STUDIES IN CARCINOGENESIS

V. METHYL DERIVATIVES OF 1:2-BENZANTHRACENE

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During the last decade a considerable amount of information has been accumulated regarding the experimental production of malignant tumors in laboratory animals. Several widely different types of agents are now known to be capable of inciting malignant growth. In addition to the well known carcinogenic action of x-rays and of radioactive substances, it is now clear, from the results obtained in a number of independent laboratories, that prolonged exposure to ultraviolet light gives rise to malignant tumors in the exposed tissues. As to biological agents, it has been recognized for some time that certain worm parasites are responsible for the genesis of malignant tumors of the urinary bladder and of the liver in man, and of the liver in the rat. Although Rous (1911) had discovered many years ago that fowl sarcoma could be transmitted by cell-free filtrates, and although Gye (1925) had been emphasizing the significance of this approach to the cancer problem, yet "the work of Peyton Rous and his collaborators has not met with its just recognition" according to Gye and Purdy (1931). The demonstration by Rous and Beard (1934) that carcinoma may be induced in rabbits with the Shope virus has,

1 The data contained in this paper were presented, in part, at the Chicago meeting of the American Association of Pathologists and Bacteriologists, March 26, 1937 (Shear, 1937 a) and, in part, at the Baltimore meeting of the Federation of American Societies for Experimental Biology, April 1, 1938 (Shear, 1938). In this collaborative study, the compounds were synthesized by Professor L. F. Fieser and coworkers in the Division of Chemistry, Harvard University, and by Dr. M. S. Newman and coworkers in the Department of Chemistry, The Ohio State University; the biological work was done in the laboratories of the U. S. Public Health Service at the Harvard Medical School. The preliminary results obtained in these biological experiments were recorded in the papers of Fieser and coworkers, and of Newman and coworkers, which described the syntheses of these compounds. A summary of the earlier findings was given in a joint report (Fieser, Fieser, Hershberg, Newman, Seligman and Shear, 1937).

2 See Roffo (1936) and Beard, Boggess and Von Haam (1936) for the bibliography of the earlier work. In this laboratory, Schereschewsky (1937) has produced malignant tumors in pure strain mice by exposure to light from the mercury-quartz lamp.
however, resulted in a revival of interest in the question of the rôle of viruses in the genesis of tumors generally.

According to Rowntree, Steinberg, Dorrance and Ciccone (1937), crude wheat germ oil, given orally, produces sarcoma in rats in a very short time.

Furthermore, substances of physiological origin have been reported to be carcinogenic. A large amount of data is now available regarding the carcinogenic action of estrone and related substances (reviewed by Loeb and others, 1937). According to Hall and Franks (1937), frequent injection of acetylcholine was followed, unexpectedly, by the development of malignant tumors in animals of several different species.

Since the 18th century, when cancer in chimney sweeps was recognized as of occupational origin, other occupations have been found to be responsible for the high incidence of tumors of the skin and of the urinary bladder in the workmen. This stimulated numerous investigators to attempt to discover the particular compound or compounds responsible for the genesis of these tumors. The early work in this field, which ultimately led to the discovery by Yoshida that 2-amino-5-azotoluene is carcinogenic, has been summarized in a previous paper (Shear 1937 b). More recently Kinosita (1937), in a summary of the work in his laboratory, reported that "butter yellow" (p-di-methyl-amino-azobenzene) is also carcinogenic. Hueper, Wiley and Wolfe (1938) have reported that commercial preparations of β-naphthylamine gave rise to carcinoma of the urinary bladder in dogs. Zinc chloride, arsenic preparations, and a styryl quinoline derivative have also been reported to be carcinogenic. As to chemical compounds as carcinogenic agents, the literature has been ably reviewed by Kennaway's group (Cook and others, 1937).

It is thus evident that agents of many different types—physical, chemical, and biological—are capable of inducing malignant new growths. Of the various aspects of the general problem of carcinogenesis, several have been under investigation in this laboratory. The present paper deals with the carcinogenic activity of certain condensed polycyclic hydrocarbons and the features of their structure which are responsible for their activity.

The pioneer work in this field was done by the London investigators, who have since been steadily and systematically contributing further important findings. In this country the work on this subject has, in certain respects, paralleled that of the English group and, in other respects, has explored different paths. In both countries most of the work has been done with mice, but with two important differences. Here pure strain mice have been employed and the compounds have usually been introduced subcutaneously, whereas in London they were usually applied to the skin in benzene solution.

Following the discovery in London (Barry and others, 1935) that methylcholanthrene prepared from deoxycholic acid was a very potent carcinogen, it was found (Shear, 1936 a) that synthetic methylcholanthrene and methylcholanthrene prepared from cholic acid were equally potent. It was next found (Shear, 1936 a; see Fieser and Seligman, 1935 a) that cholangrene, when injected into mice, had about the same carcinogenic activity as methylcholanthrene. Similar results, with the skin painting technic, were later reported from London (Cook, 1936). It was thus apparent that the methyl group of methylcholanthrene was not essential for high carcinogenic potency.
In this paper experiments are described which show that the 5-membered ring of cholanthrene (5:10-ace-1:2-benzanthracene) is not required for the presence of high potency, inasmuch as 5:10-dimethyl-1:2-benzanthracene also gave rise to subcutaneous tumors quite rapidly. Data are presented, moreover, which show that a substituent at the 5-position is not essential, for 10-methyl-1:2-benzanthracene produced subcutaneous tumors almost as rapidly as did cholanthrene and the 5:10-dimethyl derivative.

In the case of 3:4-benzpyrene, somewhat analogous results \(^3\) were obtained. This pentacyclic hydrocarbon has been regarded as structurally related to 1:2-benzanthracene, \(^4\) with carbon substituents at the 1'- and 9-positions forming a closed ring. Substitution of a methyl group, to form 9-methyl-1:2-benzanthracene, in place of a closed ring did not abolish carcinogenic potency, for the 9-methyl derivative produced subcutaneous tumors in 14 of 20 mice in six months. In an analogous fashion 5:9-dimethyl-1:2-benzanthracene has been considered as structurally comparable with 4'-methyl-3:4-benzpyrene; the former compound produced tumors much more rapidly than the latter, which has the ring at the 9-position intact.

**Method**

Unless otherwise stated, the compounds were all introduced subcutaneously, usually in the left flank. When crystalline preparations were injected, a few drops of glycerol were employed as a lubricant, as in previous experiments, to prevent the crystals from clogging the trocar. Pure strain mice \(^5\) were used exclusively; these were strain A mice except as otherwise specified. In the case of the rapidly acting compounds, the mice were given only one injection; when a compound was injected repeatedly, this is noted in the text.

The tumors referred to in this paper all occurred at the site of application of the compounds tested, except when otherwise stated. Presentation and discussion of data regarding lesions other than tumors occurring at the site of application are being deferred, for the most part, to a later date.

**Experimental: Methyl Derivatives of 1:2-Benzanthracene**

**5:10-Dimethyl-1:2-benzanthracene**

Ten milligrams of crystalline 5:10-dimethyl-1:2-benzanthracene (Fieser and Newman, 1936) were injected (6/19/36) into each of 16 male mice. Nineteen days later thickening of the tissue at the injection site was noted in

\(^3\) Presented at the Memphis meeting of the Federation of American Societies for Experimental Biology, April 23, 1937 (Shear, 1937 \(c\)).

\(^4\) For a more recent view, considering 3:4-benzpyrene as a chrysene derivative, see Fieser and Seligman (1938).

\(^5\) See Andervont (1937) for a brief description of the strains of mice used in this laboratory.
FIG. 1. SARCOMA (BIOCHEM. 1712) PRODUCED BY CRYSTALLINE 5:10-DIMETHYL-1:2-BENZANTHRACENE. × 75. TUMOR WAS TRANSPLANTED ON 75TH DAY

FIG. 2. TRANSPLANT (4TH GENERATION; A-101) OF TUMOR PRODUCED BY CRYSTALLINE 5:10-DIMETHYL-1:2-BENZANTHRACENE, SHOWN IN FIG. 1. × 75

FIG. 3. SARCOMA (BIOCHEM. 1818) PRODUCED BY CRYSTALLINE 5:10-DIMETHYL-1:2-BENZANTHRACENE. × 75. THE TUMOR WAS TRANSPLANTED ON 101ST DAY

FIG. 4. TRANSPLANT (2ND GENERATION; BIOCHEM. 2003) OF TUMOR PRODUCED BY CRYSTALLINE 5:10-DIMETHYL-1:2-BENZANTHRACENE, SHOWN IN FIG. 3. × 75
all of the mice. As in the analogous experiments with methylcholanthrene, benzpyrene, and cholanthrene (Shear, 1936 a), ulceration of the skin over the site of injection was observed in several instances. The ulceration, which was present in all of the mice by the 40th day, and in some instances was severe, became extensive in all the animals during the course of the next week. On the 75th day the first tumor (Fig. 1) that was obtained was transplanted into other strain A mice (Fig. 2). Transplantation of this sarcoma was discontinued after the fifth generation. Transplantation of another sarcoma (Figs. 3 and 4), obtained during the fourth month, was carried on for three generations and was then discontinued. Two other primary sarcomas were transplanted; in one case negative results were obtained; in the other, the transplantation was successful in 1 of 3 mice.

