Tumor Production in Rats Injected Intravenously with Oil Emulsions Containing 9,10-Dimethyl-1,2-Benzanthracene*

ROBERT P. GEYER, VIRGIL R. BLEISCH, JEAN E. BRYANT, ALICE N. ROBBINS, IRVING M. SASLAW, AND FREDRICK J. STARE

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

Emulsions of fat suitable for intravenous administration have been used in this laboratory as a source of calories, as an aid in the study of lipid metabolism, and as a vehicle for fat-soluble materials. The latter use has been of value in studies with rats on the effects of the intravenous injection of carcinogenic hydrocarbons. Several investigations have been reported in which emulsions or suspensions of carcinogenic compounds have been given intravenously to experimental animals. Andervont and Lorenz (1) reported that mice of the C3H strain developed pulmonary tumors following the intravenous injection of suspensions of 1,2,5,6-dibenzanthracene. Shimkin (6) reported that strain A mice developed pulmonary tumors after intravenous injection of 20-methylcholanthrene. No neoplasms were found by Garay and Berencsi (3) to develop in mice or rabbits after the intravenous administration of suspended 3,4-benzpyrene. The rapid excretion of intravenously administered radioactive dibenzanthracene has been reported by Heidelberger and Jones (5).

The present paper deals with the production of skin tumors in rats following the intravenous injection of emulsions of oil which contained dissolved 9,10-dimethyl-1,2-benzanthracene (DMBA).

EXPERIMENTAL

In preliminary studies the carcinogenic agent was dissolved in corn oil,1 and the mixture was emulsified by means of a high-speed blender at a 5 per cent concentration of fat and in a nitrogen atmosphere. One per cent each of a soybean phosphatide fraction (4) and a polyglycerol ester2 were used as emulsifiers and stabilizers, and 5 per cent dextrose was used for tonicity. The particles of fat were approximately 1 μ in diameter, and the emulsions were sterilized by autoclaving. The emulsions were injected twice weekly for 3 months into male 150-gm. rats of the Charles River strain (formerly Wistar). 1,2-Benzanthracene, 9,10-dimethyl-1,2-benzanthracene, 20-methylcholanthrene, 1,2,5,6-dibenzanthracene, p-dimethylaminoazobenzene, and m'-methyl-p-dimethylaminoazobenzene3 were tested in this manner. No tumors were obtained in any animals injected with any emulsion except dimethylbenzanthracene. Survival in all groups, including several in which no injections were given, was poor prior to the fifth month owing chiefly to respiratory disease. The neoplasms which developed in the animals injected with dimethylbenzanthracene were epidermoid carcinomas and papillomas, most of which appeared to arise from the sebaceous glands adjacent to one or both external ears. Infection and hemorrhage at the site of the tumors often caused death, usually after the tumor had reached the approximate size of 2×3 cm.

Several additional groups of rats were tested on the dimethylbenzanthracene emulsion with the same results as before. One of these groups received twice-weekly croton oil applications on several different locations on their backs, but, as with animals in other groups, survival was poor, and the survivors developed tumors only on the head region and not at the site of the croton oil applications.

At this point it became possible to prepare more concentrated fat emulsions by means of high pressure homogenization and to use different stabilizing materials. New experiments were set up using emulsions in which the only carcinogen studied was dimethylbenzanthracene. The emulsions were

* Supported in part by grants-in-aid from the National Cancer Institute of the National Institutes of Health, Public Health Service, the Nutrition Foundation, Inc., New York, N.Y., and The American Cancer Society.

1 A specially refined corn oil generously supplied by the Corn Products Refining Company.

2 The Demal-14 was generously supplied by the Emulsol Corporation, Chicago, Ill., and Triton-WR-1339 was generously supplied by the Rohm & Haas Company.

3 The authors wish to express their appreciation to Dr. J. A. Miller of McArdle Memorial Laboratory, University of Wisconsin, who made available to them a generous supply of m'-methyl-p-dimethylaminoazobenzene.
prepared as follows: 42.9 mg. of 9,10-dimethyl-1,2-
benzanthracene (Eastman) was dissolved in 15
gm. of warmed corn oil, and 1.0 gm. of a poly-
glycerol ester of oleic acid (Demal-14) or 0.25 gm.
of an aryl-alkyl ether polymer (Triton-WR-1339)
was added. This mixture was combined with 82
ml. of 5 per cent dextrose solution which contained
1.0 gm. of cerebrosides, and the entire mixture
was homogenized in a high-pressure homogenizer
for 60 minutes at a pressure of 3,000 pounds per
square inch and a temperature of 88° C. A nitro-
either the Demal- or Triton-stabilized emulsion.
All injections were given through the veins of the
tail while the animal was lightly anesthetized with
ether. Several groups of animals received a compar-
able number of injections of an emulsion contain-
ing Demal or Triton but no DMBA. Several
other groups of animals served as un.injected con-
trols. All the animals were maintained on stock
ration and housed in group cages. In Table 1 are
presented the data pertinent to the experiment
and the results in terms of the number of animals

