Effect of Dose and Hormones on Tumor Production in Rats Given Emulsified 9,10-Dimethyl-1,2-benzanthracene Intravenously*

ROBERT P. GEYER, JEAN E. BRYANT, VIRGIL R. BLEISCH, ELIZABETH M. PEIRCE, AND FREDRICK J. STARE

(Department of Nutrition, Harvard School of Public Health, Boston 15, Mass.)

Intravenously administered oil emulsions which contain 9,10-dimethyl-1,2-benzanthracene have been shown previously to produce a fairly high incidence of mammary tumors in female rats (5). Male rats, however, developed few tumors, and these were chiefly papillomas arising from the sebaceous gland ducts of external ears. During these and subsequent studies it became apparent that tumor incidence and dose of carcinogen were closely related, and that, at doses of 4 times the maximum previously employed, an incidence of approximately 90 per cent was achieved in 19 weeks.

The simultaneous injection of hormones such as a-estradiol was also investigated because of (a) the difference noted earlier between the sexes, (b) the possibility that the endogenous supply of such hormones was not constant between female rats nor in the same female rat, and (c) the effect of such hormones on the mammary gland. The present paper deals with some of these experiments.

EXPERIMENTAL

Emulsions of oil containing 9,10-dimethyl-1,2-benzanthracene (DMBA) were prepared by the blending technic previously described (6) and had the following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>50</td>
</tr>
<tr>
<td>Phosphatide</td>
<td>10</td>
</tr>
<tr>
<td>Demal-14</td>
<td>10</td>
</tr>
<tr>
<td>Triton-WR-1339</td>
<td>10</td>
</tr>
<tr>
<td>DMBA</td>
<td>0.44</td>
</tr>
<tr>
<td>α-estradiol (when present)</td>
<td>0.005-0.05</td>
</tr>
<tr>
<td>Diethylstilbestrol (when present)</td>
<td>0.005-0.05</td>
</tr>
<tr>
<td>Dextrose (5 per cent in water) to make 1 ml</td>
<td></td>
</tr>
</tbody>
</table>

* Supported in part by grants-in-aid from the National Cancer Institute, National Institutes of Health, United States Public Health Service, Bethesda, Md.; from the American Cancer Society through an institutional grant to Harvard University; and from The Nutrition Foundation, Inc., N.Y.

Received for publication January 31, 1953.

The emulsion was autoclaved in nitrogen-flooded sealed glass ampules at 15 pounds/square inch for 15 minutes and stored in the dark. Such preparations remained stable for months.

Female rats, descendants of the Sprague-Dawley strain, weighing 150-200 gm. were used throughout and were fed a commercial stock ration and water ad libitum. The emulsions were injected through a tail vein, while the animals were under light ether anesthesia. After receiving all their respective injections, the animals were palpated weekly to determine the appearance of both the first and subsequent tumors. Tumor-bearing animals were sacrificed when the neoplasms seriously interfered with survival. All tumors and the liver, heart, lungs, spleen, kidneys, and adrenal glands were placed in 10 per cent formalin fixative, and histological sections were prepared and stained with hematoxylin-eosin.

In previous experiments (5), the maximum total dose of DMBA given was approximately 4.5 mg/100 gm of body weight, and this was given over a period of 13 consecutive weeks. A similar dose was used in more recent experiments (Group 1) but was given according to the following injection sequence: three injections spaced 1 day apart followed 3 weeks later by a similar set of injections. Each injection of the DMBA emulsion amounted to 0.5 ml/100 gm of body weight.