At the end of three months, tumors had been obtained in 5 mice, 3 mice had died, and 8 were negative. At the end of four months, tumors had been obtained in a total of 9 mice. During the first four months 6 mice had died, showing more or less thickening under or around the extensive ulcers; the remaining mouse died during the fifth month, with an ulcer at the site of injection. These mice had died apparently as a result of the treatment; this may have been due either to the action of the hydrocarbon or to effects secondary to the severe ulceration.

This compound was then dissolved in lard, which had been melted and filtered at 38° C., in a concentration of 4 mg. per c.c. Each of 20 male mice was given 0.25 c.c. of this solution (i.e., 1 mg. of hydrocarbon). On the 57th day severe ulceration was noted at the site of injection in 15 mice. On the 78th day, 2 large tumors and 1 small one were observed. By the end of three months, tumors had been obtained in 6 mice; 6 mice which were sick, apparently as a consequence of the treatment, had been killed and the ulcerated tissue at the injection site preserved for histological study; 8 mice were still negative. During the fourth month, 2 of the mice died, showing ulceration at the injection site; the remaining 6 mice all developed tumors. When the experiment terminated at the end of four months, a total of 12 tumors had been obtained. The results of these two experiments are summarized in Table I.

5-Methyl-1:2-benzanthracene

\[ \text{CH}_3 \]

Five mg. of crystalline 5-methyl-1:2-benzanthracene (Fieser and Newman, 1936) were injected (8/24/36) into each of 20 male mice. The first tumor was noted at the end of three months; at that time no ulceration was observed in any of the mice. During the fourth month, 2 more tumors appeared; ulceration was noted in only 1 mouse. During each of the next four months

\* In all cases of transplantation, the new hosts were mice of the same strain as the bearer of the primary tumor.
**Fig. 5.** Photograph of a subcutaneous tumor, produced four months after the injection of crystalline 5-cyano-10-methyl-1:2-benzanthracene, showing the compound still present in the interior of the tumor.

**Fig. 6.** Photograph of a subcutaneous tumor, produced eight months after the injection of crystalline 7-cyano-10-methyl-1:2-benzanthracene, showing the compound still present in the interior of the tumor.

**Fig. 7.** Sarcoma (Biochem. 862) produced (Shear, 1936a) by crystalline 1:9-methylene-1:2:5:6-dibenzanthracene, showing crystal spaces in the interior of the tumor obtained nine months after the subcutaneous injection of the hydrocarbon. × 50
tumors appeared in 2, 3, 4, and 4 mice, respectively. A tumor was observed in another mouse when the 2 survivors were killed at the end of ten months.

It is thus seen that the 5-methyl derivative is a potent carcinogenic agent, for it induced subcutaneous tumors in 17 of the 20 mice. The tumors appeared much more slowly, however, than in mice treated with the 5: 10-dimethyl derivative, as can be seen from Table I (p. 509).

These results agree, in so far as ability to produce tumors is concerned, with those which the London workers (Barry and others, 1935; Bachmann and others, 1937) obtained in a skin-painting experiment with this compound. They obtained epitheliomas in 5 mice and papillomas in 2 mice; of the original 10 mice, 8 survived ten months or longer. These skin tumors began to appear after nine months had elapsed whereas 16 of the 17 subcutaneous tumors reported in the present paper had appeared by the end of the ninth month. In these particular experiments the injection technic produced tumors much more rapidly than did the painting technic.

10-Methyl-1:2-benzanthracene

Five mg. of crystalline 10-methyl-1:2-benzanthracene (Fieser and Newman, 1936) were injected (8/24/36) into each of 20 male mice. On the 37th day ulceration was noted at the site of injection in 12 of the mice; in 9 animals the ulceration was accompanied by a noticeable thickening of the tissue at the injection site; and in 1 mouse thickening was noted with no accompanying ulceration. On the 43d day, the thickening and the ulceration were more pronounced; only 4 of the mice were negative at this time. By the 57th day, the thickened tissue had developed into definite tumors in 8 mice.

On the 85th day one of these tumors was transplanted. On cutting open the tumor, a considerable amount of the crystalline hydrocarbon was seen in the center. This was a frequent finding when tumors were produced by the injection of crystals or pellets of these carcinogens. Figs. 5 and 6, for example, are photographs of subcutaneous tumors produced by crystalline 5-cyano-10-methyl-1:2-benzanthracene and crystalline 7-cyano-10-methyl-1:2-benzanthracene (Newman and Orchin, 1938), respectively, showing the solid compounds still present in the interior of the tumors. (The former compound is about as potent and as rapidly acting as 5:10-dimethyl-1:2-benzanthracene; the latter is more slowly acting.)

Since the parts of the tumors immediately adjacent to the crystalline carcinogen often contain crystals of the injected compound (Figs. 7 and 8), the areas near the carcinogen were not used for transplantation. A portion, some distance away from the hydrocarbon crystals was employed; transplantation (Figs. 9 and 10) was carried out for 5 generations and then discontinued.

Two other tumors (Figs. 11 and 12) were transplanted on the 100th day,
FIG. 8. SARCOMA PRODUCED WITHIN THREE MONTHS AFTER THE SUBCUTANEOUS INJECTION OF CRYSTALLINE 10-METHYL-1:2-BENZANTHRACENE, SHOWING CRYSTAL SPACES IN THE INTERIOR OF THE TUMOR (A-823). × 50 (ABOVE) AND × 100 (BELOW)
and after the 2nd and 5th generations, respectively, transplantation of these tumors was discontinued. Thus, in all 3 instances in which transplantation of tumors induced in this experiment was attempted, the implants grew in the new hosts.

At the end of three months, severe ulceration was observed in all mice but one; tumors had appeared in 9 animals by this time. During the next month, tumors appeared in 7 more mice. Of the survivors, 2 developed tumors during the fifth and ninth months, respectively, making a total of 18 mice with induced tumors. The 2 mice that died without tumors bore ulcers at the site of the injection. The results are summarized in Table I.

The London workers (Cook, Robinson and Goulden, 1937; Bachmann and others, 1937), using the painting technic, have also tested 10-methyl-1:2-benzanthracene for carcinogenic potency. They applied a 0.3 per cent solution in benzene twice weekly to the skin of mice. In the first experiment 20 mice were used; on the 130th day, only 4 animals were alive. One of these died bearing a papilloma and one, killed on the 260th day, bore an epithelioma. In a subsequent painting experiment with this compound, only 3 of 10 mice were alive after 135 days, at which time one bore a papilloma and 2 were negative.

Because of the divergence between the results of the first experiment with the 10-methyl derivative in this laboratory and the results obtained in London, the injection of this hydrocarbon was repeated. Ten fresh male mice each received, subcutaneously, 5 mg. of the crystalline 10-methyl derivative. The same sequence of events was noted, i.e., the ulceration and the thickening of the tissue at the site of injection occurred after the same latent period as in the
previous experiment. Small tumors were observed in 2 mice on the 66th day. By the end of the third month, tumors had appeared in 5 of the 10 mice. During the fourth month, the thickened tissue increased in amount and developed into tumors in 2 more mice, and 1 mouse died showing thickening of the tissue underneath the ulcer at the injection site. Thus, tumors were obtained in 7 of the 10 mice in the course of the first four months following the injection. Of the 2 surviving mice, 1 died during the fifth month without a tumor; the other died during the sixth month with a tumor at the injection site (see Table I).

The 10-methyl derivative was also injected in the form of a lard solution containing 5 mg. of the hydrocarbon per c.c. Each of 20 mice (10 males and 10 females) of the C, strain received 0.2 c.c. of this solution (i.e., 1 mg. of the hydrocarbon). Very little ulceration was noted in these mice even after three months. Among the 10 males, the first tumor was noted on the 66th day; tumors appeared in 2 other mice in the third month, during which time one mouse died showing thickening of the tissue at the injection site. Tumors developed during the fourth and fifth months in 3 and in 2 mice, respectively. The surviving male developed a tumor during the sixth month.

