The Conditional Biological Activity of the Carcinogens in Carbon Blacks, and Its Elimination*

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INTRODUCTION

The presence of a potent carcinogen, 3,4-benzpyrene, and six other aromatic hydrocarbons, was previously reported in some carbon blacks (3). One of these compounds (1,2-benzpyrene) is said to have mild carcinogenic activity (1), and five (pyrene, fluoranthrene, 1,12-benzperylene, anthanthrene, and coronene) either have not been tested or have been reported as inactive. Carbon blacks may be regarded as soots of controlled, usually small, particle size made by pyrolysis from various fuels such as natural gas and oils. They consist chiefly of amorphous carbon. They may contain small quantities of acetone-extractable tars which, in some soots, may reach considerable proportions. Because the anthracotic material regularly present in adult human lungs is composed in large part of inhaled soots, this discovery was of interest if not concern. Waller recently reported that 3,4-benzpyrene was regularly identified in the atmospheric dusts of eight English towns (10). We have identified the same compound in city atmospheric dust.1 Soot formed in the laboratory by burning city gas also contained 3,4-benzpyrene and other aromatic hydrocarbons like those in the carbon blacks (3).

The question at once arose whether these substances could be acting as "natural" human carcinogens on the skin and in the respiratory tract. The epidemiological and statistical studies relating frequency of lung cancer to pulmonary anthracosis, to the carbon black industry (7), and to the geography of residence (2, 8, 9) have up to now yielded conflicting evidence. A causal relationship is not readily apparent. On the other hand stands the old well-known observation of Percivall Pott on the carcinomas induced by soot in the skin—the first human cancers of specific etiology. This cause-and-effect relationship on cutaneous exposure has often been confirmed (5, 6).

Of equal importance to the observation that some carbon blacks contained carcinogenic chemicals was the fact that others contained no chemically extractable aromatic hydrocarbons (3). The difference appeared to be accounted for by the size of the carbon black particles rather than by the method of manufacture or the nature of the starting material. Blacks with an average particle diameter of under 30 mμ yielded no aromatic hydrocarbons, but those over 50 mμ usually contained all seven. The proportions of particles of small and large size in the atmosphere vary greatly from time to time and place to place.

The wide distribution of these aromatic hydrocarbons in relation to the everyday life of many people raises important questions regarding their possible danger. A related fundamental question concerns the physical state and biological activity of some of these compounds. The well known adsorptive capacity of the carbon blacks with which they are associated enters into the picture. It was previously reported that pyrene and 3,4-benzpyrene (and probably many other compounds) added to carbon blacks were only partially recovered by good eluents and incompletely by even the best (4). It was found that carbon blacks of small average particle diameter (17 mμ) adsorbed up to 1 per cent of pyrene or 3,4-benzpyrene and partly adsorbed up to 3 per cent. Only about half of such hydrocarbon could be recovered in 40 extractions (4). Some of the hydrocarbon was lost on exposure to the black. The evidence points to its having been adsorbed. This explanation appears to be clearly established by the experiment of mixing two carbon blacks, of which one contained benzene-extractable aromatic hydrocarbons and the other was free of such materials; from the mixture benzene no longer eluted the compounds. These observations raise the possibility that carbon blacks from which no aromatic hydrocarbons are extractable may actually contain some after all.

Another important question is whether ad-
sorbed carcinogens are biologically active for inducing tumors. Perhaps the body can elute these agents and make them available for carcinogenic activity slowly and over a long period of time, making them more hazardous in vivo than is indicated by the in vitro experiments.

The twelve experiments here reported were designed to study some of these problems. It was desirable to know (a) whether and under what conditions the carcinogens in carbon blacks are biologically active; (b) whether carbon blacks which contain no extractable carcinogens are also inactive when tested biologically; (c) whether a carcinogenic chemical added to carbon black is biologically inactivated; and (d) whether the carcinogens already present can be successfully inactivated and the residue rendered harmless.

