Studies of Skin Carcinogenesis in the Syrian Golden Hamster*

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SUMMARY

The carcinogens 9,10-dimethyl-1,2-benzanthracene, 20-methylcholanthrene, 3,4-benzpyrene, 1,2,5,6-dibenzanthracene, 1,2-benzanthracene, and 2-anthramine have been tested topically in the Syrian golden hamster.

The mouse skin tumor-promoting agents, croton oil and polyoxyethylene sorbitan monostearate (Tween 60), have been tested on hamster skin following a single application of 9,10-dimethyl-1,2-benzanthracene.

Melanotic tumors, similar to those previously described, were induced with 9,10-dimethyl-1,2-benzanthracene. Among the other carcinogens tested, only 20-methylcholanthrene gave rise to a single melanotic tumor.

The results are discussed in terms of chemical specificity and dose-response for the induction and progression of the melanotic tumors, in contrast to other skin tumors.

As previously reported (3), melanotic tumors are induced in the Syrian golden hamster by a single, topical application of 9,10-dimethyl-1,2-benzanthracene (DMBA) in mineral oil. The histology of these tumors has been described as similar to the cellular blue nevus of man (3) and their transplantability demonstrated (16). Since that time the occurrence of these tumors in this species, with the same carcinogen, has been reported by Horning (9) and by Ghadially (7). On the other hand, when skin carcinogenesis was studied in the hamster following repeated applications of DMBA (5, 10) or of 3,4-benzpyrene (12), the development of squamous-cell papillomas and carcinomas was reported, but not the induction of melanotic tumors.

The present investigation records the effect of the application of 3,4-benzpyrene (BP), 20-methylcholanthrene (MC), 1,2,5,6-dibenzanthracene (DBA), 1,2-benzanthracene (BA), and 2-anthramine (2-A) to the skin of the golden hamster; DMBA has been reinvestigated at other dose levels and in another solvent. Previously (3), croton oil was reported to be without carcinogenic or promoting action in the hamster. In the present study the action of croton oil has been retested under different conditions. In similar experiments polyoxyethylene sorbitan monostearate (Tween 60) has been used. Tween 60 has a strong promoting action and a weak carcinogenic action in the skin of the mouse (13, 14, 16).

MATERIALS AND METHODS

In all the experiments Syrian golden hamsters, obtained at 10–12 weeks of age from Abrams Small Stock Breeders, Chicago, were used. They were housed in plastic cages in groups of five, according to sex, and given Rockland mouse diet in pellets and tap water ad libitum. The five polycyclic hydrocarbons used were purified by column chromatography. The 2-anthramine (m.p. 239°–240°C., corr.) was kindly synthesized in this laboratory by Dr. W. Lijinsky. The Tween 60 was kindly supplied by Atlas Powder Co., Wilmington, Delaware. The croton oil, B.P., was obtained from Boots Limited, England. Mineral oil U.S.P. (Superla 34, Standard Oil of Indiana) and acetone of reagent grade, distilled before use, were used as solvents. The solutions were applied with a standard glass dropper, each drop measuring approximately 20 μl, or with a calibrated micropipette or tuberculin syringe, according to the experiment. The treated area, varying in size according to the experiment, was shaved free of hair with an electric clipper before the beginning of the experiment and reshaven periodically during the experiment.

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The animals were checked at weekly intervals, and all lesions and tumors of the skin were measured and charted on graph paper. Those pigmented lesions having a nodular appearance, raised above the skin surface, and reaching 2 mm. in their greatest diameter, were classified as melanotic tumors. Other types of skin tumors were also charted. The animals were observed until spontaneous death, were killed when moribund, or were sacrificed at the end of the experiment. All animals were autopsied except for a few lost through cannibalism. Histological study was done on the skin and on all organs showing gross pathological changes.

TABLE 1
RESULTS OF APPLICATION OF 9,10-DIMETHYL-1,2-BENZANTHRACENE TO THE SKIN OF THE SYRIAN GOLDEN HAMSTER

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. ANIMALS</th>
<th>SURVIVORS AT WEEKS*</th>
<th>ANIMALS WITH MELANOTIC TUMORS</th>
<th>CUMULATIVE TOTAL NUMBER AND SIZE OF MELANOTIC TUMORS</th>
<th>AVG. TIME OF APPEARANCE OF MELANOTIC TUMORS (WEEKS)†</th>
<th>OTHER SKIN TUMORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMBA, 1 per cent in mineral oil, 4 drops once</td>
<td>15♀</td>
<td>14 8 6 5</td>
<td>7</td>
<td>2-4 mm. 5-9 mm. 10 mm.</td>
<td>27.7 (18-43)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15♂</td>
<td>14 13 12 12 8 5</td>
<td>17</td>
<td>32 23 5 4</td>
<td>28.3 (18-75)</td>
<td></td>
</tr>
<tr>
<td>DMBA, 1 per cent in mineral oil, 4 drops, 3 times</td>
<td>15♀</td>
<td>14 13 7 2</td>
<td>14</td>
<td>50 39 8 3</td>
<td>25.4 (11-41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15♂</td>
<td>15 15 15 11 6 1</td>
<td>11</td>
<td>27 19 5 3</td>
<td>33.8 (18-74)</td>
<td></td>
</tr>
</tbody>
</table>

* Weeks from time of 1st treatment.
† Range given in parentheses.
‡ Two papillomas, one squamous-cell carcinoma, one sebaceous adenoma.
§ Four papillomas, three squamous-cell carcinomas, two sebaceous adenomas.
¶ Thirteen papillomas, three squamous-cell carcinomas, seven sebaceous adenomas, and one adnexal carcinoma.