Among the 10 females, tumors developed at the injection site in 2 during the third month and in another during the fourth month. At the end of four months, tumors had been produced at the site of injection in 3 of the 10 females as compared with 6 of the 10 males. During the fifth and sixth months tumors appeared at the site of injection in 1 and 3 female mice, respectively. The last surviving female was negative when killed at the end of seven months. These results are summarized in Table I.
### Table I: Methyl Derivatives of 1:2-Benzanthracene: Rate of Production of Tumors at Site of Injection

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mice</th>
<th>Dose</th>
<th>Total No. of Mice with Tumors (+): Negative Surviving Mice (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number and Sex</td>
<td>Strain</td>
<td>3 mos.</td>
</tr>
<tr>
<td>5:10-Dimethyl-1:2-benzanthracene</td>
<td>16 M A</td>
<td>10 mg. crystals</td>
<td>5+</td>
</tr>
<tr>
<td></td>
<td>20 M A</td>
<td>1 mg. in lard</td>
<td>6+</td>
</tr>
<tr>
<td>5-Methyl-1:2-benzanthracene</td>
<td>20 M A</td>
<td>5 mg. crystals</td>
<td>1+</td>
</tr>
<tr>
<td>10-Methyl-1:2-benzanthracene</td>
<td>20 M A</td>
<td>5 mg. crystals</td>
<td>9+</td>
</tr>
<tr>
<td></td>
<td>10 M A</td>
<td>5 mg. crystals</td>
<td>5+</td>
</tr>
<tr>
<td></td>
<td>10 M C3H</td>
<td>1 mg. in lard</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>10 F C3H</td>
<td>1 mg. in lard</td>
<td>2+</td>
</tr>
<tr>
<td>9-Methyl-1:2-benzanthracene</td>
<td>20 M A</td>
<td>1 mg. in lard</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>20 M A</td>
<td>2.5 mg. in lard</td>
<td>20-</td>
</tr>
<tr>
<td>5:9-Dimethyl-1:2-benzanthracene</td>
<td>20 M A</td>
<td>1 mg. in lard</td>
<td>8+</td>
</tr>
<tr>
<td></td>
<td>11-</td>
<td></td>
<td>0+</td>
</tr>
<tr>
<td>7-Methyl-1:2-benzanthracene</td>
<td>19 M A</td>
<td>5 mg. crystals</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>19-</td>
<td></td>
<td>19-</td>
</tr>
</tbody>
</table>

* Separate injections. ** No survivors.

Between the second and fourth months, a tumor developed in the right inguinal region in each of 2 females; in neither case did a tumor develop in the left flank, i.e., at the site of injection. These tumors were both mammary adenocarcinomas, and were probably spontaneous.

In the experiment with the lard solution of 10-methyl-1:2-benzanthracene, tumors developed somewhat more slowly than in the experiments in which the crystalline preparation had been employed. This does not appear to have been due to strain difference, inasmuch as it has been found in this laboratory that induced tumors develop more rapidly in C3H mice than in strain A mice both in the case of 1:2:5:6-dibenzanthracene (Andervont, 1934, 1935) and of 20-methylcholanthrene (Andervont, 1938). The slower development of tumors in the lard experiment may have been due to the difference in the technic of administration, although this does not seem likely in view of the rapid production of sarcomas with 1 mg. of the 5:10-dimethyl derivative in lard (see Table I).
TABLE II: 10-Methyl-1:2-benzanthracene: Rate of Development of Skin Tumors in Strain A Mice Following Skin Painting with a 0.3 Per Cent Solution in Benzene

<table>
<thead>
<tr>
<th>Technic</th>
<th>No. of Mice and Sex</th>
<th>Total No. of Mice with Tumors* (+); Surviving Mice with Papillomas (p); Negative Surviving Mice (—)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 mos.</td>
</tr>
<tr>
<td>Skin painting</td>
<td>10 M</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>0 p</td>
<td>4 p</td>
</tr>
<tr>
<td></td>
<td>8 —</td>
<td>4 —</td>
</tr>
<tr>
<td>Skin painting</td>
<td>10 F</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>2 p</td>
<td>2 p</td>
</tr>
<tr>
<td></td>
<td>8 —</td>
<td>7 —</td>
</tr>
<tr>
<td>Subcutaneous injection (5 mg. crystals)</td>
<td>30 M</td>
<td>23+</td>
</tr>
<tr>
<td></td>
<td>6 —</td>
<td>2 —</td>
</tr>
</tbody>
</table>

* This classification is based upon gross observation. The term "tumors" includes horny warts as well as large tumors. The histologic findings will be described at a later date.

Because of the small numbers of animals employed, the difference in rate of tumor production in the C₅H males as compared with the strain A males is not considered significant. As regards the C₅H females, however, the difference may possibly be found to be significant. Some evidence has already been adduced (Kreyberg, 1935; Cramer, 1936; Peller, 1936) that the development of spontaneous tumors, or even the presence of a tendency to the development of spontaneous tumors, may possibly interfere with experimental carcinogenesis.

At the same time that the injection experiment was repeated, painting of the skin of 20 other strain A mice (10 males and 10 females) was begun. A 0.3 per cent solution of 10-methyl-1:2-benzanthracene in benzene was applied to the skin three times weekly. At the end of four months no tumors were present in the 18 mice which were still alive, whereas, after this same interval, 23 of the 30 strain A mice which had been injected with crystals of this compound had developed subcutaneous tumors (see Table II).

During the third month of painting, evidence of skin damage was noted in a number of the mice. Denudation, induration, and superficial ulceration with scab formation were present in about one-fourth of the mice; in most cases these conditions persisted. Consequently the painting was discontinued for two weeks at the beginning of the fifth month, after which it was resumed, painting being done only twice weekly for the rest of the experiment. Nevertheless, 6 of the mice became progressively thinner, with no improvement of the severe skin damage even when subsequently exempted from all further painting.

At the end of five months papillomas were present on the painted areas in 6 of the 17 mice that were alive at that time. During the sixth month 5 of the emaciated mice died, bearing evidence of severe skin damage but no tumors or warts. Twelve of the mice (7 males and 5 females) survived for six months. At this time 6 of the 7 males bore tumors, warts, or papillomas, which grew
progressively during the next two months, whereas only 1 of the 5 females bore a papilloma at this time.

By the end of the eighth month tumors or horny warts had been produced in 6 of the males; the sole surviving male developed a horny wart early in the ninth month. Of the 5 females which were alive after eight months, 1 bore a small tumor, 1 bore multiple papillomas, and 3 were still negative. The experiment was terminated after nine months, by which time tumors or horny warts had been obtained in 7 males and in 4 females. These results are summarized in Table 11, which also contains, for comparison, the rate of tumor production following the subcutaneous injection of the same hydrocarbon into other mice of the same strain.

The toxic effects produced in this experiment do not appear to be attributable to the benzene, inasmuch as similar painting experiments, with benzene solutions of other materials, have been carried out for much longer periods without such harmful effects. Apparently the toxic effects were due, directly or indirectly, to the dissolved hydrocarbon.

In this experiment, too, tumors developed more slowly in the females than in the males. Because of the small numbers of animals involved, however, no generalization is drawn.

It is evident that there is no disagreement between the results obtained here and in London. Our painting experiment gave results analogous to those obtained in London, viz., skin tumors were produced but slowly in mice by a 0.3 per cent benzene solution of 10-methyl-1:2-benzanthracene. On the other hand, subcutaneous tumors in mice were produced rapidly in this laboratory; no data on this point have, as yet, been published by the London workers.

9-Methyl-1:2-benzanthracene

A lard solution containing 5 mg. of 9-methyl-1:2-benzanthracene (Newman, 1937) per c.c. was injected (12/22/36) into 20 male mice; each mouse received one injection of 0.2 c.c. (i.e., 1 mg. of the hydrocarbon) at the start of the experiment, and another injection of 0.5 c.c. (i.e., 2.5 mg.) on the 70th day. After three months all of the mice were alive and negative. During the fourth month a tumor developed in 1 mouse; the tissue at the injection site became thickened in some of the mice, with accompanying ulceration in 2 cases. The thickened tissue developed into tumor in 9 other mice during the fifth month, and in 4 additional mice during the sixth month. Tumors developed in 2 more mice in the seventh month. At the end of eight months, tumors had been obtained in 17 of the 20 mice; the other 3 mice were alive and negative. The mouse which developed a tumor at the injection site during the eighth month also bore a primary hepatoma at the margin of one lobe of the liver. The experiment was terminated after ten months, at which time the 3 surviving mice were still negative. The results are summarized in Table I.
The London workers (Bachmann and others, 1937) have in progress a skin-painting experiment with the 9-methyl derivative, but no results have as yet been reported.

*5:9-Dimethyl-1:2-benzanthracene*

A lard solution containing 5 mg. of 5:9-dimethyl-1:2-benzanthracene (Newman, 1937) per c.c. was injected (12/22/36) into 20 male mice; each mouse received only one injection of 0.2 c.c. (i.e., 1 mg. of the hydrocarbon). As early as the 24th day ulceration and thickening of the tissue at the site of injection were observed in several mice. These changes rapidly became more pronounced, and by the 45th day ulceration or thickening, or both, was present in all of the mice.

At the end of the second month the thickening under the ulcers in 2 mice was considerable; on the 69th day the first tumor (Fig. 13) was transplanted. On the 77th day a second tumor (Fig. 14) was transplanted; at this time tumorous masses were present in 5 other mice. At the end of the third month another tumor was transplanted. In all 3 instances in which transplantation was attempted, the tumor transplants established themselves successfully in the new hosts.
In the course of the first three months, tumors were observed in 8 mice. Two mice which died during the first four months showed thickening of the base of the ulcer at the injection site.

On the 111th day all 10 survivors bore large tumors at the site of injection. This hydrocarbon had thus produced tumors in 18 of 20 mice in less than four months. These results, summarized in Table I, showed that 5:9-dimethyl-1:2-benzanthracene produced subcutaneous tumors about as rapidly as 20-methylcholanthrene and cholanthrene.