The experiments will show that the carcinogens in carbon blacks are biologically active under some conditions but not in others; that a carbon black from which no carcinogens were chemically recovered also was virtually biologically inactive in carcinogenesis; that carcinogen added to a carbon black became biologically inactivated; and that the chemical carcinogens naturally present in a carbon black were rendered harmless by elution, destruction, or adsorption.

GENERAL MATERIALS AND METHODS

Carbon blacks.—Two different carbon blacks were used in the experiments which follow. Both were commercial products, and were included among the specimens reported upon in a previous paper. One sample, designated "Carbon black A," is a coarse furnace black with a surface area of about 15 square meters/gm. It will also be referred to as a "benzpyrene-containing" carbon black, because 3,4-benzpyrene and six other aromatic hydrocarbons are obtainable from it by benzene extraction—compound number 21 in a previous paper (3). It is believed to have an average particle diameter of about 80 mp.

The other specimen is designated "Carbon black B" or "non-benzpyrene-extractable" carbon black. It is a fine channel black with a surface area of approximately 300 square meters/gm. It is believed to have an average particle diameter of about 17 mp. No aromatic hydrocarbons were extracted from it with benzene and other solvents—compound number 3 in a previous paper (3). This fact does not necessarily prove that it is free from benzpyrene, and for this reason it is not so designated.

Biological tests.—All tests for carcinogenicity were made by subcutaneous injection in the interscapular region of mice of the C57BL strain, obtained from the Roscoe B. Jackson Memorial Laboratory. They varied in age from 6 to 8½ months at the time of injection. This older age assured maximum development of the skin and minimal loss of the injectate by extrusion. The mice were of both sexes, and, because no significant difference was found in response, the sex factor is not mentioned again.

The animals were fed mouse laboratory chow pellets and they had free access to water. The average weight of the males until death. There was good survival in most of the experiments, and, despite many deaths from tumors, nearly a third of the mice were still alive when the experiments were terminated early in the 20th month (589 days maximum). The mice were then about 24½ months old.

All tumors, all lesions suspected of being neoplastic, and many injection sites were examined by microscopical sections. At the termination of the experiments all survivors were sacrificed, post mortem examinations were made, and all questionable lesions were subjected to microscopical examinations. Each experiment was started with 50 mice, and the total used was 600. The effective total from which the percentage yield of tumors was calculated was the number alive at the 8th month when the first tumor deaths occurred with the most potent agent. In the table the minimum and average times for tumor comprise the period elapsing from the injection of the test material until death. Tumor "induction time" for these subcutaneous sarcomas in mice would average about 1 month less than the figures given, which are for "fatal time."

The induced tumors were polymorphous, spindle, giant-, or mixed-cell sarcomas of the types commonly found in the subcutaneous tissues at the injection site following carcinogenesis by 3,4-benzpyrene. In most instances some of the injected chemical was readily identifiable, being black, with or on the surface of the tumor. A few mice showed two independent sarcomas, but in the results these were counted as one. A few sarcomas had metastasized to the lymph nodes or lungs.

Other types of neoplasm encountered during the course of the experiments were lymphomas, hepatomas, lung tumors, angiomomas, and angiosarcomas, and a uterine sarcoma. Most of them were found in old mice. These tumors were evenly distributed among all groups, including the controls. Their frequency was not greater than that found in previous experiments in this laboratory, or than the frequencies reported for this strain in the literature. They are regarded as spontaneous and are not included in the results.

EXPERIMENTAL

Biological Activity of the Carcinogenic Chemicals in Carbon Black

Three experiments were designed to determine whether the 3,4-benzpyrene and other aromatic hydrocarbons present in some carbon blacks are carcinogenic in that condition or combination.

