthene 1.0	T	100	...	P	P	...	0	P	...	P	P	...	100
Compound X 2.8	0	100	100	T	93	...	0	P	...	0	39	39	100
1,2-Benzpyrene 2.9	T	52	...	17	41	58	0	21	21	T	21	...	100
3,4-Benzpyrene 1.7	18	82	100	41	59	100	6	23	29	18	18	36	100
1,12-Benzperylene 9.2	31	66	97	67	33	100	5	15	20	11	13	24	100
Anthanthrene 2.4	25	54	79	54	33	87	T	17	...	13	13	26	100
Coronene 10.0	40	36	76	66	26	92	12	8	20	15	10	25	100

*The symbols in this table are T for trace and P for present.

to irritant exposure are shown in Figs. 4 to 6.

Mucous secretion was also affected by exposure of the respiratory tract to irritants. Physiologically, alterations were first characterized by an increase in secretion. This response could be demonstrated in microscopic sections from the respiratory epithelium. An increase in the number of and activity of goblet cells was the first histological change noted. This progressed until there was almost complete replacement of the normal epithelium by an overgrowth of these mucus-producing cells. Subsequently, pools or lakes of mucus were formed in the epithelium, and finally, the mucous lakes emptied into the lumen of the tracheobronchial tree, with complete epithelial desquamation down to but not including the basal cells. The histopathological sequence is shown in Figs. 7 to 11. This phenomenon is of considerable interest in that it represents a step in the pathogenetic sequence in which compounds that are not in themselves carcinogenic theoretically facilitate the biological activity of compounds that are presumably endowed with the property of inducing cancer in the respiratory tract.

Human Lung Studies. Two mechanisms may explain the almost universal finding of soot-laden lungs in urban residents at autopsy —first, the action of pulmonary phagocytes in the ingestion of the particulates and second, the method previously elucidated whereby soot may be abnormally retained in the respiratory tract. An investigation was undertaken to determine the biological and chemical fate of aromatic polycyclic hydrocarbons as they occur on retained soot. We wished to learn: (1) if carcinogenic hydrocarbons remain unaltered subsequent to their deposition on the respiratory epithelium or if they change qualitatively or quantitatively; (2) if any changes that might be demonstrated are associated with the activity of exogenous substances (reactive atmospheric pollutants) or endogenous factors (metabolic degradation); and (3) if a relationship exists between the amount of soot and carcinogenic hydrocarbons recovered from human lungs and the presence of "preneoplastic" morphological changes or overt lung cancer.

3,4-Benzpyrene, a potent carcinogenic aromatic polycyclic hydrocarbon, was chosen for study as the prototype of a compound belonging to this group of hydrocarbons. Lungs for analytical study were obtained with the shortest possible postmortem delay from the autopsy service of the Los Angeles County Hospital.[10]

Of all the aromatic polycyclic hydrocarbons presumed to be present, pyrene alone was capable of quantitation. Our inability to detect the presence of the other aromatic polycyclic hydrocarbons, most notably 3,4-benzpyrene, necessitated an investigation of whether the absence reflected artifactitious destruction of 3,4-benzpyrene and related compounds incidental to our experimental methods or whether it represented their true in vivo fate.

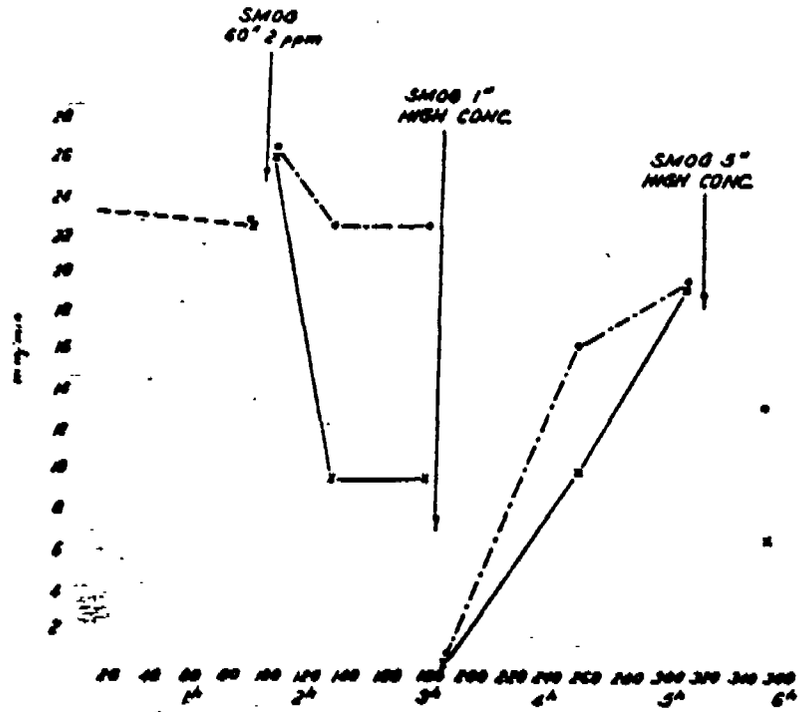


FIG. 4. Effect of smog in various concentrations on frog's esophagus. Graphic presentation of effect of irritant (smog) on movement of particles deposited on ciliated epithelium. Note greater inhibitory effect and delayed recovery in area of impingement. The lines indicate: ——— reading before exposure; x——x impinged area; and ——·—— unimpinged area.

Two methods were selected for the resolution of this problem. First, up to 20-μg. amounts of 3,4-benzpyrene were added to representative samples of lung tissue at the start during the various sequential stages of extraction procedures in a series of experiments. The presence of the added 3,4-benzpyrene was detectable at levels of 10 μg. for whole lung samples. Second, carotenoids normally present in lung tissue were identified throughout the entire procedure. In view of the extreme susceptibility of this group of compounds to oxidation, their persistence strongly suggests that benzpyrene, a less labile compound, would have survived the analytical method. The disappearance of certain aromatic polycyclic hydrocarbons, including 3,4-benzpyrene, must, therefore, be regarded as being due to factors other than our experimental method and techniques.