**7-Methyl-1:2-benzanthracene**

Each of 19 male mice received (11/4/36) 5 mg. of a specimen of crystalline 7-methyl-1:2-benzanthracene prepared by Fieser and Hershberg. The injection, this time of 10 mg., was repeated after four months, inasmuch as all of the 19 mice were alive and negative. In the course of the next few months slight ulceration and thickening were observed in some of the mice, but this ulceration healed and no tumors developed. Consequently, after eight months had elapsed, an additional 7 mg. was injected into each mouse.

In the ninth month a sarcoma (Fig. 15) was obtained at the site of injection in 1 of these male mice. In the thirteenth month, 1 mouse died bearing a primary carcinoma of the liver (Fig. 16). No other tumors developed by the

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"Unpublished work. The sample melted at 183.0–183.5° corr."
FIG. 16. PRIMARY CARCINOMA OF LIVER (A-1284) IN A STRAIN A MOUSE 13 MONTHS AFTER THE SUBCUTANEOUS INJECTION OF CRYSTALLINE 7-METHYL-1:2-BENZANTHRACENE.
× 50 AND × 100
end of sixteen months, at which time 12 of the mice were alive and negative (see Table I). This experiment was terminated in the eighteenth month by an epidemic infection.

The London group (Barry and others, 1935) applied the 7-methyl derivative to the skin of mice in painting experiments; no tumors were obtained, although 4 of the 10 mice were still alive after a year.

9:10-Dimethyl-1:2-benzanthracene

9:10-Dimethyl-1:2-benzanthracene (Newman, 1938a) is in course of being examined for potency. Each of 20 mice (10 males and 10 females) received (12/10/37) 0.2 c.c. of a lard solution containing 5 mg. of the hydrocarbon per c.c. Severe ulceration was observed at the injection site in most of the series by the 26th day. Although these animals had received only 1 mg. of the hydrocarbon, this dose appeared to be too large, for they soon became quite thin and remained small. The ulcers were healed at the end of four months. Only 8 of the 20 mice were alive after four months, at which time tumors were present in 2.

In the next experiment with this compound, each of 20 mice (10 males and 10 females) received 0.2 c.c. of a solution containing 0.5 mg. of the hydrocarbon per c.c., i.e., one-tenth of the dose employed in the preceding experiment. After three months, 18 of the 20 animals were alive and in good condition; ulceration was present in only 2. The first tumor appeared on the 84th day and another on the 100th day, at which time thickening of the tissue at the injection site was noted in 4 other mice. At the end of four months, tumors had been obtained in a total of 6 mice, and 4 of the remaining 13 showed ulceration or thickening at the site of injection.

Other Methyl Derivatives of 1:2-Benzanthracene

Fieser and Seligman (1938) have synthesized the 1'-methyl and the 1':10-dimethyl derivatives of 1:2-benzanthracene. These compounds have been injected into mice, by Seligman, in experiments which are still in progress. No tumors have been obtained after ten and six months, respectively.

The 4-methyl (Fieser and Jones) and the 8-methyl (Fieser and Johnson) derivatives are also in course of being examined for potency. Much greater evidence of carcinogenic potency has been observed following the subcutaneous injection of the 4-methyl derivative than was obtained by Barry and others (1935) in a skin-painting experiment with this compound; the first 3 tumors (20 mice) developed at the site of injection after three months.
In the first experiment (Shear, 1936b) with 4:10-ace-1:2-benzanthracene (Fieser and Seligman, 1937) each of 7 male mice received (6/19/36) 10 mg. of the crystalline hydrocarbon subcutaneously. In 19 days there was a severe local reaction in all the mice, consisting of ulceration and thickening of the tissue at the injection site. After a month the ulcers were covered over with crusts, which subsequently dropped off in most cases. With the rapidly acting carcinogens examined previously, the ulcers that formed at the injection site did not ordinarily become crusted over.

One mouse developed a tumor in the sixth month. By this time 5 mice had died, apparently as a result of the treatment, for they had become emaciated; in none of them did a tumor develop at the injection site. At the time of death, ulceration was present in all but 1, in which the ulceration had healed. In 1 mouse which survived for a year the ulceration healed and then recurred but no tumor developed.

In view of the severe ulceration and the subsequent emaciation, it seemed possible that the damage done to the subcutaneous tissues by this compound might have interfered with tumor development. Consequently 10 other male mice were given (12/29/36) one-tenth of the preceding dose; each mouse received 0.5 c.c. of a lard solution containing 2 mg. per c.c. (i.e., 1 mg. hydrocarbon per mouse). Within 2 weeks noticeable thickening of the tissue at the injection site was noted. On the 17th day, ulceration with some scab formation was seen in half of the mice. By the end of the first month the ulceration was quite severe, and the mice became thin and sickly, as had been the case with those that had received 10 mg. of the hydrocarbon.

On the 31st day, one mouse with a thickened ulcer was killed, and the new tissue beneath the ulcer was transplanted; none of the implants grew. During the second month the mice were badly ulcerated at the site of injection, and remained thin and sickly; in several cases there was considerable crust formation.

The ulcers began to heal and hair began to grow over the injection site, in many cases in the third month. Of the 2 mice that died during this month, one bore a considerable amount of newly proliferated tissue adjacent to the ulcer.

During the fourth month 3 of the 7 surviving mice were emaciated and had incompletely healed ulcers; 2 of these died, showing no tumors. A tumor developed in 1 mouse at the injection site during the sixth month, and in another during the eighth month. These were the only tumors that had been obtained by the time the experiment was terminated, in the eleventh month.

Concurrently with the preceding experiment, a parallel one was carried...
out in which 10 male mice each received (12/29/36) 0.2 c.c. of a lard solution containing 0.5 mg. of the hydrocarbon per c.c. The 0.1 mg. of 4:10-ace-1:2-benzanthracene that these mice received was one-one hundredth of the dose employed in the first experiment. As a consequence of this reduced dose, there was only a slight amount of ulceration in 4 of the mice at the end of the first month; the remaining mice were negative. After three months, 9 of the mice were negative and in good condition; the one mouse that was severely ulcerated became emaciated and died in the fifth month.

The first tumor appeared in the fifth month; tumors appeared in 3 other mice during the sixth month. One appeared in the seventh month, and another in the ninth month. The 3 negative survivors were killed in the eleventh month. These results are summarized in Table III.

In these experiments the 10 mg. dose had produced tumors in 1 of 7 mice, the 1 mg. dose had produced tumors in 2 of 10 mice, while the 0.1 mg. dose had produced tumors in 6 of 10 mice.

Morelli and Dansi (1937) have produced sarcomas in rats by the subcutaneous injection of this hydrocarbon in lard solution.

It was previously reported (Shear, 1937 c) that partial hydrogenation of certain benzpyrene derivatives did not abolish the carcinogenic potency possessed by the latter. Analogous results have now been obtained with a hydrogenated derivative of 1:2-benzanthracene.

Each of 10 male mice received (5/11/37) 5 mg. of crystalline 1':2':3':4'-tetrahydro-4:10-ace-1:2-benzanthracene (Fieser and Seligman, 1937). After the first month ulceration was observed in 7 mice; another mouse showed ulceration at the end of three months. After four months, the ulcers were healing and hair was growing over the injection site in 4 mice; 1 sick, emaciated, ulcerated mouse was killed at this time.

During the fifth month the thickening around the ulcers developed into tumors in 2 of the mice. A third mouse developed a tumor during the sixth month. Two more mice died during this time, one of them being quite thin. By the end of the ninth month, tumors had been obtained in 4 mice, and the 2 negative survivors showed healed ulcers. These 2 animals were still negative when the experiment was terminated in the twelfth month (see Table III).

As in the case of its completely aromatic analogue, reduction of the dose may be found to reduce the damage produced and to result in an increased production of tumors. It is planned to test this point when more of the compound becomes available.

Although 1':2':3':4'-tetrahydro-4:10-ace-1:2-benzanthracene produced severe ulceration and was carcinogenic, the analogous hydrogenated derivative
<table>
<thead>
<tr>
<th>Compound</th>
<th>Number and Sex</th>
<th>Strain</th>
<th>Dose</th>
<th>Total No. of Mice with Tumors (+); Negative Surviving Mice (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4 mos.</td>
</tr>
<tr>
<td>4:10-Ace-1:2-benzanthracene</td>
<td>7 M</td>
<td>A</td>
<td>10 mg. crystals</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>10 M</td>
<td>A</td>
<td>1 mg. in lard</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>10 M</td>
<td>A</td>
<td>0.1 mg. in lard</td>
<td>0+</td>
</tr>
<tr>
<td>1'-2'-3'-4'-Tetrahydro-4:10-ace-1:2-benzanthracene</td>
<td>10 M</td>
<td>A</td>
<td>5 mg. crystals</td>
<td>0+</td>
</tr>
<tr>
<td>Dehydronorcholene</td>
<td>10 F</td>
<td>—</td>
<td>6 doses 0.25 c.c. of 5% solution in lard</td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td>8 F</td>
<td>M</td>
<td>4 doses, each of 10 mg. crystals</td>
<td>0+</td>
</tr>
<tr>
<td>20-Ethylcholanthrene</td>
<td>8 M</td>
<td>A</td>
<td>10 mg. crystals</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>8 F</td>
<td>A</td>
<td>10 mg. crystals</td>
<td>2+</td>
</tr>
<tr>
<td>Aceanthrene</td>
<td>9 F</td>
<td>A</td>
<td>5 mg. crystals</td>
<td>0+</td>
</tr>
</tbody>
</table>

** No survivors.
of the strongly carcinogenic 10-methyl-1:2-benzanthracene was completely inactive in the same period. None of the 10 mice that had received injections of 5 mg. of crystalline 1':2':3':4'-tetrahydro-10-methyl-1:2-benzanthracene (Fieser and Hershberg, 1937) showed any effect after five months. Ulceration, with thickening of the base of the ulcer in 2 cases, occurred in 3 mice during the seventh month. No tumors were obtained after nine months, at which time 7 of the mice were still alive.