1 Mazóla; generously supplied by Corn Products Refining Company, Argo, Ill.
2 Phosphatides prepared as follows have proved as satisfactory as the fraction used in earlier studies (6): Four hundred gm. of grade RG lecithin generously supplied by the Glidden Company, Chicago, Ill., was dissolved in 1.6 liters of redistilled petroleum ether and filtered through an asbestos-type bacterial retaining filter. The filtrate was slowly poured into 4 liters of redistilled acetone with vigorous stirring, and the precipitate was collected by filtration. The precipitate was redissolved in petroleum ether and reprecipitated from acetone as before. The product was washed well with acetone, and the residual solvent was removed by means of a dry stream of nitrogen. The phosphatides were stored in a tightly capped bottle and kept under refrigeration.
3 The Demal-14 was supplied by the Emulsol Corporation, Chicago, Ill., and the Triton WR-1339 by the Rohm and Haas Company, Philadelphia, Pa.
4 Eastman Kodak Company, Rochester, N.Y.
5 Generously supplied by Dr. Hailman of the Upjohn Company, Kalamazoo, Mich.
6 Obtained from the Charles River Breeding Laboratories, North Wilmington, Mass.
7 Purina Laboratory Chow. Ralston Company, St. Louis, Mo.
Other groups of rats received similar injections of emulsion which furnished in addition to the DMBA a total of either 0.015 or 0.15 mg. of a-estradiol (Groups 2, 3) or diethylstilbestrol (Groups 4, 5)/100 gm of body weight. The injection sequence for animals which received the high amount of estradiol (Group 3) was as follows: two injections spaced 1 day apart, followed 2 months later by four injections spaced 1 day apart. The numbers of animals in Groups 1, 2, 3, 4, and 5 were 22, 22, 25, 23, and 22, respectively. The results of these experiments are given in Charts 1 and 3 and Table 1.

RESULTS AND DISCUSSION

Rats which received 1.32 mg. of 9,10-dimethyl-1,2-benzanthracene/100 gm body weight developed mammary tumors at a fairly slow rate, as seen from the data given in Chart 1. In 48 weeks only 75 per cent of the animals which received DMBA alone had tumors, in agreement with results published earlier (5). In the animals which received 0.015 mg of a-estradiol/100 gm body weight in addition to the 1.32 mg. of carcinogen (Group 2), tumors appeared earlier than in the control rats (Group 1), and the tumor incidence and average number of tumors per rat were higher. Unfortunately, the rats in Group 3 which received 0.15 mg of a-estradiol/100 gm body weight cannot be directly compared, because the injection sequence was unavoidably different; however, here too, the influence of the hormone was apparent. Rats which received diethylstilbestrol and 1.32 mg DMBA/100 gm body weight had an elevated incidence rate only when given 0.15 mg. of hormone (Group 5) but had a greater average number...
of tumors per rat with either the 0.015- or 0.15-mg. dose of hormone. An interesting difference between the effects of estradiol and diethylstilbestrol is that administration of the latter compound did not increase the total number of rats that developed tumors, whereas estradiol did. Others have reported experiments in which rats were given methylcholanthrene by gastric instillation (8), and estradiol was found to decrease the latent period for mammary tumor production, increase the incidence, and cause a more glandular type of tumor to develop.

As has been found in other studies (1–8), the dose of carcinogen was an important factor in the present investigations. This effect is evident by comparison of Groups 1 and 6. As shown in Charts 2A and 2B, 90 and 94 per cent of the animals in Groups 6 and 9 which received approximately 5.28 mg of DMBA/100 gm body weight developed one or more tumors within 19 weeks. The tumors were mainly mammary adenocarcinomas in contrast to the fairly large number of mammary adenofibromas, adenomas, and fibromas, and ear carcinomas found in the earlier studies (5).

As shown by the data presented in Charts 2A, 2B, and 3 (Groups 7 and 10), a-estradiol injected simultaneously with the higher dosage of carcinogen caused tumors to appear earlier, in 100 per cent of the animals, and approximately twice as many to develop as in the control DMBA group. At 10 weeks in Group 7, about 70 per cent of the animals each had an average of 2.5 tumors, and at 16 weeks all animals had tumors with an average of 4.8 tumors/rat (Chart 3). Almost without exception the tumors in this group were mammary adenocarcinomas (Table 1).

The diethylstilbestrol with high doses of DMBA likewise caused a more rapid production of tumors and a greater average number of tumors per rat, as compared to the controls, but the effect was not quite so great as that of a-estradiol. Neither estradiol nor diethylstilbestrol alone in an amount 5–7 times that used with DMBA has produced tumors even after 25 weeks (Groups 12 and 13). These compounds have been shown to cause tumors to develop in rats (4, 7), but it seems likely that much greater doses would have to be used intravenously to produce tumors in the absence of DMBA. In the present experiments the dose of hormone used in conjunction with the DMBA was chosen because that dose of hormone alone did not produce tumors, yet it did enhance tumor production with DMBA. Animals which received emulsion which contained neither DMBA nor hormones (Group 14) and those which were not injected at all (Group 15) developed no tumors.