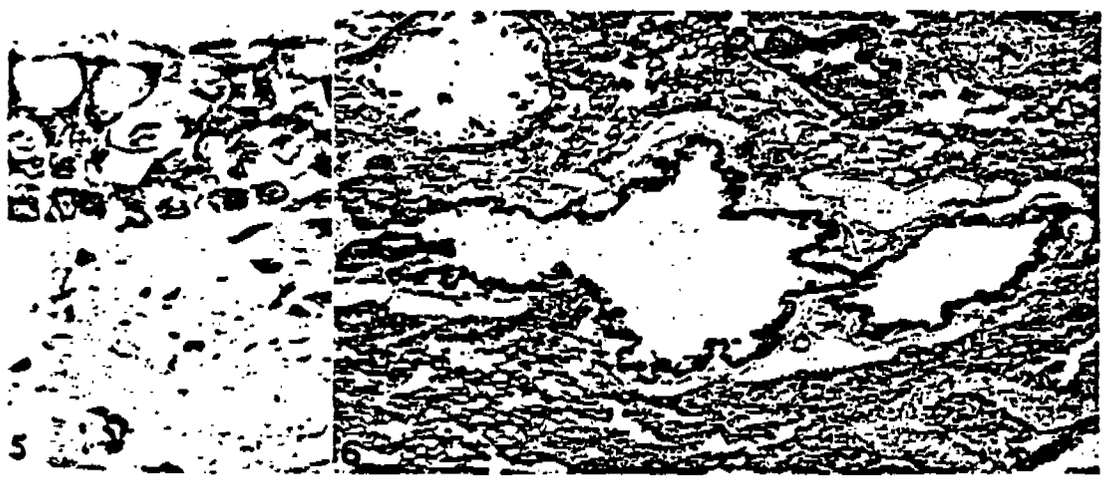


FIG. 5. Photomicrograph from section of bronchial epithelium prepared immediately after cessation of ciliary activity and flow of mucous stream. (×250.)

FIG. 6. Photomicrograph showing deposition and retention of soot on bronchial epithelium after neutralization of ciliary action and mucous flow by irritant inhalation. (×30.)

FIG. 7. Initial response of bronchial epithelium to irritant, as evidenced by increased goblet cell activity. (×125.)
FIG. 8. Later stage showing marked increase in mucous cell secretion, with mucous droplets in lumen. (×250.)
FIG. 9. Pooling of mucus in bronchial epithelium, with compression of nuclei, beginning obliteration of cell membranes, and thick static mucous stream overlying epithelium. (×250.)
FIG. 10. Beginning separation of superficial bronchial epithelium, with persistence of basal layer of cells. (×250.)

The physical characteristics, including the particle size of the soots recovered from human lungs, were studied under the electron microscope. It should be noted that the size range primarily included particles from which 3,4-benzpyrene could be readily eluted. The disappearance of aromatic polycyclic hydrocarbons from representative human lungs is shown in Table 5. The significance of these data will be discussed.

Histopathological examination of sections of respiratory epithelium removed from the lungs frequently revealed hyperplastic and metaplastic changes. These were most frequently seen at the arborizations and other impingement sites in the tracheobronchial tree (Figs. 12 and 13). This is consistent with the exaggerated experimental effect of the impingement of irritants on respirat ry epithelium when contrasted with sites of passive passage. Attempts to establish a correlation between the amount of soot recovered from the human lungs and the presence of metaplastic or neoplastic changes were unsuccessful.

Carcinogenic Studies. Painting and Injection. A necessary preliminary step in the assessment of the carcinogenic agents incidental

...ospheric contaminants is their biologi... ...ing. Extracts of exhaust products of ...soline engines, diesel engines, and the atmos...phere were bioassayed for tumorigenic prop...erties. Samples were painted on the skin or injected subcutaneously in C57BL and strain A mice. Representative tumor yields for the various materials studied are shown in Table 6.

The solvents used in the carcinogenic studies and the aerosol fraction of the atmosphere collected in Shepherd traps were used for control experiments. The studies of Shepherd et al.[33] and of Haagen-Smit[14] have shown the aerosol phase to consist primarily of the oxidation products of aliphatic hydrocarbons. We have confirmed these findings and have further demonstrated that the aerosol phase is free of carcinogenic aromatic polycylic hydrocarbons.[20]

An unexpected finding was the production of tumors after painting and injection with the control fraction, presumably a noncarcinogenic aromatic polycyclic hydrocarbon–free fraction of the aerosol phase of the atmosphere. This finding was duplicated in a second series of experiments.[28] The tumor yield with the use of these aerosol materials is shown in Table 6. An additional immediate unexpected finding was the production of a ... number of tumors in the mice painted and injected with atmospheric and vehicular exhaust extracts than could be accounted for by the presence of 3,4-benzpyrene alone. The implications of these findings will be discussed later.

Inhalation. The production of skin tumors in mice with both the aromatic polycyclic hydrocarbon fraction and the aerosol non–aromatic polycyclic hydrocarbon fraction recovered from the atmosphere made it necessary to choose the proper environment for exposure of animals in our inhalation studies. The non–aromatic polycyclic hydrocarbon fraction was selected for several reasons. First, essentially it represents a recently introduced atmospheric pollutant, the carcinogenicity of which has apparently been demonstrated for the first time. Second, in contrast to rural areas, these pollutants are present in greatest concentration in urban areas in which epidemiological studies have shown a consistently higher lung cancer mortality rate. Third, chemically related compounds have been

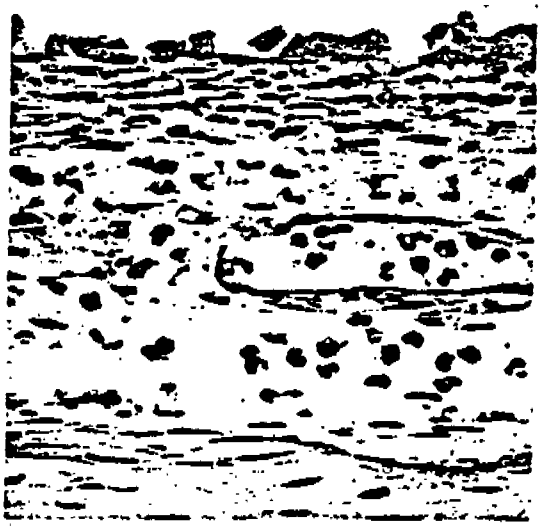


FIG. 11. Persistent basal layer of bronchial epithelium subsequent to desquamation of superficial epithelium. Note subepithelial inflammatory cell infiltration. (×250.)