*Dehydroronorcholene*

![Dehydroronorcholene](image)

Inasmuch as 20-methylcholanthrene had first been prepared from dehydroronorcholene (Wieland and Dane, 1933) it was considered of interest, both in London and here, to see whether the latter hydrocarbon was itself capable of inducing tumors.

A specimen of dehydroronorcholene prepared by Fieser and Newman by the known method was injected (12/19/34) into 18 mice. Each of 10 female albino market mice received 0.25 c.c. of a 5 per cent solution in lard; this dose was repeated five times during the next year and a half, so that each mouse received a total of 75 mg. of the hydrocarbon. Each of 8 strain M female mice was injected with 10 mg. of the solid dehydroronorcholene; this dose was repeated three times during the next year.

Only 4 of the 10 albinos lived eight months; the last mouse was killed after eighteen months. No tumors were obtained in the albinos. Of the 8 strain M mice, 7 were alive after twenty months. During the 21st month, the 6 survivors were killed (see Table III). Although the hydrocarbon was seen to be present subcutaneously in all of the mice at autopsy, no tumors had developed in any of them at the site of injection. In 2, however, hepatomas were present in the liver. Whether these liver tumors were spontaneous or were induced by dehydroronorcholene cannot be decided until this experiment is repeated with a larger number of animals.

The London group (Bachmann and others, 1937) reported negative results in a painting experiment with this hydrocarbon lasting more than two years.

*20-Ethylcholanthrene*

![20-Ethylcholanthrene](image)

Since 20-methylcholanthrene appeared to produce sarcomas in mice somewhat more rapidly than did cholanthrene, it was of interest to determine the
effect of substitution of an ethyl group at the same position in the cholangrene molecule. Accordingly, 10 mg. of crystalline 20-ethylcholanthrene (Bruce,8
1936) were injected (6/30/36) into each of 16 mice (8 males and 8 females).

Slight ulceration and thickening of the tissue at the site of injection were noted in 6 mice after one month. The first 3 tumors were obtained during the fourth month; 5 more tumors were obtained by the end of the fifth month, at which time ulceration was present in all but one of the survivors. Of the 5 mice that died during the course of the experiment, 1 bore an ulcer, 3 bore ulcers with thickened bases, while the 5th was negative. The 3 remaining mice all developed tumors, 1 each during the sixth and seventh months and 1 at the end of a year (see Table III). At autopsy the hydrocarbon was seen inside the tumors in several instances. The microscopic sections showed, in many cases, numerous crystal spaces (Fig. 17).

In this experiment 20-ethylcholanthrene produced tumors in 11 of 16 mice 8 of which developed during the first five months. Thus, substitution of an ethyl group for the methyl group in 20-methylcholanthrene did not abolish the carcinogenic activity, although it prolonged the latent period.

_Aceanthrene_

\[
\begin{align*}
\text{H}_2\text{C} &- \text{CH}_2 \\
\end{align*}
\]

Five mg. of crystalline aceanthrene, prepared by Fieser and Peters (1932), were injected (7/2/35) into each of 9 female mice. All of the animals were

*Department of Chemistry, Cornell University.*
alive and negative after fourteen months; and 5 were alive and negative after seventeen months. Not enough of the compound was available for repetition of the injection. The last 2 survivors were killed after twenty months (see Table III). No evidence of carcinogenic activity was observed.

Miscellaneous Polycyclic Compounds

2:3-Dimethyl-6:7-acechrysene

Each of 10 male mice received (6/30/36) one injection of 5 mg. of crystalline 2:3-dimethyl-6:7-acechrysene (Fieser, Fieser and Hershberg, 1936). After six months 9 mice were alive and negative. During the ninth month ulceration at the site of injection occurred in 2 of these. This ulceration persisted for several months, but no tumors were obtained although 7 of the 10 mice were alive when the experiment was terminated in the fourteenth month. No proliferation was noted at autopsy, although the crystals were still present at the site of injection in all of the mice (see Table IV).

1-Phenyl-3:4-dihydro-8:9-acephenanthrene

Ten mg. of crystalline 1-phenyl-3:4-dihydro-8:9-acephenanthrene, available from a previous investigation (Fieser and Peters, 1932), were injected (8/9/35) into each of 14 mice (7 males and 7 females). Although 9 mice survived for fifteen months, no tumors occurred in any of the animals. Not enough of the compound was available for repetition of the injection.

No evidence of carcinogenic activity was observed in the course of this experiment, which was terminated after nineteen months (see Table IV).

6:7-Dimethyl-3:4-benzphenanthrene

Since introduction of methyl groups into the 1:2-benzanthracene molecule gives compounds with marked carcinogenic potency in a number of instances,
it was considered of interest, both in London and here, to compare the behavior of other methyl-substituted tetracyclic hydrocarbons.

Crystalline 6:7-dimethyl-3:4-benzphenanthrene (Fieser, Fieser and Hershberg, 1936) was injected (11/29:35) into each of 10 male mice. After four months the 9 mice that were alive were still negative; the injection was therefore repeated. It was again repeated after eight and after twelve months, at which time 9 mice were still alive and negative. The experiment was terminated after seventeen months, no tumors having been obtained (see Table IV). The crystalline hydrocarbon was seen at autopsy at the site of injection in all of the animals.

The London workers (Bachmann and others, 1937) found that 2-methyl-3:4-benzphenanthrene produced skin tumors at a rapid rate and stated that it is evident that derivatives of 3:4-benzphenanthrene merit further attention. Other related compounds, 2:9-dimethyl-3:4-benzphenanthrene (Newman and Joshel, 1938) and 2:9-diethyl-3:4-benzphenanthrene (Newman and Joshel, 1938) are in course of being examined. No tumors have so far been obtained with these compounds after five and four months, respectively.

**2:3-Dimethylchrysene**

Each of 10 male mice received (6/30/36) 10 mg. of crystalline 2:3-dimethylchrysene (Fieser, Fieser and Hershberg, 1936); the injection was repeated two months later. After ten months, 8 of the mice were alive and negative. During the eleventh month, one mouse developed a keratin-containing spindle-cell tumor, adherent to the right salivary gland, at a distance from the crystalline hydrocarbon still present in the subcutaneous tissue in the left flank, where it had been introduced. No tumors were found in any of the other mice when the experiment was terminated at the end of a year by an epidemic of *B. piliformis* (see Table IV). No proliferation was noted, although the crystals were still present at the injection site in all of the mice.

**Triphenylbenzene**

It had previously (Shear, 1936 b) been reported that triphenylbenzene had produced no tumors in mice in thirteen months. Although the experiment subsequently ran for seven months more, no tumors were obtained.

In this experiment a specimen of symmetrical triphenylbenzene, purified
by Baxter and Hale (1936) for use in the determination of the atomic weight of carbon, was injected (8/12/35) into 20 mice (10 males and 10 females), each receiving 10 mg. of the crystalline hydrocarbon. After seven months, since all 20 of the mice were alive and negative, the injection was repeated. After fifteen months; 16 of the 20 mice were alive and negative, whereupon an additional 5 mg. of the compound was injected, making 25 mg. per mouse.

The last 2 survivors were killed at the end of twenty months (see Table IV). During the twelfth month one of the female mice developed an adenomatous tumor of mammary gland origin under the right jaw, though the hydrocarbon had been injected in the left flank. No tumors were obtained at the site of injection. At autopsy, large amounts of the hydrocarbon were seen in all of the mice at the injection site.

The London group (Bachmann and others, 1937) has reported similarly negative results following the subcutaneous injection of triphenylbenzene into 20 mice, 8 of which were alive after twelve months. They also failed to obtain tumors in any of 20 mice following application of this hydrocarbon to the skin, though 10 were alive after eighteen months.

Although the experiments in London were still in progress, they had lasted longer than the time required for the production of tumors in the experiment of Morton, Branch and Clapp (1936), in which tumors were obtained in less than nine months and in which, of the 37 mice which were alive at the time of appearance of the first tumor, 12 developed tumors by the 250th day.

Neither in this laboratory nor in London has any evidence been obtained to indicate that s-triphenylbenzene is carcinogenic.

