Carcinogenic Studies on Petroleum Asphalt, Cooling Oil, and Coal Tar

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Among the various environmental factors which during the last few decades have been incriminated in the causation of cancers of the lung and in their remarkable increase in frequency during the past 50 years, dusts, and fumes from coal tar and petroleum asphalt, as well as mists and fogs of lubricating and cutting oils inhaled either for occupational reasons or as disintegration products from tarred, asphalted, or oiled roads have frequently been cited (Kennaway and Kennaway; Kawahata; Kuroda and Kawahata; Doll; Patch: Koelsch; Schabad; Mullschitzky; Lehmann; Kling, Samssonov, and Héros). Bonne, Barnewitz, and Campbell, among others, investigated this problem with equivocal results as far as production of pulmonary cancers is concerned on animals exposed to dust from tarred roads. Epidemiologic observations demonstrated that workers exposed to the inhalation of vapors and fumes generated during the production of coke and illuminating gas, as well as during the heating of coal tar used for the construction and repair of roads and roofs, have an excessive liability to cancer of the lung. Similar observations, on the other hand, are not available on this point for workers exposed to fumes and vapors given off by heated petroleum asphalts. The experimental demonstration of carcinogenic properties of these petroleum derivatives was only very recently reported by Simmers, Podolak, and Kinosita after the completion of the studies related in this communication.

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The effluent of coke oven operations and of petroleum refineries are often discharged into bodies of water which serve as drinking-water supplies for communities located in the vicinity of such plants. These general types of bituminous substances, therefore. possess an additional interest from the viewpoint of water pollutants (Hueper and Ruchhoff).

Recent observations have shown that man and animals exposed to cutaneous contact with boiling fractions of petroleum distillation, i.e., lubricating and cooling oils, develop cancers of the skin (Shubik and Saffiotti; Eckardt; Gilman and Vesselinovitch; Mastromatteo; Cruickshank). While certain worker groups, especially those employed in metallurgical and textile factories, had for years some respiratory exposure to cooling and lubricating oils by inhaling mist of sprayed oils, this contact has been intensified during recent years by the practice of nebulizing cutting oils, thereby exposing workers employed in metallurgical operations to an oil fog which readily enters the deeper parts of the respiratory tract and thereby may create a lung-cancer hazard.

Because of the rather widespread respiratory contact not only of certain special worker groups, but also of larger portions of the general population with dust, fumes. and vapors from coal tar and petroleum asphalt and with finely dispersed lubricating oils from motors and machinery. it seemed important to obtain additional factual experimental evidence concerning the often-cited carcinogenic properties of the various bituminous substances by testing them for carcinogenic effects by inhalation in the form of fumes or fogs into the lungs, by applying them to the skin, and by injecting

them into the muscle tissue of experimental animals.

Experimental Procedures

Materials Used.—Four road asphalts oi different manufacture were obtained through the courtesy of Mr. E. F. Kelly, U.S. Bureau oi Public Roads. Three were steam distillation products and were obtained irom crudes of fields in Mississippi, California, and Venezuela, respectively, while the fourth was a steam-vacuum distillation product of a crude from a field in Oklahoma. They had flash points between 475 and 570 F and were soluble to over 99.8% in carbon tetrachloride, and, therefore, consisted essentially of a mixture of hydrocarbons.

For the carcinogenic testing of the four road asphalts, mice, rats, and rabbits were employed. The asphalts were diluted with a sufficient amount of acetone so that a drop could be applied twice weekly for a maximal period of two years to the nape of the neck oi C57 black mice, using 25 males and 25 females for each asphalt. The same material was, moreover, painted on the inside of both ears and on a shaved (2 cm. square) area of the back of six rabbits, following the same schedule as that used for the mice, except that undiluted but heated asphalts were applied. Rabbits of the New Zealand strain were employed. Animals which survived for two years were killed at the end of this period.

The asphalts diluted with equal parts of tricaprylin were injected at biweekly intervals, 6 times for mice and 12 times for rats, into the muscle tissue of the right thigh oi Strain 50C 57 black mice and 30 Bethesda black rats, using as a single dose 0.1 cc. for the mice, and 0.2 cc. for the rats. The maximal observation period was two years.