Experiment 1: Benzpyrene-containing carbon black.—Three hundred mg. of the benzpyrene-containing carbon black A, made up to 1.0 cc. with tricaprylin, was injected subcutaneously into each of 50 mice. This amount of the carbon black was used because it was believed from previous calculations to contain about 0.09 mg. of benzene-extractable 3,4-benzpyrene, which is more than a 50 per cent tumor dose in this strain of mice. The material was fairly well retained, and it quickly became encapsulated by fibrous connective tissue. Tumors began to appear in the 7th month. The first mouse died of sarcoma in 250 days, and a total of eighteen mice eventually succumbed to induced tumors in an average of 363 days. The percentage yield was 39.1 (Table 1).

Experiment 2: Tricaprylin controls.—Fifty mice were injected in the same way with 1.0 cc. of the
### Table 1

**Conditional Carcinogenicity in Carbon Blacks and Its Elimination**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Material Tested</th>
<th>No. Survivors and Sarcomas</th>
<th>Tumor Induced Yield (%)</th>
<th>Minimum Fatal Time (Days)</th>
<th>Average Fatal Time (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Benzpyrene-containing carbon black</td>
<td>Mice: 50, 44, 45, 40, 35, 30, 25, 20</td>
<td>18 39.1</td>
<td>250</td>
<td>363</td>
</tr>
<tr>
<td>2</td>
<td>Tricarboxylic control</td>
<td>Mice: 54, 48, 43, 40, 35, 30, 25, 20</td>
<td>12 39.1</td>
<td>250</td>
<td>363</td>
</tr>
<tr>
<td>3</td>
<td>Pellets, benzpyrene-containing carbon black</td>
<td>Mice: 50, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>4</td>
<td>Nonbenzpyrene-extractable carbon black</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>5</td>
<td>Pellets, nonbenzpyrene-extractable carbon black</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>6</td>
<td>Nonbenzpyrene-extractable carbon black with 0.09 mg benzpyrene added</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>7</td>
<td>Benzpyrene, 0.09 mg.</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>8</td>
<td>Pellets, nonbenzpyrene-extractable carbon black with benzpyrene added</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>9</td>
<td>Benzene extract of benzpyrene-containing carbon black</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>10</td>
<td>Carbon black residue after benzene extraction</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>11</td>
<td>Benzpyrene-containing carbon black treated with chromic acid</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
<tr>
<td>12</td>
<td>Mixture of benzpyrene-containing and nonbenzpyrene-extractable blacks</td>
<td>Mice: 50, 47, 47, 47, 47, 47, 44, 43, 42, 41, 39, 38, 37, 36, 35, 34, 33, 32, 31</td>
<td>8 43.1</td>
<td>386</td>
<td>411</td>
</tr>
</tbody>
</table>

*Calculated on the basis of number of mice surviving at 8 months.
tricaprylin. Many mice survived for 19 months, but none developed induced sarcoma (Table 1).

**Experiment 3: Pellets of benzpyrene-containing carbon black.**—In this experiment pellets made from the same specimen of carbon black as that used in Experiment 1 and weighing about 300 mg. were injected subcutaneously with a trocar into each of 50 mice. The pellets measured about 3.5 X 30 mm. They also became encapsulated, although in some mice the material was extruded. Two mice eventually died with induced sarcomas, one at 386 and the other at 436 days. The tumor yield was only 4.3 per cent.

In Experiment 1, a specimen of carbon black from which 3,4-benzpyrene and other aromatic hydrocarbons are extractable in vitro with benzene (see Experiments 9 and 10 and reference 3) induced subcutaneous sarcomas when tricaprylin was present in the injected locus, although this solvent alone in Experiment 2 caused no tumors. Under these conditions, this carbon black was carcinogenic. However, in pellet form the same carbon black was biologically almost inactive (Experiment 9). The difference between the experiments was that in one an eluent (tricaprylin) for the carcinogen was provided; in the other, any solvent present within the fibrous capsule enclosing the pellets had to be provided by the body. This it apparently did with poor success. The supernatant tricaprylin in Experiment 1 developed a strong greenish fluorescence as evidence of solvent activity. The carbon black in the pellets at necropsy appeared moist, but the interparticle phase was probably chiefly aqueous; it is probable that little carcinogen was eluted from the carbon black particles and carried to the encapsulating connective cells or beyond to exert carcinogenic action. It may be concluded that this specimen of carbon black was carcinogenic under certain conditions but much less so under others—the biological activity was conditional. In subsequent experiments, other conditions will be described in which the biological activity was entirely lost.