**Fluoranathene**

Ten mg. of crystalline fluoranthene were injected (8/8/35) into each of 14 mice (7 males and 7 females). The injection was repeated four times during the experiment. Although 6 mice survived for eighteen months, no tumors were obtained in this experiment, which was terminated after 19 months (see Table IV). This is in agreement with the negative results obtained by the London workers (Barry and others, 1935) in a skin-painting experiment.

**1:6-Dimethylpleiadene**

Ten mg. of crystalline 1:6-dimethylpleiadene, available from a previous investigation (Fieser, 1933), were injected (10/3/35) into each of 10 male
TABLE IV: Miscellaneous Polycyclic Compounds: Rate of Production of Tumors at Site of Injection in Strain A Mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number and Sex</th>
<th>Dose</th>
<th>6 mos.</th>
<th>9 mos.</th>
<th>12 mos.</th>
<th>15 mos.</th>
<th>18 mos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 : 3-Dimethyl-6 : 7-acechrysene</td>
<td>10 M</td>
<td>5 mg. crystals</td>
<td>0+</td>
<td>9-</td>
<td>0-</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>1-Phenyl-3 : 4-dihydro-8 : 9-acephenanthrene</td>
<td>14(7M; 7F)</td>
<td>10 mg. crystals</td>
<td>0+</td>
<td>14-</td>
<td>13-</td>
<td>9-</td>
<td>4-</td>
</tr>
<tr>
<td>6 : 7-Dimethyl-3 : 4-benzphenanthrene</td>
<td>10 M</td>
<td>7 mg. crystals*</td>
<td>0+</td>
<td>9-</td>
<td>0+</td>
<td>0+</td>
<td>**</td>
</tr>
<tr>
<td>2 : 3-Dimethylchrysene</td>
<td>10 M</td>
<td>9 mg. crystals*</td>
<td>0+</td>
<td>8-</td>
<td>8-</td>
<td>3-</td>
<td>**</td>
</tr>
<tr>
<td>s-Triphenylbenzene</td>
<td>20 (10 M; 10 F)</td>
<td>10 mg. crystals*</td>
<td>0+</td>
<td>20-</td>
<td>20-</td>
<td>17-</td>
<td>4-</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>14 (7 M; 7 F)</td>
<td>5 injections each of 10 mg. crystals</td>
<td>0+</td>
<td>14-</td>
<td>14-</td>
<td>8-</td>
<td>6-</td>
</tr>
</tbody>
</table>
mice. The injection was repeated after two months and again after five months. Although 6 of the mice survived for fifteen months, and 2 of the mice were still alive when the experiment was terminated, after nineteen months, no tumors were observed at the site of injection (see Table IV).

1:6-Dimethylthiopleiadene

Ten mg. of crystalline 1:6-dimethylthiopleiadene, available from a previous investigation (Fieser, 1933), were injected (8/9/35) into 14 female mice. The injection was repeated after two months and again after seven months. None of the mice which died during the course of the experiment, and none of the 5 survivors, which were killed after twenty-one months, developed a tumor at the injection site, although the compound was present subcutaneously in all the mice at autopsy, fourteen months after the last injection (see Table IV).
Each of 10 male mice received seven injections, of 10 mg each, of fluorene over a period of sixteen months. No tumors had been obtained at the site of injection in any of the mice by the time the experiment was terminated, in the nineteenth month, when 7 of the mice were still alive (see Table IV). This is in agreement with the negative results obtained in London and elsewhere (see Kennaway, 1930) in skin-painting experiments with this compound.

**Fig. 18. Ulcer at the Site of Injection of 5:9-Dimethyl-1:2-Benzanthracene in a Mouse One Month Following Subcutaneous Administration of 1 mg. of the Hydrocarbon in Lard Solution**

**Fig. 19. Subcutaneous Tumor Produced by Subcutaneous Injection of 5:9-Dimethyl-1:2-Benzanthracene, Growing at the Margin of the Previously Formed Ulcer**

**Ulcration and Trauma**

**Ulcration**

It has long been suspected that “chronic irritation” plays a part in the induction of malignant growths. Evidence which did not appear to fit into this theory was discussed in a previous communication (Shear, 1936 b). Yet it was a frequent finding that the subcutaneous injection of potent carcinogens into strain A mice resulted in such severe local tissue damage that the skin over the injection site ulcerated. These ulcers usually did not heal and, when tumors subsequently arose, they developed at the base or at the margins of the ulcers. This was noted with methylcholanthrene, benzpyrene, and chloanthrene (Shear, 1936 a), and it has since been observed regularly with a large number of rapidly acting carcinogens.

Fig. 18 illustrates the type of ulceration produced. In this case, the tissue damage was produced one month after the subcutaneous injection of 1 mg. of **Fluorene**

![Fluorene Structure](image)
5:9-dimethyl-1:2-benzanthracene (in 0.2 c.c. lard). Fig. 19 is a photograph of another mouse in this same experiment, showing a subcutaneous tumor growing at one margin of the ulcer. This type of ulceration is not to be confused with the kind which often occurs when a previously non-ulcerated subcutaneous tumor becomes large and breaks through the overlying skin, as in the tumor shown in Fig. 20. The ulceration produced by the rapidly acting carcinogens is often pronounced by the end of the first or second month and occurs before the development of tumor.

The London workers (Barry and others, 1935) noted ulceration at the site of injection of methylcholanthrene in at least one experiment but, inasmuch as most of their work was done with the skin painting technic, ulceration was not a prominent feature in their animals. In our work, largely with the injection technic, it has been so regular an occurrence that if a newly synthesized polycyclic compound produces ulceration at the injection site in the first few months, it has come to be expected that tumors will usually develop shortly thereafter. Tissue damage has, moreover, been noted in our skin painting experiments, also.

This does not mean, however, that tissue damage is necessarily responsible for tumor genesis. There are many compounds which produce ulceration at the site of injection and yet do not produce tumors. Furthermore, the ulceration is dependent not only on the compound but on the dosage. In fact, as is shown in Table III, the large doses of 4.10-ace-1:2-benzanthracene which produced severe ulceration also produced few tumors. As the dose was decreased, the visible damage became less and the tumor production became greater. The gross damage may merely indicate the presence in high concentration of a biologically active material which, in lesser amounts, may not be destructive.

It is also pointed out that these gross signs of tissue damage have been ob-

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Some of these experiments have been carried out in collaboration with Prof. S. B. Wolbach of the Department of Pathology, Harvard Medical School, who has obtained histological evidence of destructive action (see preliminary report of Wolbach, 1937).
<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Total No. of Mice with Tumors (+); Negative Surviving Mice (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 months</td>
</tr>
<tr>
<td>20-Methylcholanthrene</td>
<td><img src="image" alt="Structure" /></td>
<td>18+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12−</td>
</tr>
<tr>
<td>Cholanthrene</td>
<td><img src="image" alt="Structure" /></td>
<td>11+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17−</td>
</tr>
<tr>
<td>5 : 10-Dimethyl-1 : 2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>11+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16−</td>
</tr>
<tr>
<td>5-Methyl-1 : 2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19−</td>
</tr>
<tr>
<td>10-Methyl-1 : 2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>19+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30−</td>
</tr>
<tr>
<td>9-Methyl-1 : 2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>0+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20−</td>
</tr>
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</table>
TABLE V—Continued

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Total No. of Mice with Tumors (+) and Negative Surviving Mice (−)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 months</td>
</tr>
<tr>
<td>5:9-Dimethyl-1:2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>8+</td>
</tr>
<tr>
<td>7-Methyl-1:2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>0+</td>
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<tr>
<td>9:10-Dimethyl-1:2-benzanthracene</td>
<td><img src="image" alt="Structure" /></td>
<td>2+</td>
</tr>
</tbody>
</table>

Obtained largely in strain A mice. Other strains of mice, both in these experiments and in those of Andervont in this laboratory, have not usually responded in this way to these hydrocarbons.

**Trauma**

To see whether mechanically induced damage promoted the action of 20-methylcholanthrene, 10 mg. of the crystalline hydrocarbon were injected (10/7/35) subcutaneously in 10 strain A female mice. Three days later the tissue at the site of injection was subjected to mechanical injury, which was repeated four and nine days later. The first 2 tumors developed after two and a half months, and another after three months. When the experiment was terminated, after four months, only 1 of the 6 remaining mice was negative.

On comparing these results with those obtained in analogous experiments with 20-methylcholanthrene, in which strain A mice were not mechanically injured, no evidence of promotion of tumor induction following trauma was noted.

A similar experiment was carried out, also in 10 strain A female mice, with crystalline deoxycholic acid instead of 20-methylcholanthrene. The site of injection was traumatized in the same way. When the experiment was terminated, after four months, no tumors were present in any of the 10 survivors; the skin lesions produced early in the experiment were all healed by this time.
These exploratory experiments gave no indication either of a promoting or of a retarding effect of tissue damage on this type of carcinogenesis. From other experiments, however, the impression was gained that tissue damage had interfered with tumor genesis. Further information on this point may perhaps be obtained by repeating the above experiment with a dose of methylcholanthrene which is near the minimum effective amount.