TABLE 5

DISAPPEARANCE OF AROMATIC POLYCYCLIC HYDROCARBONS FROM SOOT IN HUMAN LUNGS*

Lung no.	Pt. age, yr.	Wet wt. lung, gm.	Amount of: Soot, mg.	Ash, mg.	Pyrene: Found, μg.	Pyrene: Expected, μg.	Benzpyrene & benzperylene: Found, μg.	Benzpyrene & benzperylene: Expected, μg.
2	90	950	720	480	0.9	6-27	...	34-172
3	71	1,335	360	200	1.9	3-13	...	17-86
4	70	1,570	660	770	1.9	6-24	...	31-158
5	74	1,570	360	170	3.6	3-13	...	17-86
6	71	710	390	1,070	1.3	3-14	...	18-93
7	81	1,260	190	150	2.3	2-7	...	9-45
8	62	1,120	540	510	4.4	5-20	...	25-130
9	70	910	830	310	5.1	7-31	Trace	38-200
11	65	1,420	5,010	3,460	1.3	45-185	...	233-1,200

*Data are reprinted from *Cancer* 11: 486, 1958.

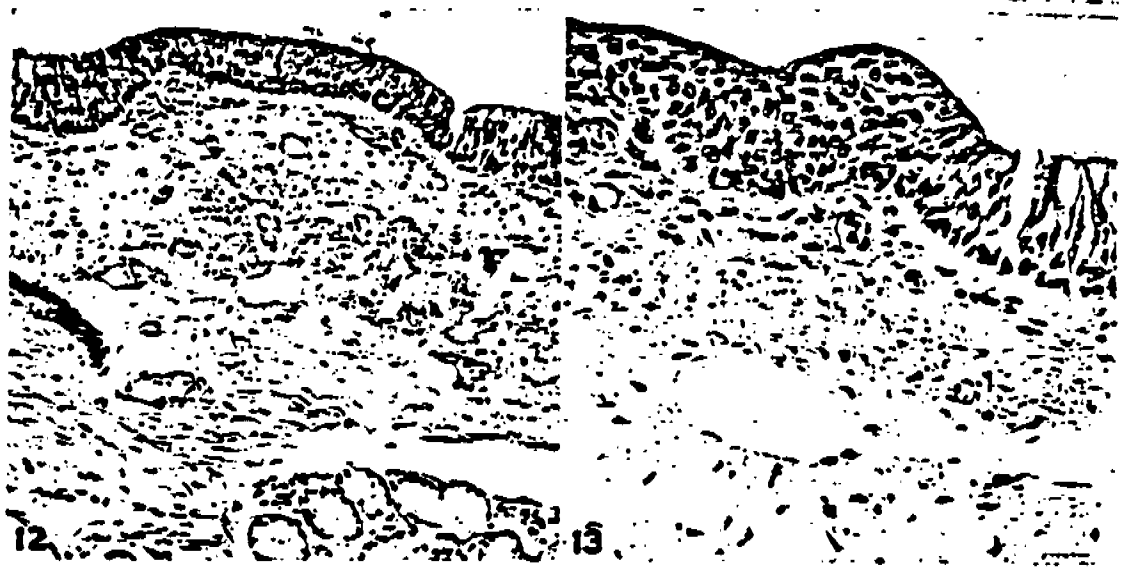


FIG. 12. Focal area of metaplasia presumably at site of impingement. Note ciliated epithelium on either side of metaplastic area. (×125.)

FIG. 13. Higher power of area adjacent to squamous metaplasia adjacent to site of ciliated bronchial epithelium. (×250.)

demonstrated to be carcinogenic in injection studies by workers in Great Britain[33, 34] and by Fieser et al.[18] in their injection studies on the carcinogenicity of oxidation products of cholesterol. Data from British studies indicated that these compounds are capable of intracellular activity.[33, 34] Fourth, general agreement exists that the latent development period of lung cancer is approximately 2 decades, and these aliphatic phase materials were introduced into the atmospheric environment sufficiently long ago to have allowed for any carcinogenic influence present to have become manifest at the time of the recorded epidemiological increases. Finally, from a technical viewpoint the synthetic reproduction of these materials in inhalation chambers is more feasible than is the use of aromatic polycyclic hydrocarbons adsorbed on soot.

Analytical studies by others and by ourselves have shown the aliphatic phase of hydrocarbons in the atmosphere to consist primarily of the oxidation products of straight chain, branched, and cyclic olefinic hydrocarbons. Gasoline provided a convenient and realistic source of these hydrocarbons, and it was reacted with oxides of nitrogen using either sunlight or artificial ultraviolet light as sources of photochemical energy for the production of ozone. For the major portion of the study, however, gasoline was directly reacted with ozone. The resultant oxidation products were similar with both methods. Mass spectrometer[35] and infrared spectroscopic studies[37] were used to demonstrate the essential similarity between the naturally occurring pollutants and those reproduced in exposure chambers (Figs. 14 and 15).