### Table 1

<table>
<thead>
<tr>
<th>Duration of experiment (weeks)</th>
<th>No. of animals examined microscopically</th>
<th>Adenocarcinoma of breast</th>
<th>Adenoma of breast</th>
<th>Fibroadenoma of breast</th>
<th>Fibroma of breast</th>
<th>Fibrosarcoma, dermal</th>
<th>Keratinising squamous-cell carcinoma of sebaceous gland of external ear</th>
<th>Keratinising squamous-cell papilloma of sebaceous gland of external ear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>52</td>
<td>18 (8)</td>
<td>9 (4)</td>
<td>5 (2)</td>
<td>0</td>
<td>0</td>
<td>4 (4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>20 (11)</td>
<td>9 (4)</td>
<td>5 (2)</td>
<td>1</td>
<td>0</td>
<td>4 (4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>22 (12)</td>
<td>8 (3)</td>
<td>5 (2)</td>
<td>2</td>
<td>0</td>
<td>5 (5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>21 (12)</td>
<td>8 (3)</td>
<td>5 (2)</td>
<td>2</td>
<td>0</td>
<td>5 (5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>16 (9)</td>
<td>8 (3)</td>
<td>5 (2)</td>
<td>2</td>
<td>0</td>
<td>5 (5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>114 (64)</td>
<td>8 (3)</td>
<td>5 (2)</td>
<td>2</td>
<td>0</td>
<td>5 (5)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Larger figure indicates total number of tumors, figure in parentheses refers to number of animals with tumors.
Tumor production by means of the method employed in the present paper offers the following advantages in the study of mammary cancer: (a) Rapid and reproducible tumor production, (b) tumor incidences of 100 per cent, (c) range in number of tumors per rat, (d) homogeneity of the type of tumors produced, and (e) a means for the simultaneous administration of other lipid-soluble materials. The latter has the advantage that each particle of such a fat emulsion is in itself a separate nonaqueous solution which carries with it some of each of the dissolved materials and thus, if absorbed by tissue cells, may furnish all the lipid-soluble components of the emulsion at a given instant. This circumvents the reliance on chance that is ordinarily involved when various lipid-soluble materials are given singly or together by other means and fairly comparable rates of absorption are assumed for each. It should be pointed out, however, that, although the primary target in the present experiments proved to be the mammary gland, other tissues, especially the liver, initially take up large quantities of the emulsified lipids and may then act as sources of the carcinogen and hormone for later redistribution, provided that such tissues do not completely destroy these compounds. It is of interest that few tumors were found in tissues other than the mammary gland.

SUMMARY

Tumor production in female rats by means of intravenously administered 9,10-dimethyl-1,2-benzanthracene (DMBA) has been further studied from the standpoint of the dose of carcinogen given and the influence of simultaneously administered α-estradiol or diethylstilbestrol. Administration of approximately 5.2 mg of DMBA/100 gm body weight produced a tumor incidence of approximately 90 per cent in 19 weeks, in contrast to an incidence of 80 per cent in 47 weeks with a dose of 1.25 mg. The rats which received the higher dose of carcinogen had twice as many tumors per rat as the others.

The simultaneous administration of 0.6 mg of estradiol caused an earlier appearance of tumors, increased the tumor incidence to 100 per cent in about 16 weeks, increased the number of tumors per rat, and yielded mammary adenocarcinomas almost exclusively. Six-tenths milligrams of diethylstilbestrol was almost as effective as estradiol. Neither hormone alone in doses up to 4 mg/100 gm body weight produced any tumors.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Janice Holland and Thomas Faherty, who prepared all the histological material, and of Claudine Zighera, who aided with some of these experiments.

REFERENCES

Effect of Dose and Hormones on Tumor Production in Rats Given Emulsified 9,10-Dimethyl-1,2-benzanthracene Intravenously


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/13/7_Part_1/503

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.