**DISCUSSION**

Whether carcinogenic hydrocarbons such as those reported in this paper are involved in the genesis of spontaneous human tumors is still an open question. While it is true that the isolation of 3:4-benzpyrene from coal tar and the synthesis and study of a large number of related compounds were the outcome of attempts to ascertain the mechanism of the genesis of certain types of occupational cancer, their significance may perhaps be limited merely to those types of cancer.

While it is of interest to note that 20-methylcholanthrene was first obtained from a bile acid, and that the normal animal organism contains substances which are composed of related condensed ring systems, it should nevertheless be borne in mind that the transformation of desoxycholic acid to 20-methylcholanthrene was effected by drastic laboratory procedures, and that the biologically occurring phenanthrene compounds are hydrogenated to a considerable extent. It may well be that substances analogous to the synthetic compounds found to be carcinogenic in London and in this laboratory are involved in human carcinogenesis, but this is at present only a conjecture.

Attention is also called to the fact that the dramatic production of tumors by these synthetic materials has been effected chiefly in mice and rats. Other animal species are much less susceptible. Indeed, it has been found difficult to produce malignant tumors with hydrocarbons even in other rodents. Rabbits and guinea-pigs have, unexpectedly, been found to be refractory to the action of carcinogens which are quite potent in mice (Lacassagne, 1933; Sanní, Oberling and Guérin, 1935; Boyland and Burrows, 1935; Miescher, 1935; Schürch and Winterstein, 1935; Haagensen and Krebbiel, 1936a and b; Lacassagne and Nyka, 1936; Lambret, Driessens and Cornillot, 1937 and Klinke, 1937). No tumors were obtained in fourteen months by Warren in guinea-pigs given 1:2:5:6-dibenzanthracene in cholesterol. In Menkin's laboratory, in the Department of Pathology of the Harvard Medical School, no tumors were obtained in rabbits which had been injected subcutaneously with 3:4-benzpyrene in sesame oil or with crystalline 20-methylcholanthrene, or which had received repeated intravenous injections of an aqueous solution of 20-methylcholanthrene-choleic acid; negative results were also obtained after repeated painting of the ears of rabbits with 3:4-benzpyrene in benzene solution.

In experiments in this laboratory, no tumors have so far been observed: (1) in rabbits, each of which received, subcutaneously, 50 mg. of crystalline 3:4-benzpyrene in sesame oil or with crystalline 20-methylcholanthrene, or which had received repeated intravenous injections of an aqueous solution of 20-methylcholanthrene-choleic acid; negative results were also obtained after repeated painting of the ears of rabbits with 3:4-benzpyrene in benzene solution.

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10 Personal communication from Prof. Shields Warren, Department of Pathology, Harvard Medical School, and the Deaconess Hospital, Boston.
11 These experiments were carried out, in part in collaboration with the author, by Dr. Valy Menkin, with the same specimens of carcinogens used previously in these studies (see Shear, 1936 a).
20-methylcholanthrene nine months ago; (2) in dogs each of which received, subcutaneously, 10 mg. of crystalline 20-methylcholanthrene nineteen months ago (in collaboration with Dr. F. C. Turner); (3) in a cat which survived for ten months after receiving one pellet of 20-methylcholanthrene in the brain and another subcutaneously (in collaboration with A. M. Seligman). In other collaborative experiments,\(^{12}\) 5 mg. of crystalline 3:4-benzpyrene were injected subcutaneously into each of 24 rats and 23 guinea-pigs. The first guinea-pig tumor appeared in the fourteenth month, whereas tumors had been obtained much earlier in 17 of the rats, which were all dead by this time. This guinea-pig tumor grew quite slowly and did not result in the death of the host until the twenty-third month after the injection of the hydrocarbon. In the twentieth month, a small tumor was noted in a second animal, at which time 19 of the 23 guinea-pigs were still alive.

It is thus clear that guinea-pigs and rabbits are much more refractory\(^{13}\) to the action of these carcinogenic hydrocarbons than are mice and rats. Neither the difference in life span of these species nor the difference in dose, calculated on the basis of body weight, which have at times been advanced to explain this resistance to tumor genesis, appears to account satisfactorily for the difference in susceptibility. Whether these compounds are capable of inducing malignant growth in man is still unknown. It is true that certain tars and oils produce clinical carcinomas, and readily produce epitheliomas in rabbits. It is all the more unexpected that 3:4-benzpyrene, which is present in coal tar in very low concentration, should have so little effect on rabbit skin even in concentrations apparently much higher than those in tar.

This raises the question as to whether there is present in coal tar some other material which, acting either alone or in conjunction with 3:4-benzpyrene, is responsible for its ability to produce tumors readily in rabbits. According to Rous there is an "unknown agent present in tar" which is the real carcinogen. Some information which may have bearing on this point has been obtained in experiments begun early in 1936.

In the course of an investigation\(^{14}\) of the possible retarding effect of creosote oil preparations on the induction of skin tumors in mice painted with 3:4-benzpyrene, it was found that certain fractions of creosote oil delayed the appearance and reduced the yield of skin tumors. Unexpectedly, however, the basic fraction appeared to expedite tumor genesis, as compared to controls. This was noted both with 0.2 per cent and 0.05 per cent solutions of 3:4-benzpyrene in benzene.

Since facilities were not available for repetition and extension of these painting experiments, no further work has been done with the retarding fractions. The effect of the basic fraction on the induction of subcutaneous tumors was investigated, however. It was found that, whereas a borderline dose of

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\(^{12}\) With Professor Percy R. Howe and Dr. Mark Elliott of the Forsyth Dental Infirmary for Children, Boston.

\(^{13}\) Putschar, who found (Putschar and Holtz, 1930) that ultraviolet light produced malignant tumors in rats, obtained negative results with guinea-pigs similarly irradiated (personal communication).

\(^{14}\) Undertaken at the suggestion of Mr. Samuel Cabot, and carried out in collaboration with him and with Mr. Nathaniel Shear, with materials prepared in the laboratories of Samuel Cabot, Inc., Boston.
3:4-benzpyrene produced subcutaneous tumors slowly in only about half the mice, tumors appeared rapidly in 90 per cent when the basic fraction was added. The painting experiment with the basic fraction was recently repeated, and it was again found that it hastened the production of skin tumors by 3:4-benzpyrene.

The basic fraction alone has not given rise to tumors in either the painted or the injected control mice. Since it does not appear to be carcinogenic itself and yet enhances the activity of 3:4-benzpyrene, it has been termed, for convenience, a "co-carcinogen." No further information is as yet available regarding its chemical nature but, from its presence in this fraction of creosote oil, this co-carcinogen may be a cyclic compound with basic properties.

As stated previously, the data presented in this paper refer, except when otherwise stated, only to the presence or absence of tumors at the site of application of the substances examined. In some cases changes were seen in other tissues; these will be described in later communications.

Experiments such as these are to be considered only exploratory in nature. They constitute a broad-meshed sieve to distinguish, by means of simple procedures, the powerful carcinogens from relatively inactive compounds. Since the examination of these substances for potency has been carried out in mice, the conclusions drawn apply only to mice. Some compounds which are carcinogenic in mice may be completely inactive in other species; the reverse is also conceivable. Furthermore, the routine tests are restricted in London to the skin and, in this laboratory, to the subcutaneous tissues. Since several instances have been found (Shear, 1937b, 1938) in which compounds were negative as far as the subcutaneous tissues are concerned but in which primary malignant growths were produced at other sites, the generalizations here drawn regarding carcinogenic potency are restricted to the tissues actually tested.

The experiments described in this paper have not been designed for the purpose of quantitative comparisons of minimum effective dose or of latent period. For such quantitative comparisons larger numbers of mice and different technics are required than were used in the qualitative sifting of the wheat from the chaff. Moreover, insufficient supplies of strain A mice have been available up to the present time even for the qualitative type of experiment. As a consequence, various other pure strains of mice have been employed when strain A mice were not available. Since Andervont (1934, 1935, 1938) has shown that the latent period for a given hydrocarbon in mice varies from strain to strain, only data obtained in the same strain are strictly comparable.

It is therefore pointed out that the value for the "average time" of the latent period of a carcinogen, as used previously (Fieser and others, 1937a), is not to be considered as an invariable property inherent in the hydrocarbon; it is merely a convenient summary of the results of particular experiments and is valid only for animals of the strain, sex, age, etc., of the particular species employed, as well as for the dose, menstruum, mode of application, and site of application of the carcinogen under consideration.

With the foregoing limitations of the concept "average time" clearly understood, values so calculated for the different carcinogens serve a useful pur-

\footnote{In collaboration with Mr. Robert Sall of the Harvard Medical School.}
pose. Even for the specific conditions enumerated above, however, the actual numerical values assigned to the different compounds may not have an accuracy of better than about one half of a month in many cases up to the present. One of the uncertainties implicit in such data is the precise time at which a malignant tumor may be said to have appeared. In many cases the newly formed tissue at the injection site becomes progressively greater in amount, from a "slight thickening" in a small area to "pronounced thickening" in a much larger area and, by a gradual transition, develops into a well defined mass. Even in the case of a rapidly acting carcinogen these various stages may be spread over a month or more. Consequently the fixing of the time of "appearance" of a subcutaneous tumor depends upon arbitrary criteria, which are estimated to have an accuracy of about half a month in these experiments.