TABLE 6

PRODUCTION OF SKIN TUMORS IN MICE BY PAINTING 3 TIMES A WEEK*

Mouse strain No. mice	Sample material	Appearance of tumors in gp.† Appear. 1st. tumor, days	No. mice surv.	Tumor-bearing mice No.‡	%	No. with mult. tum.
Painting with aromatic hydrocarbons						
C57BL 76	Partic. phase atmos.	465	31	13 (9)	42	4
C57BL 108	Partic. phase gas. exh.	420	86	41 (22)	48	15
A 25	Partic. phase dies. exh.	402	20	17 (11)	85	8
Painting with oxidation products of aliphatic hydrocarbons						
C57BL 50	Aerosol phase atmos.	421	35	7 (2)	20	

*Data are reprinted from *Cancer* 9: 906-907, 1956.

†Eleven per cent of the mice that developed tumors had malignant lymphomas.

‡Numbers in parentheses indicate the number of mice with malignant tumors.

...rain A mice, possessing a high spontaneous pulmonary tumor incidence along with presumably a low threshold of pulmonary reactivity to tumorigenic agents, were exposed to an atmosphere of ozonized gasoline for a tumor-dose study.[21] In contrast, C57BL mice, a strain possessing a low spontaneous pulmonary tumor rate with presumably a high resistance threshold to the induction of pulmonary neoplasms, were used for the primary induction of tumors.[23] Two control environments were used for contrasting the strains of mice under study. One consisted of an atmosphere washed by passage thr ugh successive banks of mechanical and activated carbon filters. The other environment was an open room in which the atmosphere was unaltered. The human experience of exposure to daily variation in pollutant concentration was thus duplicated.

As noted earlier, a modified tumor-dose-exposure study was undertaken with the strain A mice. The significance of experimental induction of pulmonary tumors in strain A mice in terms of extrapolations of the data to humans is but one facet of the much larger problem concerned with the application to man of data from carcinogenic studies in animals. While discussion of this problem is beyond the scope of this presentation, certain facts relative to spontaneously occurring and induced pulmonary tumors are pertinent. The true neoplastic character of these tumors has been established by the observation of metastases both in regional lymph nodes and in distant organs and by successful transplantation.[17, 37] Pathogenetic and histological differences notwithstanding, the commonness of spontaneous development of lung tumors especially in mice and man adds to rather than militates against the extrapolation of data from the former to the latter. Finally, it has been shown that the response of pulmonary tumor formation to meticulously controlled doses of carcinogens given intravenously is so sensitive that lung nodule formation has been advanced as a method for bioassay of suspected carcinogenic agents.[38]

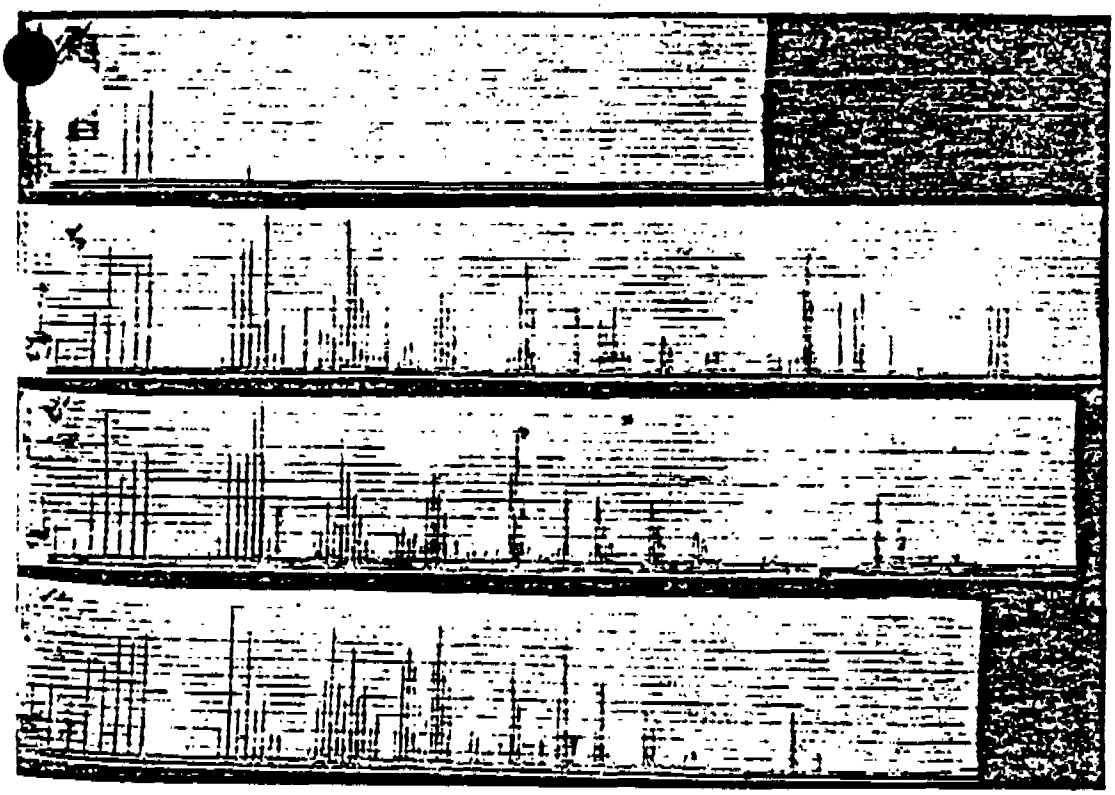


FIG. 14. Mass spectrometer studies showing similarity between naturally occurring and synthetically reproduced ...idant type smog. The first spectrogram is that of a concentrate from 150 liters of air sampled at El Rancho del ... Lindo, located on the desert near Palmdale, Calif., about 60 miles from Los Angeles. The second spectro... that of a Los Angeles smog sample, taken on Oct. 19, 1950, at −15° C.; the third is that of a Los Angeles ...le taken on Nov. 7, 1950, at −35° C., and the fourth is that of "witches' brew" synthetic smog, taken