One roofing asphalt was procured from a commercial source. It was a blown petroleum asphalt produced from residual oil of asphaltic type through which for several hours air had been blown at elevated temperatures (Abraham) causing a~oxidation of hydrocarbons. This particular blown asphalt had a flash point of 393 F.

The coal tar used was obtained from a major processer of coke oven tars. Similar products this source had previously been tested on mice and rabbits and had been found to be carcinogenic to the skin of these animals. Observations on the Occurrence of cancers of the skin and, possibly, of the lung among distillers of tars as well as among their industrial users were on record.

For the purpose of the inhalation experiment, between 400 and 1,000 gm. of coal tar and irom 700 to 1,000 gm. of roofing asphalt was placed in a large evaporating dish, which was placed in an electric heater and heated to about 250 to 275 F, i.e., rather close to the flashpoint. The temperatures were controlled by a rheostat. Under these conditions a considerable amount of brownish fumes developed from the tar and asphalt. Since these apparatus were placed directly into the exposure chamber, it was necessary to introduce sufficient amounts of cooled air to keep the temperature within the chamber at around 75 F. The cooled air was delivered into the chamber from an air-conditioning unit, the operation of which was controlled by a thermocouple placed within the exposure chamber. Between 10 and 30 gm. of the coal tar and between 2 and 10 gm. of the petroleum asphalt were volatilized every day during five hours of operation. Fresh tar and asphalt were placed into the evaporating dish once a week. On other days only the lost mount was replaced. The operation was carried on for four days a week during a total period of two years.

The fumes and vapors, after having passed through the chambers. were sent through two Fiberglas filters for the removal of a larger portion of the particulate phase in the effluents before they were discharged into the general exhaust system. A greasy gray-brown material, moreover, condensed from the fumes in the pipes connecting the chamber with the filters. This phenomenon was especially marked for the coal tar operation. The chambers were kept under negative pressure controlled by an alarm system connected with a flow meter.

Into each chamber were placed 30 guinea pigs, Strain 13, 2 months old, and 65 Bethesda black rats, females, 2 months old.

The Fiberglas filters were renewed at intervals of one month. For testing the coal-tar fume condensate for carcinogenic properties at one occasion the filters used in the coal tar operation were extracted with dichlorornethane in a Soxhlet apparatus until a clear solvent was obtained. This process was repeated first with benzene and then with acetone to be certain that all of the extractable material had been removed. After the solvent had been evaporated from the extract, the condensate was used in a bioassay for determining whether the fumes and vapors given off by the heated coal tar contained carcinogenic material retained by the filters after having been in contact with the animals in the exposure chambers. This filter extract condensate will be referred to as coal tar filter extract. This material was diluted with olive oil at a ratio of 1 part extract to 3 parts olive oil, since the undiluted extract proved to be too toxic to mice upon intramuscular injection. One hundred C57 black mice. Line 6, equally distributed as to both sexes, about 8 weeks old, received six biweekly injections of 0.15 cc. of the diluted material into the muscle tissue of the right

TABLE 1.—*Neoplastic Reactions in Mice and Rats Receiving Cutaneous and Intramuscular Administration of Road Asphalts*

Type of Asphalt	Route of Administr.	Species	No.	Neoplastic Reactions						
				Skin				Lymph Node		
				Carc.	Papil.	Thigh Sa.	Leukemia Lymphoma	Liver	Sarcoma	Uterus Carc.
Venezuela	Skin painting	Mouse	100				4			
	Thigh intramuscular	Mouse	50			1	1			
		Rat	30			2		2		
Mississippi	Skin painting	Mouse	50	1	1		1			
	Thigh intramuscular	Mouse	50			1	1			
		Rat	30			1		3	1	1
Oklahoma	Skin painting	Mouse	50		1		2			
	Thigh intramuscular	Mouse	50					1		
		Rat	30			1				1
California	Skin painting	Mouse	50							
	Thigh intramuscular	Mouse	50	1		1		1		
		Rat	30			6		4	1	1
Control	Normal	Mouse	200				1			
	Tricaprylin subcutaneous	Mouse	144							
	Normal	Rat	60					4	1	3

length. The maximal observation period was two years.

Coal tar as well as petroleum roofing asphalt were, moreover, tested for carcinogenic properties on 50 C57 black mice (25 of each sex) by applying these materials, sufficiently heated to make them liquid, twice weekly to the nape of the neck for a maximal period of two years.