RESULTS
9,10-dimethyl-1,2-benzanthracene in mineral oil.
—Two groups of fifteen female and fifteen male hamsters each were given 4 drops of a 1 per cent solution of DMBA in mineral oil either once only or 3 times at weekly intervals. The drops were applied with a glass dropper, evenly spaced down the midline of the entirely shaved back. In both groups, during the first few weeks following the treatment, slight desquamation and redness of the treated area were observed. Within the 10th week from the beginning of the experiment, numerous minute black spots appeared scattered throughout the treated area. Around the 15th week of the experiment some of these black spots acquired a nodular form, became raised on the skin surface, and were recorded as melanotic tumors if and when they had reached a diameter of 2 mm. As shown in Table 1, most of these melanotic tumors remained essentially unmodified throughout the experiment, increasing in size very slowly up to 4 mm. in the greatest diameter. Some of them, 21 out of a total of 121, progressed further up to 9 mm. in the greatest diameter; they still maintained an oval or rounded shape, with the shortest diameter varying from 2 to 5 mm. Ten out of the 121 melanotic tumors grew larger, exceeding 10 mm. in the greatest diameter and assuming an irregular shape. In a male hamster given a single application of DMBA and killed at the 65th week of the experiment, one of the melanotic tumors reached a size of 40 X 35 X 20 mm. One female, given three applications of DMBA, had a melanotic tumor which appeared at the 22d week, grew...
tions had approximately the same number of melanotic tumors; the females of the first group had fewer tumors because of their poor survival; the females given three applications of DMBA developed a significantly higher number of tumors than did the females receiving one application or the males of the corresponding group, in spite of a lower survival rate. It is noteworthy that, as in many other experiments performed in this laboratory, the life span of the female hamsters was considerably shorter than that of the males.

The average latency, calculated for all melanotic tumors at the time at which they reached 2 mm. in diameter, varied from 25 to 33 weeks. However, tumors appeared as soon as at the 11th week and as late as at the 74th week; thus, the standard deviation was too high to permit any significant conclusions on the average latent period.

In addition to the melanotic tumors, squamous-cell papillomas and carcinomas and adnexal tumors of the skin were observed (Table 1). They appeared at a late stage of the experiment, explaining the larger number in the male hamsters, which lived for a longer period. There was a clear dose-response effect for these tumors, particularly notable in the male groups, which developed 24 epidermal tumors when given three applications of DMBA and only four when given one application. The only internal tumor seen in the group given one application was a cholangiocarcinoma in a female; in the groups given three applications, there were an adenocarcinoma of the rectum in a female and two generalized malignant lymphomas of histiocytic type among the males.

3,4-Benzpyrene in acetone.—A group of five female and five male hamsters was given a single application of 50 μg. of DMBA in 50 μl. of acetone. The solution was applied with a micropipette to a circular area of 3.46 sq. cm., delineated by means of a wire ring between the dorsal scent glands. Fifteen weeks later, the treated area of all animals had many minute black spots; some of them progressed to a nodular form, but only two in the females and two in the males became raised on the surface of the skin and were recorded as melanotic tumors when they reached a diameter of 2 mm. One, in a female, was 6 × 5 × 2 mm. in size at the death of the animal at the 85th week. Two females and three males were alive at the 50th week, the last female dying at the 71st week and the last two males being killed at the 85th week. No other types of tumors of the skin or internal organs were seen.

3,4-Benzpyrene in mineral oil.—Fifteen female and fifteen male hamsters received 4 drops of a 0.8 per cent solution of BP in mineral oil to the midline of the entirely shaved back. The treatment was applied once weekly for 3 weeks and then for an additional 5 weeks after an interval of 30 weeks. At the 33rd week, prior to the second course of treatment, fourteen females and twelve males survived; these animals did not have any grossly visible melanotic lesions or other skin tumors. Microscopical accumulations of pigmented cells were seen occasionally in the dermis of the animals dying before the 33rd week. At the 50th week, six females and nine males were surviving and had a few minute black spots in the treated area. The last female died at the 68th week and the last male at the 99th week. One male hamster dying at the 89th week had a small nodular melanotic lesion which measured 1 mm. in diameter; histological study showed that this lesion was identical to the smallest melanotic tumors seen in the DMBA-treated groups. Additionally, in the male group there were two malignant lymphomas of histiocytic type, one involving principally the skin and the superficial lymph nodes, the second localized to the mesenteric lymph nodes and intestine. No tumors were seen in the female groups.