TABLE 7

PRODUCTION OF LUNG TUMORS IN STRAIN A MICE*

Wk. of study	Sex of mice	No. studied†	Tot. no.	%	Tumor-bearing mice: % with mult. tumors	No. with sing. tumors	No. with mult. tumors: 2 tumors	3 tumors	4 tumors	Total tumors: No.	Total tumors: %‡
Ozonized gasoline–washed air chamber											
16	♀	15	2	13	...	2	...	...	...	2	13
20	♀	15	4	27	...	4	...	...	...	4	27
24	♀	15	4	27	...	4	...	...	...	4	27
28	♀	45	11	24	4.4	9	1	1	...	14	31
32	♀	45	8	18	4.4	6	2	...	...	10	22
36	♀ ♂	75	19	25	4.	16	3	...	...	22	29
40	♀ ♂	89	19	21	1.1	18	1	...	...	20	23
52	♀ ♂	39	16	11	10.	12	3	1	...	21	54
TOTAL		338	83			71	10	2	...	97	
PERCENTAGE				24							29
Aliphatic hydrocarbon–polluted air chamber											
16	♀	14	1	7	...	1	...	...	...	1	7
20	♀	14	5	36	...	5	...	...	...	5	36
24	♀	15	6	40	13.3	4	1	1	...	9	60
28	♀	44	21	48	11.3	16	3	1	1§	32	73
32	♀	45	20	45	11.1	15	5	...	...	25	56
36	♀ ♂	75	40	53	17.3	27	12	1	...	54	72
40	♀ ♂	90	57	63	31.1	29	21	5	2	94	104
52	♀ ♂	39	31	80	48.6	12	9	8	2	62	159
TOTAL		336	181			109	51	16	5	282	
PERCENTAGE				54							84

*Data are reprinted from *Cancer* 9: 911 and 913, 1956.
†Mice were removed at 4 week intervals for study, in the numbers indicated. At the end of the fifty-second week, all remaining mice were removed for study.
‡This percentage represents the total percentage of tumors in the total number of surviving mice.
§This mouse had 7 tumors.

The accumulated knowledge serves to re-emphasize certain pre-experimental considerations. First, the target tissue selected for study was the respiratory tract. Epidemiological data relating to cancer of this system served as the basis for this investigation. Second, the method of exposure chosen was that of inhalation. Third, the spontaneous pulmonary tumor formation observed in the strain A mice under study provided a sensitive target for the rapid procurement of data.

The results of exposing strain A mice to a washed air control atmosphere and polluted air test atmosphere are shown in Table 7.

The C57BL mice were exposed in inhalation chambers for a maximum of 92 weeks at which time surviving mice were killed. The incidence of lung tumors and certain data relating to them are shown in Table 8.

Two associated findings of marked interest were observed in the C57BL mice. The first was a consistent and often intense hyperplasia and metaplasia in the test chamber mice as compared to the controls. The second finding of interest was a significant decrease in the incidence of extrapulmonary tumors in the test mice. The changes observed in the mice exposed to the daily variati ns in air pollution as they naturally occur were essentially similar to those seen in the test chamber mice. The data relating to these experiments have been previously reported.[22]

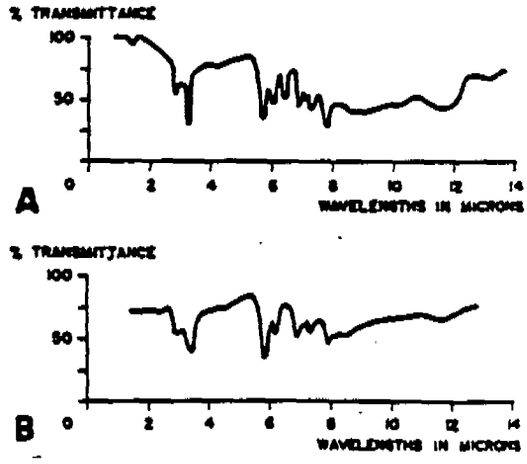


FIG. 13. Similarity between naturally occurring an synthetically reproduced ether-soluble aerosols. A Typical infrared spectrogram of synthetic aerosols. B Typical infrared spectrogram of natural aerosols.

FIG. 16. Intrabronchial papillary tumor in C57BL ... subsequent to inhalation of synthetic smog. (25.)

... readily apparent that the ozonized ...sphere atmosphere had a significant tumori...nic effect on the respiratory epithelium of ...e exposed mice. The tumors produced were ...entical with those referred to as classical ...veologenic carcinomas, although our find...gs indicated that these tumors were probably ...rminal bronchiolar rather than alveolar in ...rigin. Representative tumors are shown in ...gs. 16 and 17A and B. Several observations ...lating to these tumors are worthy of note. ... A significant number of tumors were pres...nt in association with intensive surrounding ...neumonitis. (2) In areas noncontiguous with ...mor areas and frequently in non–tumor-...volved lobes, hyperplastic and so-called ...etaplastic changes could be seen in the ...ronchial and bronchiolar epithelium. (3) ...reater pleomorphism and invasiveness were ...monstrable in the induced tumors than ...re seen in the tumors among the controls.

COMMENT

The identification of carcinogenic agents in ...llutant sources and in the atmosphere does ...t inevitably connote an adverse biological effect. Nevertheless, despite significant omissions in our data and despite the limitations imposed by the absence of or deficiencies in certain analytical and biological techniques, our chemical, physical, and biological data unite to form a constellation that strongly implicates the atmosphere as one dominant factor in the pathogenesis of lung cancer. The data are accorded additional significance by virtue of their congruity with the epidemiological pattern of lung cancer.

The following considerations are presented in sequence to support our concepts.

Carcinogenic agents have been identified in several ubiquitous sources of atmospheric pollution. Materials identified include 3,4-benzpyrene, aliphatic hydrocarbon oxidation products, and specific inorganic materials such as chromium and nickel compounds. Ever increasing pollution of the atmosphere with carcinogenic agents has been a concomitance of the industrialization and urbanization of society. One of the most ubiquitous of the carcinogenic agents belonging to the aromatic polyclic hydrocarbon group of chemicals is 3,4-benzpyrene. It is emitted into the air from many sources including gasoline and diesel engine exhausts, soot secondary to the incomplete combustion of organic matter, soot incidental to rubber tire wear, tear, and degradation, and, in isolated areas, soot from specific industrial effluents in the manufacture of coal tar and its derivatives.