For the inhalation of cutting oils a commercially used mixture of neutral paraffin oil and prime lard oil was used, blended at a ratio of 10:1 by volume. This mixture was injected, through a commercial type jet (Milford Atom Lube) operated by compressed air, into the exposure chamber where it delivered a scarcely visible oil fog. During six hours of operation from 80 to 100 gm. of this oil were discharged. The jet was operated for six hours per day, four days per week, for a total period of two years. A total of 30 guinea pigs and of 105 rats were thus treated. Of the guinea pigs, 34 animals, and of the 105 rats, 41 animals survived this treatment for more than a year.

The petrolatum (paraffin) oil was painted on the skin of the nape of the neck of 50 C57 black mice, equally divided as to sex, twice a week for 24 months, when the last surviving animals were killed. One hundred mice of the same strain were, moreover, injected twice with 0.3 cc. of the paraffin oil into the muscle tissue of the right thigh, with an interval of two months between the two injections.

The skin at the nape of the neck of 25 male and 25 female mice of the C57 black strain was painted twice a week for a maximum period of 22 months with the condensate of a benzol extract of an activated carbon filter, through which large amounts of the effluent waters of an oil separator of a petroleum refinery having only a thermal cracking unit were passed.

An identical experiment was performed with the condensate of a benzol extract of an activated carbon filter through which water from a coking plant was passed. These materials were supplied by the Sanitary Engineering Center, U.S. Public Health Service, Cincinnati, and had a tarry appearance.

Postmortem examinations were performed on all animals. Histologic examinations were made of all tissues which exhibited gross abnormalities.

Observations and Results

The principal results obtained with the various test materials and by the different routes of administration to the several species of animals used are summarized in Tables 1-3.

The papillomas and carcinomas of the skin in mice after painting with asphalts and the sarcomas of the thigh following intramuscular injection of these materials into mice and rats are anatomic reactions to the carcinogenic constituents present in the asphalts (Figs. 1-3). It is probable that the four leukemias seen in 100 mice painted with Venezuela asphalt and the two leukemias and one Kupffer-cell sarcoma found among 100 mice treated with asphalt from Oklahoma crude are also causally related to these agents. Whether any of the other leukemias found among the asphalt-treated

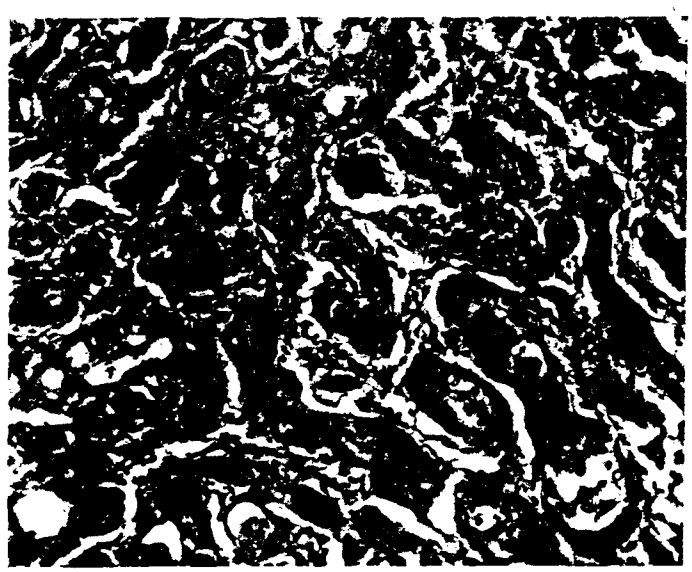

Fig. 1.—Squamous-cell carcinoma of the skin consisting in part of small nests of squamous cells with abortive cornified pearls. Mouse was painted with petroleum asphalt. × 205.