| TABLE 1 |

| INCIDENCE OF TUMORS IN RATS FOLLOWING THE INTRAVENOUS INJECTION OF EMULSIONS CONTAINING 9,10-DIMETHYL-1,2-BENZANTHRACENE (DMBA) |

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Emulsion no.*</th>
<th>No. of weekly injections</th>
<th>Total DMBA (mg.)</th>
<th>No. of rats at 19 wks.</th>
<th>Animals with tumor(s) at 19 wks.</th>
<th>Total tumors at 35 wks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>38I</td>
<td>B</td>
<td>13</td>
<td>6.4</td>
<td>30</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>38II</td>
<td>A</td>
<td>12</td>
<td>5.5 (4.8–6.2)</td>
<td>30</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>39I</td>
<td>A</td>
<td>12</td>
<td>5.5 (3.6–6.2)</td>
<td>30</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>39II</td>
<td>A</td>
<td>10</td>
<td>4.1 (3.7–4.8)</td>
<td>22</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>40A‡</td>
<td>A</td>
<td>13</td>
<td>3.7 (3.6–3.9)</td>
<td>20</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>40B‡</td>
<td>A</td>
<td>13</td>
<td>3.3 (3.0–3.5)</td>
<td>20</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td>152</td>
<td>94</td>
<td>36</td>
</tr>
</tbody>
</table>

* Emulsion No. A contained 15 per cent corn oil, 1 per cent cerebrosides, 1 per cent Demal-14, 0.0450 per cent 9,10-dimethyl-1,2-benz-
thane, and 4.8 per cent dextrose.

† Emulsion No. B was similar to No. A, except that the 1 per cent Demal-14 was replaced with 0.85 per cent Triton.

‡ Weanling rats.

Female rats of the Sprague-Dawley strain weighing about 150 gm. were used in all the follow-
ing experiments except one in which weanling rats of the same strain and sex were used. The rats were
given approximately twelve weekly injections of

A purified cerebroside preparation kindly supplied by Dr. R. J. Vander Wal of the Armour Company, Chicago, Ill.

Model Junior 50 Viscolizer, Cherry-Burrell Company, Chicago, Ill.

Obtained from the Charles River Breeding Laboratories, Boston, Mass.

which developed tumors and the total number of tumors observed.

For histological purposes, some biopsies were taken of various tumors, and at necropsy sections of all tumors and of many normal-appearing tissues were obtained. The tissue was fixed in Zenker's fluid or 4 per cent formaldehyde solution, and the mounted sections were stained with hematoxylin and eosin, Masson's trichrome, phosphotungstic acid hematoxylin, or Sudan IV. A listing of location and kinds of tumors produced is given in Table 2. Figures 1, 2, and 3 are photo-
micographs of some of these tumors.

RESULTS AND DISCUSSION

A fairly high incidence of skin tumors in rats resulted when 9,10-dimethyl-1,2-benzanthracene was administered intravenously in the form of emulsions. As shown by the data in Table 1, approximately 88 per cent of all the animals injected with these emulsions developed one or more tumors adjacent to the ears or on the ventral trunk.

† Gaines Krunchon, Gaines Division, General Foods, Kankakee, Ill.
When based on the number of surviving animals at the time the first tumor was recognized, the percentage of rats with tumors was approximately 55 per cent. Death in most cases was caused by a respiratory infection which also was responsible for the fatalities in the control groups that received no injections of emulsion. The majority of fatalities occurred prior to the fifteenth week. Within the range of total amount of carcinogen given in these experiments, little can be said regarding correlation between dose and response. In several additional experiments, weanling female rats that received a total of 1 mg. of dimethylbenzanthracene in six injections had a tumor incidence of 10 per cent in 32 weeks. No change in kind or incidence of tumors resulted from substituting the polyglycerol ester (Demal-14) for the aryl-alkyl ether (Triton WR-1559) as part of the stabilizing system. In preliminary experiments in which the remainder of the stabilizing system was phosphatide instead of cerebroside, a low incidence of tumors was obtained with this carcinogenic agent, and most of these arose in the region of the ears. Other normally potent agents such as methylcholanthrene were without effect when contained in the phosphatide-Demal stabilized emulsions. This difference between the phosphatide- and cerebroside-stabilized emulsions may be due (a) to an influence of these substances on tumor formation, (b) to an effect of these materials on the carcinogenic agents prior to injection (e.g., the phosphatides might participate in a coupled oxidation with the carcinogen), (c) to an influence which these substances might have on the extent and speed of emulsion removal by the skin or sebaceous glands, or (d) to a difference in the strain and sex of rats used in the two different sets of experiments. Studies are now in progress to elucidate which, if any, of these possibilities is responsible. The carcinogens which were tested with the phosphatide-stabilized emulsions are now being retested in cerebroside-stabilized emulsions.

