Induction of Tumors in Rats by Carcinogens in Various Lipids

H. A. Davenport, M.D., John L. Savage, M.D., Morris J. Distine, M.D., and F. B. Queen, M.D.

(From the Department of Anatomy* and the Patterson Cancer Research Laboratory, Northwestern University Medical School, Chicago, Ill.)

(Received for publication July 7, 1941)

The experiments to be described were begun about two years ago, and were prompted by the unsettled status of the effect that different solvents for carcinogens might have on the induction of tumors. Since then, Shimkin and Andervont (14) and Sall and Shear (11) have placed the knowledge of the effects of solvents on a more comprehensive basis. We wish, therefore, to record our experiments on the production of tumors in rats and to indicate why the original objective of showing effects of solvents was not attained.

In 1935 Peacock (9) reported that 1,2,5,6-dibenzanthracene was effective as a tumor-producing agent when dissolved in lard and injected into the breast muscles of fowls, but was ineffective when dissolved in chicken fat. The possibility that the carcinogen might be more readily eliminated when dissolved in species homogenous fat was considered and the next year Chalmers and Peacock (4) studied the elimination of polycyclic hydrocarbons by means of fluorescence. They found that colloidal water suspensions of the hydrocarbons injected intravenously were eliminated by way of the bile, but no specific light was thrown on the question of the fats as solvents. Coincident with their work, Berenblum and Kendall (2) failed to produce any tumors by 30 weekly intravenous injections of colloidal carcinogen in water totalling 20 mgm. of 1,2,5,6-dibenzanthracene given to each of 20 fowls. Later, with mice, Peacock and Beck (10) used 3,4-benzpyrene in powder form, dissolved in ether, in lard, in olive oil alone or olive oil mixed with paraffin, and in mouse lipids. They concluded that the effect of the solvent was that of keeping the carcinogen in place rather than having any effect in itself, and that the powder, ether solution, and mouse fat solution were more readily absorbed and eliminated than the oil solution. Doses of 0.5 to 1.0 mgm. per mouse were used. Oberling and co-workers (8) performed similar experiments with benzpyrene in rats. Olive oil, lard, and rat fat were used for solvents with two levels of dosage, 21 to 25 mgm. per rat in one and 1 mgm. in the other. No appreciable difference in the number and evolution of the tumors produced by the three solutions of each dosage level was observed. Morton and Mider (7) found, however, that 0.25 mgm. of benzpyrene injected subcutaneously into mice gave tumors in only 2 per cent of the animals within 30 weeks when dissolved in mouse fat, but gave tumors in 78 per cent when dissolved in sesame oil and in 50 per cent when used in the colloidal condition.

Twort and Twort (15) painted mice with solutions of dibenzanthracene, benzpyrene, and methylcholanthrene dissolved in chloroform, oleic acid, mineral oil, and liquid paraffin. The chloroform solutions were found to be the most potent. In similar experiments, Crabtree (5) used 0.3 per cent benzpyrene solutions in ether and benzene, both with and without the addition of 2 per cent liquid paraffin. Papillomas appeared 2 to 3 weeks earlier in the mice treated with the solutions which contained the paraffin oil. In a second paper he reported the inhibitory effect of the basic solution by monochloracetal and other chlorinated organic compounds.

Burrows, Hieger, and Kennaway (3) observed the production of a few tumors in rats (but not in mice) by means of lard injections alone. Andervont (1) concluded originally that the solvent medium played no significant role in his tests on 8 different strains of mice, but later, Shimkin and Andervont (14) were able to show that the solvent affected the time of induction when the dose of the carcinogen was near minimal. Observations made by Sall and Shear (11) on the accelerating effect of the basic fraction of creosote oil indicate that a high concentration of carcinogen in the solvent may obscure any effect of substances associated with the solvent itself.

**EXPERIMENTAL**

Eighty-five white rats of heterogeneous ancestry whose weights ranged between 85 and 290 gm. were used. There were 35 males and 50 females, and these were divided into 10 groups as shown in Table I. All except the 9 rats in group 6 received approximately 8 mgm. of carcinogen administered subcutaneously into the right flank as a warm 2 per cent solution in the different lipids used. A control injection of lipid alone was put into the left flank. The rats of group 6 received 3 injections each, in the right axillary region, lower thoracic region, and flank, 0.25 cc. of a 2 per cent solution of methylcholanthrene in each site with linseed oil, lanolin, and lard respectively as solvents. Similar injections of lipids alone were placed on the left side.

The first six groups received methylcholanthrene. Groups 7-10 received dimethylbenzanthracene. Each rat received one subcutaneous injection except those in group 6, which received three at the same time. The ration was the same for all and consisted of Purina Dog Chow plus weekly supplements of lettuce (or carrots) and beef liver. The lard, lanolin, and sperma-

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1 Purchased from the Eastman Kodak Co., Rochester, N. Y.
2 Gift of Dr. W. E. Bachmann.
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ceti were melted and allowed to cool to approximately body temperature before injection. A short 22 gauge needle was needed for the spermaceti since it solidified much more quickly than the other two solvents. The linseed oil and rat fat were liquid at room temperature. The rat fat was prepared originally by extracting adipose tissue from the abdominal cavity with acetone, then with petroleum ether. The extracts were combined after evaporation of the solvents, and the whole process carried through at room temperature.

The rats were examined once a week until the tumors began to grow, then twice a week thereafter until sacrificed. The time of the beginning of a tumor was taken as the semi-weekly date immediately preceding the one at which the first certain measurable increment was followed by other progressive increments in size. Fluctuations in size not followed by progressive growth were disregarded. Fluctuations in size not followed by progressive growth were disregarded.