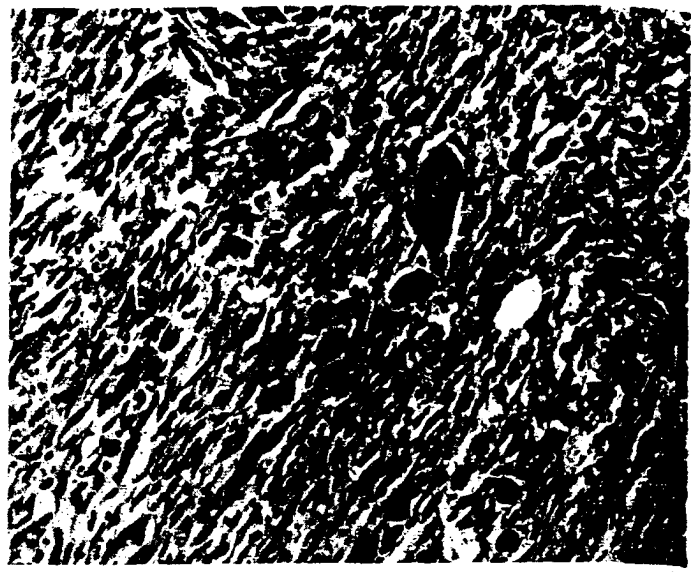

Fig. 2.—Spindle-cell sarcoma with multinucleated giant cells in the thigh of a mouse receiving intramuscular injections of petroleum asphalt. × 205.

mice have such associations appears doubtful, since such reactions occur spontaneously among animals of this strain. The sarcomas of the liver and ileocecal lymph nodes, as well as the carcinomas of the uterus seen in rats, on the other hand, are evidently unrelated to the administration of the asphalts, because similar incidence rates of these tumors were observed among the control animals. It can be considered as established that under the experimental conditions used, road asphalts obtained from crudes of four different fields contain carcinogenic substances active in mice and rats.

The results obtained with coal tar fumes, coal tar distillate, and petroleum roofing asphalt clearly demonstrate the distinct carcinogenicity of the coal tar distillate to the skin and soft tissues of mice, yielding a cancer rate by both routes of about 50% of the treated animals (Fig. 4). None of the animals which inhaled coal tar or petroleum asphalt fumes developed any cancers of the lung or of the skin. All cancers seen

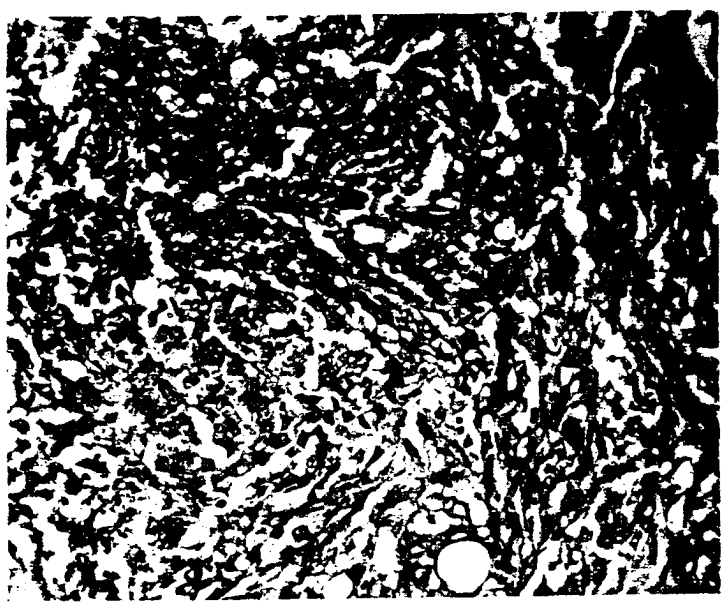

Fig. 3.—Spindle-cell sarcoma invading skeleton muscle of the thigh of a rat following intramuscular administrations of petroleum asphalt. × 205.

TABLE 2.—*Neoplastic Reactions in Rats and Guinea Pigs Inhaling Fumes from Coal Tar and Petroleum Roofing Asphalt and in Mice, Rats, and Rabbits Receiving Cutaneous Administrations and Intramuscular Injections of These Materials*

Substance	Route of Administration	Species	No.	Neoplastic Reactions						
				Skin			Leukemia	Lymph Nodo		
				Carc.	Papil.	Thigh Sa.	Lymphoma	Liver	Sarcoma	Uterus
Coal tar	Inhalation	Rat	75				1	6	1	
		Guinea pig	42							
Coal tar Distillate	Skin painting	Mouse	50	22	4	a				
	Thigh intramuse.	Mouse	100							
Petroleum roofing tar	Inhalation	Rat	65					3	2	1
		Guinea pig								
	Skin painting	Mouse	50	17						
		Rabbit	6							

in rats exposed by this route are evidently of "spontaneous" origin. Petroleum roofing tar, in contrast to coal tar, was also noncarcinogenic to the skin of mice and rabbits. The apparent noncarcinogenicity of roofing asphalt may be due to the fact that the aeration of this material resulted in an oxidative destruction of any carcinogenic material which might originally have been present.