The tumor-inducing agents demonstrated in Experiments 1 and 3 may be regarded as "natural" carcinogens in the sense that they are chemicals which were not especially prepared but are normally present in materials which have a rather wide distribution in the environment. However, they were only conditionally active. This principle appears to provide an explanation for the action of soot in causing human skin cancers but few apparent lung cancers. On the skin the body provides an eluent in the form of sebum, whereas in the lung no good lipid solvent is normally present. Later, an alternative interpretation will be given.

**Carcinogenic Activity of a Carbon Black Which Contains No Extractable Aromatic Hydrocarbons**

This experiment was designed to test whether failure to demonstrate the presence of chemical carcinogens in a carbon black by solvent extraction was paralleled by absence of carcinogenicity on biological testing.

**Experiment 4: Nonbenzpyrene-extractable carbon black**.—Carbon black B, a specimen from which no aromatic hydrocarbons were obtained by solvent extraction, was tested in mice for carcinogenic activity by subcutaneous injection. Three hundred mg. of the material made up to 1.5 cc. with tricaprylin was injected into each of 50 mice as in Experiment 1. The survival times are shown in Table 1. No sarcomas were induced. The early fibrous reaction to the material resembled that in Experiment 1, but retention of the material was poorer. Nevertheless, enough was retained so that the material is regarded as having been tested.

**Experiment 5: Pellets of a nonbenzpyrene-extractable carbon black**.—The same specimen of carbon black as that used in Experiment 4 was tested also in pellet form. Each of 50 mice was injected with 800 mg. of the black made into pellets averaging about 3.5 X 45 mm. The survival time of the mice was good. One sarcoma was found in relation to a pellet at 524 days (Table 1).

Experiments 4 and 5 show that a specimen of carbon black from which no aromatic hydrocarbons could be eluted with benzene was nearly noncarcinogenic on biological test. The supernatant tricaprylin did not become fluorescent on standing, unlike that with carbon black A in Experiment 1. Only one sarcoma was induced after a long induction time among 95 mice that survived for 5 months. The results demonstrated that the chemical and biological tests for carcinogenicity were nearly parallel, with the latter slightly more sensitive. Failure to demonstrate carcinogen by chemical methods did not prove its complete absence. We have previously reported that 3,4-benzpyrene which is added to some carbon blacks cannot be quantitatively recovered (4), and this was interpreted as indicating adsorption. If that interpretation was correct, Experiment 5 may show that prolonged elution in vitro was better than short extraction in vitro by the methods used. An alternative explanation for the one induced sarcoma is that a carcinogen of a type not detected by the
Adsorption of Carcinogen by Carbon Black

These experiments were designed to test whether 3,4-benzpyrene added to a carbon black of small average particle size would retain its activity as a carcinogen. It had previously been reported that considerable quantities of 3,4-benzpyrene could be adsorbed from benzene solution by carbon blacks of small average particle diameter and that such chemicals could be eluted only with great difficulty or not at all (4). Does this adsorbed state nullify carcinogenic activity?

Experiment 6: Carbon black with added 3,4-benzpyrene.—To carbon black B (the same specimen as that used in Experiment 4) which contains no benzene-extractable aromatic hydrocarbons, 3,4-benzpyrene was added to the amount of 0.09 mg/300 mg of the black, and the mixture was made up with tricaprylin to a volume of 1.5 cc. This amount was then injected into each of 50 mice. The local fibrous reaction resembled that observed in Experiment 4. The survival time of the mice is shown in Table 1. No sarcomas were induced by this mixture.

Experiment 7: 3,4-Benzyrene control.—Benzyrene from the same specimen used in Experiment 6 was injected in the amount of 0.09 mg/mouse, dissolved in 1.0 cc. of tricaprylin. The first mouse with induced sarcoma died at 142 days, and 95.1 per cent of the animals ultimately died with tumors at an average of 233 days (Table 1).