RESULTS AND DISCUSSION

No tumors developed at the site of the injections of lipids alone. These injections appeared to cause a greater tissue reaction than the lipids which contained carcinogen, but the palpable masses disappeared during the first three or four weeks and did not reappear. At injection sites of the lipids which contained carcinogen, palpable masses were slow to appear and did so after about a month. Usually the masses remained as soft to moderately firm 1.0 to 1.5 cm. subcutaneous plaques for several months until progressive growth started. Ulcers did not occur on the control side but occurred occasionally at the site of the injections of methylcholanthrene (less than 10 per cent) and rather frequently (20 to 25 per cent) at the site of the injections of dimethylbenzanthracene. In several instances the formation of an ulcer together with the sloughing out of the area of injection appeared either to delay or prevent tumor formation.

<table>
<thead>
<tr>
<th>Table I: Time Required for Induction of Malignant Tumors in Rats by Methylcholanthrene and Dimethylbenzanthracene Dissolved in Rat Fat and Other Lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group no.</td>
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<tr>
<td>-----------</td>
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<tr>
<td>Methylcholanthrene series, groups 1 to 6.</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
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<tr>
<td>6</td>
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<tr>
<td>Total</td>
</tr>
<tr>
<td>Dimethylbenzanthracene series, groups 7 to 10.</td>
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<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Grand Totals</td>
</tr>
</tbody>
</table>

* Adenocarcinomas of the breast. All other tumors were sarcomas.
In listing the number which survived the induction period, the rats which died before 80 days were not counted, since the first tumor started at that time. Two rats in the methylcholanthrene series and 7 in the dimethylbenzanthracene series died without tumor at intervals from 98 to 310 days after injection. One rat of group 6 (Table I) developed tumors at all three sites of injection, two developed two tumors, and the remaining three succumbed to a single tumor.

There was a nearly complete overlap of the periods of induction in all groups, both with regard to the solvents and carcinogens. There was a trend, however, for the tumors induced by dimethylbenzanthracene to start somewhat sooner than those induced by methylcholanthrene. When the time of occurrence of progressive tumor growth for the two carcinogens is plotted (Fig. 1) a shorter induction period for dimethylbenzanthracene is indicated. A comparison of the averages of the intervals required for induction is inconclusive.

About two-thirds of the total number of tumors were examined histologically and their malignant morphology verified. Three of the 71 tumors were sarcomas. Transplants were made of 51 tumors into other rats. The results on transplantation, together with certain genetic effects seen, will require a separate report since the work is still in progress. Suffice it to say that the percentage of takes with the sarcomas into animals of heterogeneous ancestry was low (approximately 20 per cent). All attempts to transplant the carcinomas failed beyond the first transplant, and very few transient growths were seen in the first passage.

It seems that the amount of carcinogen used, and particularly its concentration in the solvent, has been the chief factor which has been responsible for different results with regard to the influence of the solvent. Morton and Mider (7) used 0.25 mgm. of benzpyrene in a 0.1 per cent solution per mouse. Oberling et al. (8) used both large and small doses (21 to 25 mgm. and 1 mgm.) for rats but in solutions of about 2 per cent concentration. The dosage is not stated in the article by Chalmers and Peacock (4), but Peacock and Beck (10) used 0.5 mgm., 0.75 mgm. and 1.0 mgm. per mouse as a 1 per cent solution. Dobrovolskaia-Zavadskaiia (6) showed that the size of the dose of 1,2,5,6-dibenzanthracene (0.04 per cent in olive oil) affected the incidence of tumor formation, and that female mice were more susceptible than males. Shear and Ilfeld (12) and Shear and Lorenz (13) found that a concentration of at least 1 per cent of carcinogen in cholesterol pellets was necessary to produce tumors with regularity. Shimkin and Andervont (14) found that, in mice, the solvent exerted an effect on both the latent period and the incidence. Methylcholanthrene (0.5 mgm. in 0.25 cc. of solvent) produced tumors in less than half the time when dissolved in tricaprylin than it did when dissolved in butyl phthallate. Differences were noted also among different lots of lard. Sall and Shear (11) have suggested 0.02 mgm. of methylcholanthrene in 0.2 to 0.3 cc. of solvent as a threshold dose for demonstrating the influence of factors in the solvent. In view of these observations, it appears that our dosage of 8 mgm. per rat administered as a 2 per cent solution was too large and too concentrated to reveal any influence of the solvent.

**SUMMARY**

Methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene produced malignant growths in 80 to 297 days in rats when the carcinogens were dissolved in rat fat or other lipids. Of the 71 tumors produced in an original number of 85 rats, all were sarcomas except three adenocarcinomas of the mammary gland. The dosage of 8 mgm. of carcinogen per rat given in 0.4 cc. of lipid appears to be too large and concentrated to reveal any effects either of the homologous fat or other lipids on the induction time or incidence of tumor formation. Transplantation of the tumors into rats of heterogeneous ancestry gave only 20 per cent progressively growing takes for the sarcomas and none for the carcinomas.

The average induction time for 50 tumors induced by methylcholanthrene was 146.6 days while that for 21 induced by dimethylbenzanthracene was 136.1 days. Rat fat, linseed oil, lard, lanolin, and spermaceti were used as solvents for the carcinogens.

No tumors occurred at the sites of injection of lipids alone.
REFERENCES


9. PEACOCK, P. R. Leeuwenhoek-Vereniging, Amsterdam. 1935. (Quoted from Chalmers and Peacock (4)).


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*Cancer Res* 1941;1:821-824.

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