Mention may be made of the fact that both guinea pigs and rats inhaling fumes from coal tar and petroleum asphalt showed, in some instances, extensive chronic fibrosing pneumonitis with peribronchial adenomatosis, associated in the rats with squamous-cell metaplasia of the bronchial mucosa or of frequently noted bronchiectatic lumens. In none of the animals have these changes assumed a cancerous character. They are, moreover, found not infrequently in both rats and guinea pigs after respiratory or intravenous administration of various substances or as the result of nonspecific chronic pneumonitis.

The observations made indicate that the paraffin oil contained in the cooling oil blended with lard was mildly carcinogenic to the skin of mice (Fig. 5) and to the lung of rats (Fig. 6), and may, moreover, have elicited lymphomatous or leukemic reactions in mice.

As in the inhalation experiment with coal tar and petroleum asphalt, the lungs of guinea pigs and rats sometimes exhibited peribronchial adenomatosis and proliferations of the bronchiolar epithelium. In the guinea pigs these lesions were often associated with foreign-body granulomas surrounding clear, reflecting deposits (Fig. 7).

None of the 50 mice painted with adsorbates of the water of a coke oven plant and of an oil refinery developed tumors of any kind.

Comment

The general character and significance of the neoplastic reactions obtained with the four road asphalts tested and found at the sites of administration are similar to those

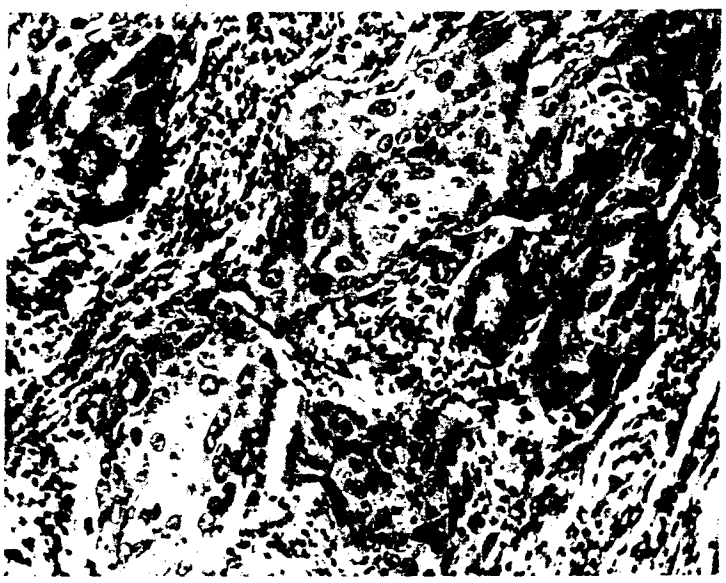

Fig. 4.—Cornified squamous-cell carcinoma of the skin of a mouse painted with condensate of coal tar fumes. × 205.

reported by Simmers et al. in 1959. It is perhaps noteworthy that asphalt from California crude was the most potent one of the four studied and that it came closest in this respect to the mixture of several California asphalts tested by Simmers et al. There exists, moreover, the suspicion that constituents of asphalt absorbed into the organism stimulate and/or promote the development of leukemia and lymphomatosis in mice through exerting a systemic effect. It thus appears that petroleum asphalts prepared from crudes of various fields possess mild to moderate carcinogenic properties for the skin and soft tissues of mice and rats. Since the top layer of asphalted roads is usually cured by the addition of lower boiling fractions of petroleum distillates, ranging from heavy fuel oil ior slow curing, to kerosene or crude gasoline, for rapid curing, dust from asphalted roads originating from such a processed top layer may possibly be somewhat more carcinogenic than the asphalts proper. From experimental observa-