Experimental studies have shown that the aromatic polycyclic hydrocarbons vary in their stability in the atmosphere, with 3,4-benzpyrene belonging to the group with maximum relative stability. The destruction rate of aromatic hydrocarbons in the atmosphere indicates that their survival in the atmosphere is consistent with their being inhaled by exposed populations. Even in a strong oxidizing atmosphere, such as occurs in Los Angeles, the rate of destruction of 3,4-benzpyrene is lower

TABLE 8

LUNG TUMOR INCIDENCE IN C57BL MICE*

Mouse gp.	Appear. 1st. tumor, wk.	No. mice surv.	Tumor-bear. mice	
			No.	%
Washed air chamber	56	376	6	1.6
Smog chamber	71	155	15	9.6

*Data are reprinted with correction from *Cancer* 11: 474, 1958.

FIG. 17. A, Papillary pulmonary tumor in C57BL mouse adjacent to site of intensive pneumonitis. (×125.) B, High power of marked area of Fig. 17.A, showing stream of neoplastic tissue into surrounding pneumonic lung. Note arrow. (×750.)

than that of many other hydrocarbons. In others areas, in which the oxidizing atmospheric property is less significant, the stability of this carcinogen is greater.

In addition to the traditional carcinogenic agents belonging to the group of aromatic polycyclic hydrocarbon compounds, we have characterized two heretofore unidentified polycyclic hydrocarbons in the atmosphere (Fig. 18). The structure of these compounds warrants their bioassay for carcinogenicity. Should they indeed be carcinogenic, these compounds will help explain the discrepancy observed by ourselves and by other investigators between tumor yield and benzpyrene content of samples from various environmental sources. [A third aromatic polycyclic hydrocarbon that has been recently identified is 3,4-benzfluoranthene. Subcutaneous injection of 5-mg. doses of this compound into C57BL mice has resulted in a tumor yield of more than 30% in experiments currently in progress.]

The aliphatic materials are primarily introduced into the atmosphere as a result of pollution by raw gasoline vapors. These vapors under certain meteorological conditions react in the presence of sunlight and oxides of nitrogen to form a broad spectrum of hydrocarbon oxidation products. These agents have become significant atmospheric pollutants simultaneous with intensive industrialization. Their temporal presence coincides with th epidemiological increase in lung cancer. The are capable of direct cellular entry, and thu the necessity for concern with the mechanism relating to the elution of aromatic polycycli hydrocarbons from soot is obviated. As lun cancer, in common with all neoplastic disease must surely have many agents involved in it inception, the simultaneous or sequential ac tion of both the traditional carcinogenic arc matic polycyclic hydrocarbons and the ali phatic oxidation products in the pathogenesi of lung cancer is a reasonable possibility Chromium and nickel though present only i infinitesimal amounts must also be considered by virtue of the occupati nal and/or experi mental data that incriminate them as carci nogenic agents.[1]

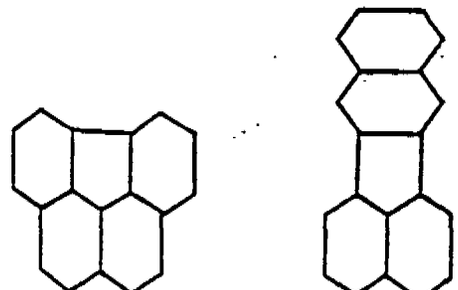
A critical factor in the elicitation of th carcinogenic response is the concentration a which the etiological agents are present. It i our belief that as of the m ment we must a sume that any exposure is hazard us.[19] As th carcinogenic dose is the product of the con centration of the environmental carcinogeni agent and the exposure time, current dat strongly suggest that in general we are bein exposed to low dosages of carcinogens. Whil little is really known concerning carcinogeni dosages in man, the exposure to a "low dose over a long period of time is compatible wit the induction of the neoplastic state. Abun

perimental evidence exists to indicate this is so. Further, both epidemiological and clinical data indicate that the development of cancer usually requires a prolonged interval. In the instance of human lung cancer, a period of from 10 to 20 years is postulated. This seems entirely reasonable when one considers that experimentally approximately 25% to 50% of the life span of an animal is usually required for the induction of cancer. Spontaneous tumors in experimental animals usually occur in the second half of the life span. This is similar to the experience in man.

The physical state of the carcinogenic hydrocarbons present in the atmosphere is one of adsorption on soot particles in a size range compatible with the deposition of and retention of a portion of the breathed carcinogen-laden particles. The anatomical site of deposition is a specific reflection of the size of particles. The relative infrequency of primary tracheal carcinoma is perhaps, among other factors, the result of the rarity of particle deposition on the tracheal epithelium. Those particles destined, on the basis of their size, to settle on tracheal epithelium are the ones that do not readily pass beyond the nose, accessory sinuses, and pharynx. Further, the adsorptive bond between particles settling on the bronchial and bronchiolar epithelium and the carcinogenic hydrocarbons is such that elution of the latter readily occurs in the presence of tissue and plasma proteins.[12]

Respiratory epithelial changes facilitating the deposition of and retention of particulates occur in response to a broad spectrum of irritants present in the atmosphere. The assembled data from studies suggest the following: (1) An apparent purposefully exaggerated activity of ciliated cells and goblet cells is the initial response of the respiratory epithelium to the deposition of foreign particulate irritants. (2) Persistence of the insulting agents rapidly leads to a neutralization of the protective factors, with resultant slowing of particle movement; this, in turn, leads to progressive accumulation of particles at selected sites in the respiratory tract. (3) A demonstrable difference in the effect on the epithelium can be observed, depending upon whether the respired material directly impinges on or passively flows over tracheobronchial epithelium. In the instance of the former, the effect is more intense and of greater duration. (4) Reversibility of the adverse effect of atmospheric environmental agents persists for an unanticipatedly long period, although restoration to base line levels of activity seldom occurs. (5) Denudation of the superficial epithelium permits the immediate apposition of inhaled particulate carcinogenic matter to the persisting layer of basal cells, which presumably may give rise to hyperplastic, metaplastic, and ultimately neoplastic change.