Experiment 8: Benzyrene-carbon black pellets.—Pellets containing 300 mg. of carbon black B were made and injected as in Experiment 5 except that, in addition, 0.09 mg. of 3,4-benzyrene (same specimen as Experiments 6 and 7) was added to each pellet. The survival time of the mice was excellent, but no sarcomas were induced (Table 1).

The results in Experiments 6 and 8 show that 3,4-benzyrene added to a carbon black of small average particle diameter was lost as far as carcinogenic activity was concerned, although the same amount of carcinogen injected in tricaprylin solution was highly active (Experiment 7). No eluent was provided to the mice in Experiment 8, in which pellets were tested. In Experiments 6 and 7, however, an eluent was used, and the size of the injected locus was nearly the same, so that the amount of exposure of each encapsulating cell should have been approximately equal if equal amounts of the 3,4-benzyrene were in solution. The inactivation of the carcinogen by the presence of carbon black is interpreted as most probably caused by its adsorption. The same phenomenon (adsorption) is illustrated also by Experiment 12.

Elimination of the Carcinogenic Activity in Carbon Blacks by Solvent Extraction, by Destruction, and by Adsorption

The question arose whether the biological activity shown by the carcinogen(s) in carbon black A (the 3,4-benzyrene-containing specimen) could be eliminated and the black rendered harmless. This was attempted by three methods.

Experiment 9: Solvent extraction of carbon black.—Carbon black A was extracted 10 times with redistilled benzene. The extracts were pooled, taken to dryness, and dissolved in tricaprylin so that each cubic centimeter contained the extract from 300 mg. of the black. One cc. was then injected into each of 50 mice. The results (Table 1) show that this extract had the same or a slightly greater degree of carcinogenic activity (49 per cent tumor yield) as possessed by the original black in Experiment 1 (80 per cent sarcoma yield).

Experiment 10: Test of the carbon black residue.—The carbon black residue remaining after ten benzene extractions in Experiment 9 was tested in mice, each animal receiving 300 mg. of the residue made up to 1 cc. with tricaprylin. Only one sarcoma was induced in this experiment (Table 1), showing that most of the activity had been removed. It is known by reference to the elution curve of benzyrene that complete removal is not achieved by only ten extractions (4). Furthermore, the supernatant tricaprylin on standing became fluorescent, showing that the benzene extraction had been incomplete. However, the results of this experiment show that for practical purposes most of the biological activity was eliminated by prior solvent extractions. Additional extractions would probably have removed most of the residual carcinogen or reduced it below biologically effective levels.

Experiment 11: Elimination by chromic acid treatment.—A specimen of carbon black A was treated with strong chromic acid for 3 hours on a steam bath. It was then injected in the same quantity as before, namely, 300 mg. made up in tricaprylin to a volume of 1 cc. Although many mice lived through the test period no sarcomas were induced. The carbon black, which before treatment was biologically active, became inactive after the exposure to chromic acid. It is probable that the carcinogen was destroyed. This black underwent a change in physical characteristics after chromic acid treatment resembling that produced by sol-
vent extraction. It had a smaller volume and was unstable in tricaprylin suspension, tending to flocculate.

Experiment 12: Elimination of activity by mixing a carcinogen-containing with a noncarcinogen-extractable carbon black.—Equal parts by weight of carbon blacks A and B were mixed, and 600 mg. of the mixture made up to a volume of 1.5 cc. with tricaprylin was injected into mice. Although survival of the animals was fair, no sarcomas were induced (Table 1). It is clear that the mixture was biologically inactive although half of the material under similar conditions of testing had induced tumor in 39 per cent of the test mice (Experiment 1). It is probable that the carcinogenic chemical(s) eluted by the solvent was adsorbed by the large amount of surface of the adjacent small particles so that it was not available for carcinogenic action on the enclosing connective tissue cells. If this explanation is correct, this is another example of inactivation of carcinogenic activity by adsorption, somewhat resembling that in Experiments 6 and 8. It had previously been shown that, from a similar mixture, no chemically identifiable aromatic hydrocarbons could be eluted (3).