The data reported here and in the previous papers are based chiefly upon gross observations. The histologic findings will be described at a later date, but it may be mentioned that, occasionally, "thickened tissue" not classified as "tumor" in these tabulations was seen upon microscopic examination to be malignant; occasionally, however, a nodule in the subcutaneous tissue classified as "tumor" in these tables was found to consist of tissue not yet definitely malignant. Only in the case of a few selected compounds were the mice sacrificed at frequent intervals and the newly formed tissue examined histologically and by transplantation.

In order to obtain more precise information regarding the first appearance of malignancy in such induced new growths, it would be necessary to set up a different type of experiment with much larger numbers of mice. The sacrifice of a sufficient number of mice at frequent intervals would give the material required for the histologic determination of the first appearance of malignancy. This type of experiment would give precise data on the latent periods of the various carcinogens. Up to the present time, however, data of this kind have not been considered of sufficient importance to warrant such experiments in this laboratory.

Another point which merits discussion is that of dosage. In both the painting and the injection experiments the different polycyclic compounds have been tested, for the most part, with one arbitrary dose or concentration. With some compounds too high dosages produce local tissue damage, generalized toxic effects and loss of some of the animals; too low dosages produce tumors in only a small percentage of the mice and only after a prolonged latent period. The effect of varying the dosage has been studied in the case of only a few compounds; with most compounds but one dose, or one level of dosage, has been employed. Where tumors have been obtained with different doses of the same compounds, attempts at quantitative comparisons of latent periods should include mention as to whether the comparisons are of results obtained at equal doses or at the optimum dose for each compound. In the case of 4:10-ace-1:2-benzanthracene, for example, 0.1 mg. produced more subcutaneous tumors in a given time than did 1 mg. of this hydrocarbon (see Table III), whereas 0.1 mg. of 3:4-benzpyrene produced fewer subcutaneous tumors in given time than did 1 mg. (unpublished experiments).

In this connection the question also arises as to the interpretation of the unexpectedly slow production of tumors in certain experiments with carcino-
gens that in other experiments are rapidly acting. For example, when 1.25 mg. cholanthrene in lard solution (5 mg. hydrocarbon per c.c.) was injected subcutaneously into 10 strain D female mice, in an experiment not previously reported, no tumors were observed during the first three months either in the 4 mice that died during this time or in the 6 survivors. The first tumor was obtained at the end of six months, at which time the injection was repeated; tumors were obtained in 4 other mice eight to thirteen months after the start of the experiment. The slow production of tumors by cholanthrene did not appear to be due to the strain employed (strain D) or to the sex, inasmuch as crystalline cholanthrene had produced tumors rapidly in other females of this strain (see Fig. 3, Shear, 1936 a). The London workers (Bachmann and others, 1937) have reported a somewhat analogous refractoriness to the production of tumors in one of the painting experiments with 20-methylcholan-
threne; in this case the marked prolongation of the latent period was ascribed to the mouse strain employed (Simpson strain).

Since there is no evidence that the lard used as solvent interferes with tumor production, the question arises as to whether some other factor, such as sex, has a retarding effect, particularly when comparatively small doses of carcinogens are employed. In the painting experiment with the 10-methyl derivative the tumors appeared later in the females than in the males (see Table II). In the C,H mice injected with 1 mg. of 10-methyl-1:2-benzanthracene in lard, the tumors seemed to develop more slowly in the females than in the males (see Table I).

In the earlier experiments male mice were used whenever available, in order to avoid complications arising from the development of spontaneous tumors in the females. In many instances in which both males and females were employed in the examination of a given compound, no pronounced differences were noted which appeared to be ascribable to sex. In some experiments, however, tumors appeared more slowly in the females (see discussion, pp. 509–510); in others, the tumors arose more slowly in the males. These differences may well have been fortuitous in view of the small numbers of mice employed for each compound. Further information on this point might be obtained by using borderline doses of carcinogens in much larger numbers of pure strain mice of both sexes. During the last year equal numbers of males and females have been employed, when available, in the routine tests. Furthermore, in reporting the results of the bioassays, data on sex are henceforth being recorded.

For the routine examination of these compounds for carcinogenic potency the subcutaneous technic has been employed, chiefly because the labor involved is far less than with the skin-painting technic. The possible deleterious effect of solvents such as benzene is also avoided. Incidentally it has been found that the subcutaneous tissues of the mouse are apparently somewhat more sensitive to the action of some of these compounds than is the skin. This may be due, at least in part, to the protective action of the thin layer of dead skin formed early in painting experiments with active compounds (Wolbach; see page 527).

In this laboratory, as in London, it has been found that increasing the complexity of the molecule of substances related to 1:2:5:6-dibenzanthracene, to
3:4-benzpyrene, and to 20-methylcholanthrene, usually results in a diminution or complete loss of carcinogenic potency. Simplification of the molecule has, however, given substances of high activity. Substitution of simple groups at certain positions in the 1:2-benzanthracene molecule is sufficient to confer carcinogenic properties of a high order. While 9-methyl-1:2-benzanthracene is potent, it acts more slowly than 3:4-benzpyrene. The 5:9-dimethyl and 5:10-dimethyl derivatives act about as rapidly as cholanthrene. The 10-position appears to be more important for the rapid production of subcutaneous tumors than the 9-position, inasmuch as 10-methyl-1:2-benzanthracene acts almost as rapidly as the 5:9-dimethyl and the 5:10-dimethyl derivatives. For ready comparison, the results obtained with the cholanthrenes and with the methyl derivatives during the first four months are summarized in Table V.

The significance of the 10-position is further attested by the potency of 10-ethyl-1:2-benzanthracene. This hydrocarbon produced tumors in a high proportion of mice, although the latent period was longer than in the case of the 10-methyl derivative. A few tumors have also been obtained with the 10-methoxy derivative and with a number of other 10-substituted compounds.16

Methyl substitution at the 5-position, when a methyl group is also present at either the 9- or the 10-position, increases the speed of tumor production. It is interesting to note that the 5-cyano-10-methyl derivative (Newman, 1938b) has been found to be about as rapidly acting as the 5:10-dimethyl derivative. The 5-chloro-10-methyl derivative (Newman, 1938b) is also carcinogenic.

It is thus clear that the 5-membered ring of the cholanthrenes is not essential for the rapid production of subcutaneous tumors. Whether this has any bearing on the possible role of 20-methylcholanthrene or closely related compounds in the genesis of spontaneous human tumors is still an open question.

Of interest in connection with the question as to whether condensed polycyclic compounds related to these synthetic substances may be involved in the genesis of spontaneous neoplasms is the finding that at least two hydrogenated compounds are carcinogenic. Both 1′:2′-dihydro-4′-methyl-3:4-benzpyrene and 1′:2′:3′:4′-tetrahydro-4:10-ace-1:2-benzanthracene have been found to be carcinogenic (Shear, 1937c). These are of especial interest because related compounds which are known to occur physiologically are also hydrogenated, to an even greater degree.

Those compounds which produce tumors in a high proportion of the mice in a very short time have received considerable attention because of their dramatic action. If, however, spontaneous tumors are induced by polycyclic carcinogens, the weakly active carcinogens and the slowly acting ones may possibly be found to be of equal or even greater significance, especially if they contain polar groups. The results obtained with compounds having polar groups will be reported at a later date.

**SUMMARY**

1. Of 21 compounds examined for carcinogenic activity by subcutaneous injection into pure strain mice, 10 were found to produce tumors at the site of injection.

2. Subcutaneous tumors were produced in mice by 5:10-dimethyl-1:2-benzanthracene about as rapidly as by cholanthrene, showing that the pentacyclic system of the latter is not essential for high carcinogenic potency.

3. Subcutaneous tumors were produced by 10-methyl-1:2-benzanthracene almost as rapidly as by the 5:10-dimethyl derivative.

4. The production of skin tumors by 10-methyl-1:2-benzanthracene, with the skin painting technic, was slower than the production of subcutaneous tumors with the injection technic.

5. Tumors were produced by 5:9-dimethyl-1:2-benzanthracene about as rapidly as by cholanthrene.

6. The 9-methyl derivative was also found to be a potent carcinogen, but the latent period was longer than in the case of the 5:9-dimethyl derivative.

7. The 4:10-ace derivative was found to be carcinogenic, especially in small doses which did not produce severe local tissue damage.

8. The 1′:2′:3′:4′-tetrahydro derivative of 4:10-ace-1:2-benzanthracene was also found to be carcinogenic.

9. 20-Ethylcholanthrene produced tumors in a high proportion of the mice but was more slowly acting than 20-methylcholanthrene or cholanthrene.

10. No tumors were produced by s-triphenylbenzene even after 20 months.

NOTE: Technical assistance was given by Mr. Adrien Perrault. The animals were cared for by Mr. John Francis Linnell.

BIBLIOGRAPHY