The tumors in the preliminary groups in which phosphatide was present in the emulsion represented keratinizing squamous-cell tumors of sebaceous gland type, arising adjacent to the external auditory canal. These tumors were similar to those induced by oral 2-acetylaminofluorene (8) and by oral administration of benzidine or by subcutaneous injection of benzidine in unemulsified olive oil (8). Twelve per cent of 88 animals injected with dimethylbenzanthracene in phosphatide-stabilized emulsions developed these tumors, one of which was bilateral. Forming beneath the skin just anterior and inferior to the external ear, the tumors grew to large sizes, extending over the necks of the animals internal to the mandible, and along the base of the skull. When small, the tumors were coarsely lobulated and semi-firm. All which were of moderate or large size showed puriform liquefaction, and most of them contained masses of gray-white cheesy material. Histologically, the tumors were cystic, papillary lesions with bands of keratinized squamous epithelium, chublike rete pegs, and masses of sebaceous cells. There were groups of atypical prickle and basal cells with nuclear and cytoplasmic variation, many mitoses, and stromal invasion. The cystic spaces were filled with lipid-rich sebum or keratin. These tumors were invasive and histologically malignant, and two tumors had metastasized to the lung.

Ten per cent of 177 rats which received dimethylbenzanthracene in cerebroside-stabilized emulsions also developed keratinizing squamous-cell tumors of sebaceous gland type (Fig. 1). Thirty-five per cent of these were bilateral. This can be compared with the 9 per cent incidence of bilaterality in the preliminary experiments and the 10.6 per cent incidence in the report of Spitz et al. (8). In none of these animals were metastases demonstrated. Thirty-two per cent of these rats also developed numerous tumors over the course of the "mammary line" on the ventral surface of the trunk. Six per cent of animals developed both types of tumors, and 57 per cent of animals with "ear" tumors also presented mammary tumors. Some of the mammary tumors were bilateral, and a number were multiple. The tumors arose in the corium and spread in the subcutaneous tissue, growing to a size of more than 6 cm. and to a weight of more than 50 gm. Most of those which

<table>
<thead>
<tr>
<th>Tissue of origin</th>
<th>Histological characterization of tumor</th>
<th>No. of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland, left side</td>
<td>Adenocarcinoma</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Adenosarcoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adenoma</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Fibroadenoma</td>
<td>1</td>
</tr>
<tr>
<td>Mammary gland, right side</td>
<td>Adenocarcinoma</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Adenosarcoma</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Sebaceous gland, left ear duct</td>
<td>Adenoma</td>
<td>1</td>
</tr>
<tr>
<td>Sebaceous gland, right ear duct</td>
<td>Epidermoid carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Sebaceous gland, right ear duct</td>
<td>Epidermoid carcinoma</td>
<td>6</td>
</tr>
<tr>
<td>Subcutaneous tissue, left groin</td>
<td>Fibroma</td>
<td>1</td>
</tr>
<tr>
<td>Liver, spleen, nodes</td>
<td>Monocytic leukemic infiltrate</td>
<td>2</td>
</tr>
</tbody>
</table>
were more than 1 cm. in diameter had invaded underlying muscle, and many were ulcerated. No distant or lymph node metastases were found. It is probable that some of the skin tumors were metastatic satellites, e.g., the flank tumor which was on the same horizontal level with the tumor over the left lower abdomen in rat 38II-3, described below.

Some tumors produced by cerebroside-stabilized emulsions grew into massive neoplasms. One animal (38II-3) developed six large tumors: (a) 3X2X1.5 cm. in the right clavicular region, (b) 5X3X2 cm. over the right chest wall, (c) 7X4X2 cm. over the right inferior costal border and abdomen, (d) 9X6X4 cm. over the entire left chest and upper abdomen, (e) 2.5X1.5X1.0 cm. over the left flank, and (f) 1X0.8X0.6 cm. over the left abdomen. This represented a total of 148 gm. of tumor in a 368-gm. rat.