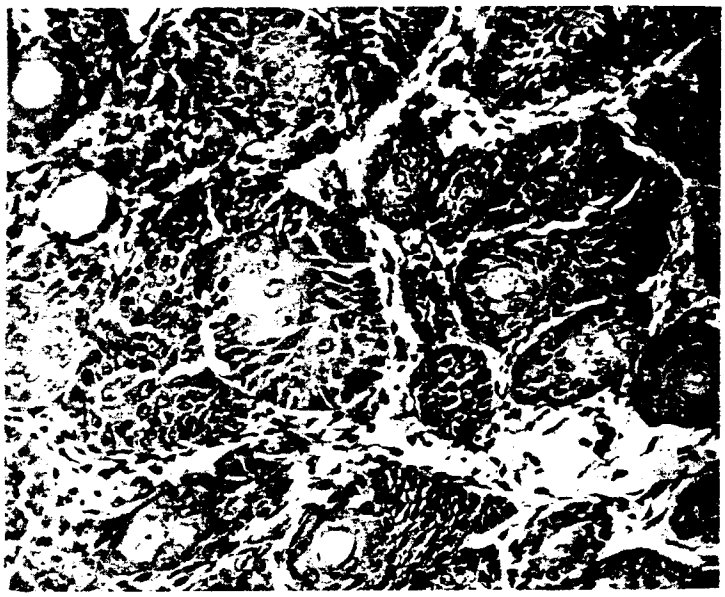

Fig. 5.—Cornified squamous-cell carcinoma of the skin of a mouse painted with paraffinic cutting oil. × 205.

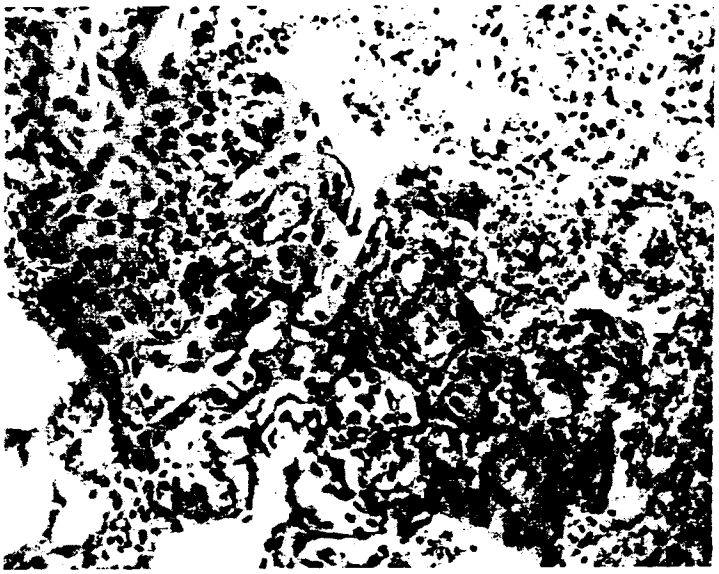

Fig. 6.—Small squamous-cell carcinoma in the subpleural portions of the lung in a rat inhaling a blend of paraffinic cooling oil and lard. × 205.

tions of Woodhouse and Irwin it appears that high-boiling oily fractions of petroleum seem to be more carcinogenic than tarry and pitchy fractions when tested on the skin of mice. This phenomenon may in part be owing to the fact that the latter contain polymerized and therefore chemically and carcinogenically rather inert constituents. Burrell recently supplied circumstantial epidemiologic human evidence which incriminated alcoholic liquor brewed in barrels retaining some of their original contents of cut-down asphalt, a product consisting of asphalt with a variable proportion of spilt-over fuel oil, as the suspected cause of an excessive frequency of esophageal cancer among inhabitants of several Bantu villages in South Africa consuming this beverage.

The experimental and epidemiologic evidence on hand thus indicates that occupational and environmental exposure to petroleum road asphalts may be associated with cancer hazards to the tissues of contact. The observations made with one sample of petroleum roofing asphalt subjected to oxidative processing, on the other hand, do not **sup**