Soot recovered from human lungs has been shown to be free of the carcinogen 3,4-benzpyrene. Pyrene, although demonstrable, is present in significantly less than anticipated amounts. A gradient of disappearance exists, varying from partial in the case of pyrene to total in the case of 3,4-benzpyrene. The difference between the total disappearance of the carcinogen, 3,4-benzpyrene, and the partial disappearance of the noncarcinogen, pyrene, may be a reflection of the carcinogenicity of the former and innocuousness of the latter.



BENZ-(a,a,o)-FLUORANTHENE BENZ-(k)-FLUORANTHENE

FIG. 18. Two aromatic polycyclic hydrocarbons recently identified in atmosphere and now under bioassay for carcinogenicity.

SUMMARY AND CONCLUSIONS

The carcinogenic implications of atmospheric pollutants reside in at least two indispensable factors relating to the pathogenesis of lung cancer. The first is, of course, the environmental presence of and the host entry of agents proved experimentally to be tumorigenic and epidemiologically to be associated with an increased liability to the development of the disease. The second factor is the atmospheric existence of host-modifying factors that, by virtue of their effect on the ciliated epithelium of the tracheobronchial tree, make possible the abnormal deposition and retention of particulate matter in the lungs. In the instance of carcinogenic aromatic polycyclic hydrocarbons, the elution of benzpyrene from

soot by intracellular proteins is thereby facilitated. A significant local concentration of desorbed aromatic polycyclic hydrocarbons, including 3,4-benzpyrene, results. Atmospheric irritants may, in addition, periodically and intermittently cause denudation of the superficial epithelium, so that the basal cell layer is directly exposed to the carcinogenic stimulus. This periodic epithelial desquamation and regeneration in the presence of an abnormal growth stimulus is regarded as providing a favorable environment for subsequent abnormal growth patterns.

Carcinogenic aliphatic oxidation products present in the atmosphere in aerosal form are experimentally capable of direct cellular entry, so that the respiratory epithelial cells are subjected to simultaneous or sequential exposure to tumorigenic agents. An endogenous environment favorable to the biological activity of the carcinogenic hydrocarbons results.

Bronchogenic carcinoma represents one of the current critical problems in the field of pulmonary disease. Laboratory investigation can contribute much information to the ultimate solution of this problem. In the physical science area, finite analytical data are possible. In the biological realm, strong supporting data can be secured, despite the fact that experimental investigations are necessarily limited to nonhuman animal species. It is necessary, of course, to remember certain deficiencies inherent in the biological studies. Choice of species, selection of appropriate animal strain, duration of exposure, concentration of test material, and routes of administration are all variables that modify the extrapolation of experimental data from other animals to man. Despite these shortcomings, past experience has shown a high index of meaningfulness of animal experiments for the human species. The broad spectrum of agents carcinogenic for visceral organs in experimental animals and apparently for those in man should make one proceed with caution in attributing the absolute dominance of any one agent over another.

It is perhaps safest to regard the development of lung cancer as the end stage of a series of sequential changes that require the presence of a carcinogenic agent, environmental host-modifying factors, and the innate susceptibility of the host. All of the links in the chain are not of equal magnitude. Rather than searching exclusively for a "cause" of lung cancer, it might well be more rewarding to investigate the series of changes that constitute its pathogenesis.

REFERENCES

1. BAETJER, A. M.: Pulmonary carcinoma in chromate workers; I, review of literature and report of cases. *Arch. Indust. Hyg.* 2: 487-504, 1950.

2. CAMPBELL, J. M., and KREYBERG, L.: Degree of air pollution in Norwegian towns. *Brit. J. Cancer* 10: 481-484, 1956.

3. CLEMO, G. R., and MULLER, E. W.: Carcinogenic action of city smoke. *Chem. & Ind.* [1955]: 38, 1955.

4. DAUTREBANDE, L.; BECKMANN, H., and WALKENHORST, W.: Lung deposition of fine dust particles. *A. M. A. Arch. Indust. H.* 16: 179-187, 1957.

5. DIKUN, P. P.; SRABAD, L. M., and NORKIN, V. L.: [Some new data on pollution of atmosphere of industrial cities by 3,4-benzopyrene.] *Gig. i Sanit., Moskva* 21: 6-11, 1956.

6. DOLL, R., and HILL, A. B.: Lung cancer and other causes of death in relation to smoking: second report on mortality of British doctors. *Brit. M. J.* 2: 1071-1081, 1956.

7. DOLL, R.; HILL, A. B., and KREYBERG, L.: Significance of cell type in relation to aetiology of lung cancer. *Brit. J. Cancer* 11: 43-48, 1957.

8. DUNN, H. L.: Lung cancer in twentieth century. *J. Internat. Coll. Surgeons* 23: 326-342, 1955.

9. DURAN-REYNALS, F.: Neoplastic infection and cancer. *Am. J. Med.* 8: 490-511, 1950.

10. FALK, H. L.; KOTIN, P., and MARKUL, I.: Disappearance of carcinogens from soot in human lungs. *Cancer* 11: 482-489, 1958.

11. FALK, H. L.; MARKUL, I., and KOTIN, P.: Aromatic hydrocarbons: IV, their fate following emission into atmosphere and experimental exposure to washed air and synthetic smog. *A. M. A. Arch. Indust. H.* 13: 13-17, 1956.

12. FALK, H.; MILLER, A., and KOTIN, P.: Elution of 3,4-benzpyrene and related hydrocarbons from soot by plasma proteins. *Science* 127: 474-475, 1958.

13. FIESER, L. F.; GREENE, T. W.; BISCHOFF, F.; LOPEZ, G., and RUPP, J. J.: Carcinogenic oxidation product of cholesterol. [Communications to the Editor.] *J. Am. Chem. Soc.* 77: 3928-3929, 1955.