Although there was relatively great variation in histologic picture, two major types of tumor were produced by the emulsions containing cerebroside (Table 8). One was of epidermoid and sebaceous gland type, the other of apparent mammary gland derivation. It should be noted that both these types represented tumors of skin and dermal adnexae. The variations represented transition from one type of breast tumor to another, differences in apparent malignancy, and variations in stromal activity. Two animals with leukemic infiltrates in liver, spleen, and lymph nodes were also identified. On microscopic examination atypical acini and small cysts, sheets, and clumps of cells were found in a generally active, fibroblastic stroma. Many of the acini were thyroid-like in size and contained colloid-like material which did not include lipid. There were many mitoses, and there was invasion of stroma and adjacent muscle. Some of the tumors exhibited apparent transition from fibroadenoma to adenoma to adenocarcinoma (Fig. 4). In some there were squamous-lined ducts and sheets of prickle cells.

The animals in the preliminary experiments died within a few weeks of the development of tumors. Necrosis and fatty change of spleen and liver, pneumonia, and other infections were found at necropsy. Rats that received a cerebroside-stabilized emulsion, however, lived for months after development of tumors of both types, and some lived for weeks after ulceration of these tumors. Some were finally killed. A few appeared to die of "cancer cachexia." Some died of respiratory or generalized infection.

The cutaneous localization of these tumors, induced by a parenteral agent in a stable emulsion is striking. Simpson and Cramer (7) demonstrated that, after topical administration, 20-methylcholanthrene is concentrated in sebaceous glands. Suntzeff et al. (9) showed that the development of epidermal carcinomas depends upon the presence of dermal adnexae. It remains to be determined whether the activity of intravenously administered dimethylbenzanthracene is concentrated in sebaceous glands, whether the carcinogen is modified or activated in sebaceous glands, or whether there is a special susceptibility of dermal organs to the carcinogenic effect of these fat-soluble agents administered in stable emulsion.

It is obvious that the studies reported here are incomplete. Such fundamental aspects as the responses of tumor-susceptible strains of animals versus nonsusceptible strains remain to be studied. However, the experiments reported in this paper demonstrate clearly the practicability of using properly prepared fat emulsions as vehicles for the intravenous administration of fat-soluble carcinogenic compounds. The results of these experiments demonstrate the desirability of the use of such emulsions in the study of tumor production; and, conceivably, fat emulsions also offer a practical route of administration of compounds of possible chemotherapeutic value in the field of tumors.

SUMMARY

The intravenous administration of fat emulsions which contained 9,10-dimethyl-1,2-benzanthracene caused a high incidence of skin tumors in female rats of the Sprague-Dawley strain. Fifty-five per cent of the surviving animals developed keratinizing squamous-cell tumors of the sebaceous gland type and/or adenocarcinomas which were usually located over the course of the "mammary line" on the ventral surface of the trunk. Only two animals developed neoplasms which were not of skin or dermal adnexae derivative.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. G. W. Goddard for assistance with the pathological studies, to T. Faherty, J. Pendergast, and G. S. Yee for technical assistance.

REFERENCES

5. Heidelberger, C., and Jones, H. B. Distribution of Radio-

Downloaded from cancerres.aacrjournals.org on May 30, 2017. © 1951 American Association for Cancer Research.
activity in Mouse Following Administration of Dibenzanthracene Labeled in the 9- and 10-Positions with C\textsuperscript{14}, *Cancer*, 1:289–90, 1948.


---

**Figure 1.**—Epidermoid carcinoma arising in the sebaceous gland adjacent to the external ear. Rat 3911, No. 15; dimethylbenzanthracene, cerebroside stabilizer, and Triton.

**Figure 2.**—Secretory type adenocarcinoma, invasive, low grade of malignancy, of right pectoral region. Rat 381, No. 22; dimethylbenzanthracene with cerebroside stabilizer and Triton.

**Figure 3.**—Adenocarcinoma of right breast. Rat 381, No. 12; dimethylbenzanthracene, cerebroside stabilizer, and Triton. Note invasion of skeletal muscle.

**Figure 4.**—Adenoma of secretory type arising adjacent to atypical adenomatous hyperplasia of breast. Rat 3811, No. 3; dimethylbenzanthracene, cerebroside stabilizer, and Triton.
Tumor Production in Rats Injected Intravenously with Oil Emulsions Containing 9,10-Dimethyl-1,2-Benzantracene