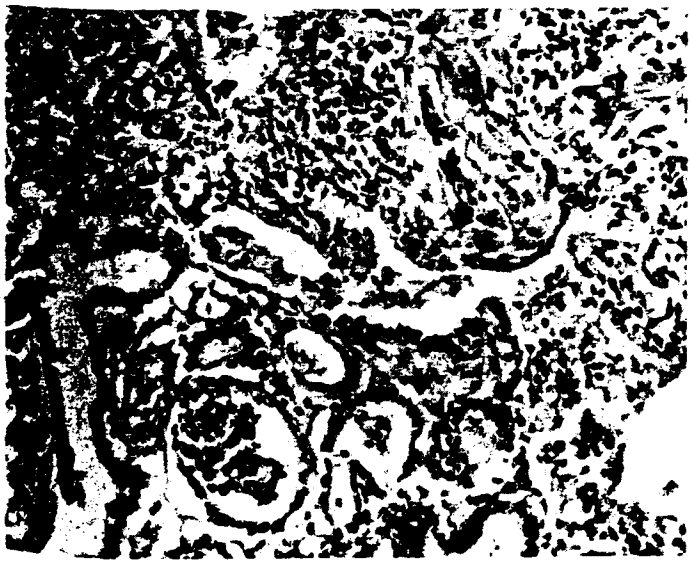

Fig. 7.—Adenomatosis of the lung in a guinea **pis** inhaling cooling-oil fog, with faintly stained, reflecting rod-like deposits in lumen. × 205.

port at present a similar effect for this type of asphalt, since not a single tumor was produced with this material in mice, rats, guinea pigs, and rabbits.

While the absence of any lung tumors in rats and guinea pigs exposed to the inhalation of roofing tar may thus be attributed to an apparent noncarcinogenicity of this material, such an explanation fails completely to explain the absence of any carcinomatous pulmonary reactions in members of these two species which were inhaling fumes of coal tar, because these proved when condensed to be highly carcinogenically potent in mice and rats when cutaneously and intramuscularly administered.

The few observations on the successful production of pulmonary cancers in experimental animals following the inhalation of various carcinogenic dust, fumes, or vapors (beryllium, nickel, radioactive material, gasoline engine exhaust) continue to emphasize the disappointing fact that the respiratory tract of experimental animals appears to be much more resistant to inhaled carcinogenic matter than the human bronchial tree even when carcinogenic material is administered in doses which are much higher than those ordinarily encountered under the worst occupational conditions by man. This observation contrasts distinctly, on the other hand, with the demonstration that the lung tissue of various species is not refractory to the action of various carcinogens, since pulmonary cancers have readily and repeatedly been elicited when such materials were introduced by other methods and routes into the lung. It, therefore, seems as if the degree of exposure of the lung tissue to inhaled carcinogens is, as a rule, inadequate for eliciting cancerous responses in the lungs of experimental animals.

The cancers produced in mice and rats with paraffinic cooling oil confirm previously reported observations on the carcinogenic action of such oils on the skin of man and animals. They provide additional evidence concerning a probable existence of distinct cancer hazards associated with an occupational inhalation of cooling oil fogs in indus-

TABLE 3.—*Neoplastic Reactions in Rats and Guinea Pigs Inhaling Cooling Oil and in Mice Painted and Intramuscularly Injected with Cooling Oil*

Substance	Route of Administr.	Species	No.	Neoplastic Reaction							
				Lung	Skin		Thigh Sa.	Leukemia Lymphoma	Lymph Node		Uterus
					Carc.	Pap.			Liver	Sarcoma	
Cooling oil	Inhalation	Rat	105	1					1		
		Guinea pig	65								
Paraffin oil	Skin painting	Mouse	50		1	1		2			
	Thigh Intramuscular	Mouse	100					4			

try and possibly also with an inadvertent medicinal introduction of such paraffinic oils into the lung. Such a hazard should be especially pronounced whenever insufficiently refined oils enter the human body (Santé; Wood; Foe and Bigham; Proudfit, Van Ordstrand, and Miller; Hueper; Huguenin, Fauvet, and Bourdin). This suspicion should be maintained despite the essentially negative outcome of the inhalation experiments reported here and the similarly unsuccessful efforts of Lushbaugh et al. in this respect.

The negative results obtained with the two water adsorbates obtained fro121 effluents of a coking plant and of an oil refinery extend previous experiences with such materials reported some time ago (Hueper and Ruchhoft). Since the Sanitary Engineering Center, U.S. Pupil Health Service, has recently installed equipment through which large amounts oh such adsorbates become available for testing on a comprehensive scale and by various routes, the significance of the positive, as well as negative, findings made on experimental animals with such absorbates for man consuming drinking water obtained from industrially polluted sources will then be determined in a more reliable and thorough manner than was possible heretofore.