DISCUSSION

The results of these experiments appear to be clear-cut. Their direct interpretation is also fairly obvious. Their possible implications and broad significance, however, merit some discussion, because they seem to concern important principles in experimental carcinogenesis as well as in human experiences.

The carcinogenic chemicals present in carbon blacks were biologically active under some conditions but not under others. Thus, they exerted carcinogenic effects when an adequate eluent was present, but they were inactive in the absence of proper solvent and in the presence of an adsorbent. These agents, therefore, were conditional carcinogens. The conditions determined whether the chemicals were available to expose the cells.

Of the two factors, the adsorption phenomenon appeared to be more potent than the solvent factor in determining whether a carcinogen would be biologically active, under the conditions used in testing. Thus, in Experiment 6, 3,4-benzpyrene in carcinogenic dosage levels, added to a highly adsorbing carbon black, became biologically ineffective even in the presence of an eluent which was adequate under other experimental conditions (Experiment 1). The great importance of the solvent in the carcinogenic response has long been recognized in experimental oncology, and it may be of importance under natural conditions of exposure in man. The adsorption phenomenon, however, has had little or no experimental recognition, although it may be of even greater importance in the laboratory and the clinic. Apart from its obvious industrial applications, it appears to be an important general principle applying to entire populations.

Moreover, it was demonstrated that carcinogenic activity which is "naturally" present in a material could be entirely eliminated or greatly reduced by three methods, namely, by prior solvent extraction, by chemical destruction, and by adsorption. These methods appear to have importance in occupational and public health, and in the experimental laboratory.

The clinical, statistical, epidemiological, and experimental evidence relating carbon blacks and soots to cancer induction was reviewed in a previous paper (3). It was pointed out that the accumulated evidence does not unequivocally indicate a hazard in the respiratory tract, but there is clearly some danger on the skin from prolonged exposure to soots, that the experiments by several different investigators with extracts of soots have clearly shown carcinogenic activity, and that the presence of 3,4-benzpyrene has been demonstrated in atmospheric dusts and soots in several reports. These apparent inconsistencies in the biological hazard are now probably explained by the presence or absence of an adequate solvent at the anatomical site of exposure, and by the phenomenon of adsorption which may make carcinogens unavailable despite the presence of what is, under other circumstances, a good solvent.

It is probable that the lung does not normally have as good a solvent for this class of compounds as skin. Pathological lungs, however, seem to be abnormally predisposed to the development of cancer, despite the fact that the lesions are of several different varieties. Such lungs have one feature in common and possibly two: they have abnormal accumulations of anthracotic pigment, and they would appear, on morphological evidence, to have an increase in lipids of either cellular or exogenous origin. It is possible that these two factors combined induce some tumors that neither alone would produce, the lipids acting as eluting agent for carcinogens in the soot. Although old tuberculous lesions are not currently widely accepted as giving rise to pulmonary carcinomas, a collection of cancers which appear to have originated in tuberculous foci exists in this department. They have in common, in addition to the tuberculosis, large amounts of anthracotic materials and lipids. A possible "co-carcinogenic" action for these substances must be seriously considered. Some of the waxes
and lipids derived from the tubercle bacillus are by themselves strong stimulants to cell proliferation (although for mesoblastic rather than epithelial cells), but these cellular aggregates do not go on to prothrombosis. They regress. These lipoidal substances might, however, be carcinogenically important as eluents for carcinogens in the vicinity rather than as direct tumor cell evocators.