14. HAAGEN-SMIT, A. J.: Chemistry and physiology of Los Angeles smog. *Indust. & Engin. Chem.* 44: 1342-1346, 1952.

15. HAMMOND, E. C., and HORN, D.: Smoking and death rates—report on 44 months of follow-up on 187,783 men; II, death rates by cause. *J. A. M. A.* 166: 1294-1308, 1958.

16. HOFFMAN, E. F., and GILLIAM, A. G.: Lung cancer mortality, geographic distribution in United States for 1948–1949. *Pub. Health Rep.* 69: 1035-1042, 1954.

17. KLEIN, M.: Transplantability and growth of urethan-induced pulmonary adenomas of mouse. *Cancer Res.* 17: 655-658, 1957.

18. KORTEWEG, R.: Mortality from lung cancer in Netherlands during and after last war. *Brit. J. Cancer* 8:34-39, 1954.

19. KOTIN, P.: Experimentally weak carcinogens. [Guest edit.] *Cancer Res.* 18: 1-3, 1958.

20. KOTIN, P., and FALK, H. L.: Production of tumors in C57BL mice with atmosphere-extracted al-

...drocarbons. [Abstr.] *Proc. Am. A. Cancer* ... 1955.

...IN, P., and FALK, H. L.: II. Experimental ...duction of pulmonary tumors in strain-A mice after ...ir exposure to atmosphere of ozonized gasoline. ...cer 9: 910-917, 1956.

22. KOTIN, P.; FALK, H. L.; MADER, P., and THOMAS, ...: Aromatic hydrocarbons; I, presence in Los Angeles ...mosphere and carcinogenicity of atmospheric extracts. *A. M. A. Arch. Indust. Hyg.* 9: 153-163, 1954.

23. KOTIN, P.; FALK, H. L., and MCCAMMON, C. J.: III. Experimental induction of pulmonary tumors and ...anges in respiratory epithelium in C57BL mice following their exposure to atmosphere of ozonized gasoline. *Cancer* 11: 473-481, 1958.

24. KOTIN, P.; FALK, H. L., and THOMAS, M.: Aromatic hydrocarbons: II, presence in particulate phase ... gasoline-engine exhausts and carcinogenicity of exhaust extracts. *A. M. A. Arch. Indust. Hyg.* 9: 164-177, 1954.

25. KOTIN, P.; FALK, H. L., and THOMAS, M.: Aromatic hydrocarbons; III, presence in particulate phase ... diesel-engine exhausts and carcinogenicity of exhaust extracts. *A. M. A. Arch. Indust. H.* 11: 113-120, 1955.

26. KOTIN, P.; FALK, H. L., and THOMAS, M.: I. Production of skin tumors in mice with oxidation products of aliphatic hydrocarbons. *Cancer* 9: 903-909, 1956.

27. [LARSON, G. P., Director]: Second Technical and Administrative Report on Air Pollution Control in Los Angeles County; 1950–1951. Los Angeles, Calif. Air Pollution Control District, County of Los Angeles. 1951.

28. LOMBARD, H. L.: Increase in lung cancer in Massachusetts. *Cancer* 9: 667-670, 1956.

29. LYONS, M. J., and JOHNSTON, H.: Aromatic ...drocarbons from vehicular exhausts. *Brit. J. Cancer* ... 1957.

...NCISO, T. F., and COULTER, E. J.: Cancer mortality among native white, foreign-born white, and ...nwhite male residents of Ohio: cancer of lung, larynx, bladder, and central nervous system. *J. Nat. Cancer Inst.* 20: 79-103, 1958.

31. NIELSEN, A., and CLEMMESEN, J.: Bronchial carcinoma—pandemic; II, incidence and tobacco consumption in various countries. *Danish M. Bull.* 1: 194-199, 1954.

32. PASCUA, M.: Increased mortality from cancer of respiratory system. *Bull. World Health Organ.* 12: 687-703, 1955.

33. ROSS, W. C. J.: Biological action of X-rays, nitrogen mustards, diepoxides and peroxides. [Letter to the Editor.] *Nature, London* 165: 808-809, 1950.

34. ROSS, W. C. J.: Chemistry of cytotoxic alkylating agents. *Advances Cancer Res.* 1: 397-449, 1953.

35. SHEPHERD, M.; ROCK, S. M.; HOWARD, R., and STORMES, J.: Isolation, identification, and estimation of gaseous pollutants of air; examination of Los Angeles County, Calif., smog. *Anal. Chem.* 23: 1431-1440, 1951.

36. SHIMKIN, M. B., and MCCLELLAND, J. N.: Induced pulmonary tumors in mice; IV, analysis of dose response data with methylcholanthrene. *J. Nat. Cancer Inst.* 10: 597-603, 1949.

37. STEWART, H. L.; GRADY, H. G., and ANDERVONT, H. B.: Development of sarcoma at site of serial transplantation of pulmonary tumors in inbred mice. *J. Nat. Cancer Inst.* 7: 207-223, 1947.

38. STOCKS, P.: Lung cancer, tobacco and atmospheric pollution. *In* OGILVIE, H., Ed.: British Empire Cancer Campaign; Thirty-First Annual Report Covering the Year 1952. London, England. British Empire Cancer Campaign. 1953; pp. 410-412.

39. STOCKS, P.: Statistics of cancer of lung. *J. Fac. Radiologists* 6: 166-173, 1955.

40. STOCKS, P., and CAMPBELL, J. M.: Lung cancer death rates among nonsmokers and pipe and cigarette smokers; an evaluation in relation to air pollution by benzpyrene and other substances. *Brit. M. J.* 2: 923-929, 1955.

41. WALLER, R. E.: Benzpyrene content of town air. *Brit. J. Cancer* 6: 8-21, 1952.

42. WINTERNITZ, M. C.; WASON, I. M., and MCNAMARA, F. P.: The Pathology of Influenza. New Haven, Conn. Yale University Press. 1920; p. 48.