The observations made in the various experiments finally provide pertinent evidence relative to the recently often advanced claim that sarcomas produced in rats at the site of the subcutaneous introduction of test material of any kind represent by themselves not adequate proof for the carcinogenicity of the substances eliciting such cancers. In fact, the claim has been made that these sarcomas in rats are even not real cancers but merely some anatomical reaction having a histologically cancer-like appearance, since they were said not to grow infiltratively into the surrounding tissue and not to produce metastases (Brunner). These allegations have become, during the past years, of more than purely scientific importance, because they have been employed to discredit the significance of cancers produced in rats by chemicals present in consumer goods by this method, and to remove thereby existing legal objections against their use as food additives.

Such contentions through which sarcomas, arising from the connectif-e tissue of rats upon the introduction of some chemical, are assigned an exceptional position apart from any other cancer, however, are scientifically unsound because they are based on incorrect or biased arguments. The present experiments have clearly shown that petroleum asphalts and cooling oil capable of producing fibrosarcomas and spindle-cell sarcomas of the thigh in rats elicit identical reactions in the soft tissues of the thigh of mice and, in addition, produce squamous-cell carcinomas of the skin of mice. Similar correlations between the carcinogenic responsiveness of epithelial and connective tissues of rats and mice have been demonstrated for methylcholanthrene, various polymeric plastic films (Oppenheimer et al.), for chromium compounds (Hueper and Payne), nickel and nickel salts (Hueper: Gilman), shale and Bergius oils (Hueper), and paraffin (Bonser et al.; Meigs). The majority of the sarcomas produced in rats by these and other agents, moreover. showed encapsulation only toward the skin, while they were growing infiltratively into the muscle tissue and bone at their base (Hueper). A not inconsiderable number of them, moreover, formed metastases in distant organs. It is noteworthy in this connection that dunng a recent study of cancers produced in mice by the subcutaneous injection of 3,4-benzpyrene dissolved in tricaprylin, none of the sarcomas formed in 200 mice thereby (measuring up to 2 cm. in diameter) produced a metastasis, although all were invading the tissue at their base. It would be most unwise to consider the sarcomas thus produced as insufficient evidence attesting to a carcinogenic property of 3,4-benzpyrene. Experiences based on carcinogenic investigations with numerous chemicals and many thousands of rats of several strains provide, moreover, scientifically sound and adequate proof of the

fact that the connective tissue of rats does not respond to the introduction of practically all chemical irritants with the development of a sarcoma.

The adoption of the discriminating claims concerning the unsuitability of the subcutaneous introduction of test material into rats for determining carcinogenic properties, in fact, would in the final analysis result in discrediting the scientific soundness of the numerous carcinogenic experiments performed on rats. It scarcely can be maintained that the objections advanced can be applied to connective tissue of the subcutaneous region only. They cannot plausibly be limited to other types of mesenchymatous or epithelial tissues, such as muscle cells, periosteal cells, liver cells, epidermal cells, and mucosal epithelium of the bladder, for which in part similar differences in species-specific susceptibility have been observed.

The large amount of reliable and valid experimental evidence on this subject provides adequate justification for refuting the claim for the establishment of a special position for sarcomas elicited in the connective tissue of rats. Whatever variations do exist between rats and other species, they can well be accounted for by the well-known species-specific differences in the susceptibility of various species and of specific tissues to carcinogenic substances.

Conclusions

1. Four road asphalts applied to the skin of and injected intramuscularly into mice and rats elicited cancers at the sites of application in some of these animals, attesting to the presence of carcinogenic constituents in these materials.

2. Fumes from heated coal tar and petroleum roofing asphalt did not produce cancers of the lungs in rats and guinea pigs inhaling such fumes for a period of up to two years, although the administration of condensates of the coal tar fumes to the skin and muscle tissue of mice demonstrated the high carcinogenic potency of this matter. A roofing asphalt tested, on the other hand, proved to be noncarcinogenic to the skin of mice and rabbits.

3. Paraffinic cooling oil fog inhaled by rats and guinea pigs elicited mainly multifocal adenomatosis in the lungs of these animals. Only one small squamous-cell carcinoma of the lung was found in a rat. A few benign and malignant tumors appeared in the skin and muscle tissue of mice following the administration of the cooling oil to these tissues, indicating a weak carcinogenic property of this paraffih oil to the tissues of mice.

4. The various observations reported with these materials on mice and rats provide additional evidence in refutation of the allegation that sarcomas elicited in the subcutaneous tissue of rats through introduced chemicals are not genuine cancers and, therefore, do not provide proof of the carcinogenic property of the chemicals tested.

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