The pneumoconioses may also predispose to carcinoma of the lung, especially of the peripheral forms. All of them are characterized morphologically by the accumulation of anthracotic pigment and by lipids in addition to the specific lesions. Some of the pneumoconiosis-producing materials represent classes of chemicals which are remote to the recognized carcinogenic compounds (e.g., asbestos). Their activity has sometimes been attributed to nonspecific effects as, for example, from chronic irritation. The experiments here reported provide the principles to an alternative and more attractive explanation which brings these clinical observations in relation to the experimental work. It is possible that the role of the inhaled pneumoconiotic material is that of providing a chronic lesion in which carcinogenic agent and an eluent are accumulated and brought together, the former being provided by soot and the latter released locally.

One important question regarding the existence of carcinogens in carbon black remains inadequately answered by these experiments. This is the problem whether failure to elute these agents from a carbon black indicates their actual absence. An attempt was made to solve this problem by biological tests. In one experiment (Experiment 5) a single tumor was induced by a carbon black from which no aromatic hydrocarbons could be extracted, but in another test (Experiment 4) the same material was inert. When 3,4-benzpyrene was added to this black before injection, it was still inert; the carcinogen was lost with respect to carcinogenic activity. The deductions must be made that the original black might have contained carcinogen which was detected neither by chemical nor biological tests, that the biological test was little if at all inducible by exposure to soot, so that only in the greatly exaggerated conditions of exposure do tumors occur ("chimney sweeps cancer"). In the lung the situation is different. Practically every human lung gradually accumulates anthracotic pigment. The question arises why many more pulmonary cancers do not occur. In this paper we have pointed out two possible protective principles: First, an efficient eluent may be absent; and, second, the adsorptive capacity of particles of small average diameter may make harmless such carcinogen as is released from the larger particles. The retained anthracotic material is a mixture of particles of various sizes, and, as in Experiment 12, some degree of protection may be provided by adsorption. Considering these phenomena together, perhaps it is only under conditions— singly or collectively—of exceptionally large exposure, of an associated pathological condition providing eluent and co-carcinogenic effects, or of an overwhelming of the protection provided by the absorption phenomenon that threshold carcinogenic dosage levels are reached and tumors induced by this class of carcinogens. Also, the possibility of additive or summation effects with other types of agent must be kept in mind.

SUMMARY AND CONCLUSIONS

1. A carbon black from which 3,4-benzpyrene and other aromatic hydrocarbons could be eluted with benzene was carcinogenic on subcutaneous test in mice when it was injected with tricaprylin but practically inactive in pellet form. This difference in result is interpreted as showing that the subcutaneous tissues had no eluent adequate to make the chemical available for carcinogenic action on the adjacent cells. These experiments illustrate three principles in carcinogenesis, namely, (a) the existence of "natural" carcinogenic compounds; (b) the occurrence of conditional carcinogens; and (c) the importance of the solvent in the carcinogenic response.

2. A carbon black whose benzene extracts contain no recognized carcinogenic hydrocarbons was slightly carcinogenic on biological test. It is probable that adsorbed carcinogenic chemicals were present which were not eluted in vivo but which became available for carcinogenic activity in vivo, indicating that the biological test for carcinogenicity was better than the chemical.

3. When the potent carcinogenic aromatic hydrocarbon, 3,4-benzpyrene, in an amount which when tested alone gave a 95 per cent tumor yield, was added to a noncarcinogenic carbon black of small average particle diameter prior to injection, no tumors were induced by the combination. This inactivation is attributed to adsorption, and it is interpreted as signifying that in the adsorbed state

*P. E. Steiner, and C. Huggins, unpublished data.
this carcinogen was rendered biologically inactive.

4. The carcinogenic activity of a carbon black was eliminated by destruction and by adsorption; it was nearly eliminated by solvent extraction.

5. The phenomenon of adsorption was a more potent factor than the solvent in determining the carcinogenic response under the experimental conditions used.

6. The principles of natural and conditional carcinogens, of solvent elution, and of adsorption are advanced to explain some clinical and epidemiological observations on human skin and lung cancers, and on the role of preceding pathological lesions in predisposing to pulmonary tumors.

ACKNOWLEDGMENTS

The chemical studies in the first part of these experiments were made by Dr. Hans Falk to whom thanks are due.

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