9,10-Dimethyl-1,2-benzanthracene in mineral oil.—Fifteen female and fifteen male hamsters received 6 drops of a 0.01 per cent solution of BP in acetone for 40 weeks. The 6 drops were applied with a glass dropper, evenly spread over a 6 × 3-cm. area located in the lumbar region, circumscripting the dorsal scent glands. All the animals were alive at the 50th week, and three females and four males at the 70th week. In the treated area there were few minute black spots, but no melanotic tumors or other skin tumors were observed. One female, killed at the 70th week, had a hepatoma and a thyroid adenoma.

20-Methylcholanthrene in mineral oil.—Fifteen female and fifteen male hamsters received applications of 0.8 per cent solution of MC in mineral oil under conditions identical to those for the corresponding BP experiment, i.e., for 3 and 5 weeks, with an interval of 30 weeks. At the 33rd week from the beginning of the experiment, twelve females and all the fifteen males were alive and had multiple minute black spots scattered over the entire back. At the 50th week, nine females and twelve males were surviving; the last female died at the 67th week; five males were still alive at the 85th week, the last dying at the 101st week from the beginning of the experiment. No melanotic tumors were observed, although in the older animals focal collections of melanocytes were seen in the dermis. In the female group the only tumor was a rhabdomyosarcoma of a foreleg. In the male group there were four papillomas of the keratoacanthotic
type and one sebaceous adenoma, all of which arose in the treated skin. In addition, the males had one cortical adenoma of the adrenal and two malignant lymphomas of the histiocytic type; one of these lymphomas was observed in an animal dying at the 47th week and apparently had one single localization in the skin and no involvement of other organs; the second lymphoma found in an animal killed at the 87th week had extensively involved the skin and the superficial lymph nodes, while spleen and liver showed no involvement.

20-Methylcholanthrene in acetone.—As in the BP experiments, a second group of five females and five males were treated with MC in acetone, 6 drops of a 0.01 per cent solution twice weekly for 40 weeks, to a 6 × 3-cm. area in the lumbar region. At the 50th week, all the ten hamsters were surviving; at the 70th week, one female and four males were still alive; the female had a small melanotic tumor which had reached a 2-mm. size at the 33d week, remaining practically unchanged throughout the experiment. All the other animals had multiple black spots or minute melanotic nodules first appearing at the 20th week, but never reaching a diameter of 2 mm. The continuous treatment produced some desquamation of the skin; at the 19th week one female had a small papilloma; at the 40th week, at the end of the treatment, all animals had marked desquamation and one or two sessile papillomas or epidermal cysts. In the following weeks more epidermal tumors appeared; at the 70th week, a total of seventeen epidermal tumors were observed in the females and 50 in the males.

1,2,5,6-Dibenzanthracene.—Five female and five male hamsters were given twice-weekly applications of a 0.9 per cent solution of DBA in mineral oil. Each application consisted of 8 drops which were evenly spread over the entire back. The treatment lasted 10 weeks, up to a total of twenty applications. At the 30th week, four females and four males were surviving, and at the 50th week two females and three males; at the 75th week one female and one male were still alive and were sacrificed. In none of the ten animals were melanotic tumors or other skin and internal tumors observed. At the 50th week of the experiment, the surviving animals had a few minute black spots which did not show any appreciable modification up to the end of the experiment. At histological examination they showed the usual pattern of focal intradermal accumulation of melanocytes.

1,2-Benzanthracene.—A group of five females and five males were given BA in a manner identical to that for the DBA, except that the concentration was 0.5 per cent in mineral oil. One female died during the treatment and one male at the 14th week; no other deaths occurred up to the 40th week; at the 50th week three females and three males were alive; the last female was killed at the 61st week and the last male at the 85th week. As in the DBA group, no tumors were observed; several minute black spots were seen in the animals surviving after the 40th week of the experiment.

2-Anthramine.—Ten female and ten male hamsters were given twice-weekly applications of a 1 per cent solution of 2-A in acetone to a 6 × 3-cm. area in the lumbar region. The solution was freshly prepared immediately before treatment. Each treatment consisted of 4 drops for the first 10 weeks; it was then increased to 8 drops for the following 40 weeks; ultimately it was reduced again to 4 drops for a final period of 5 weeks. The animals suffered little general toxic effect from the treatment; the weight of the animals was checked at monthly intervals and did not show any remarkable variation. One male died at the 27th week, a second at the 28th week, and a third was killed at the 47th week; three females died between the 42d and the 47th week; thus, at the 50th week from the beginning of the experiment, seven females and seven males were still alive. At the end of the 60th week, all females were dead, and five males were surviving. The last male was killed at the 80th week.

Starting from the 15th week, the treated skin of both males and females showed epilation, slight desquamation, and a diffuse increase of pigmentation with numerous minute black spots. Eventually a few of these spots slowly progressed to small melanotic lesions which, however, never reached the size of 2 mm. in their largest diameter. Histologically, these nodules were identical to the larger tumors seen in the DMBA experiments. After the 40th week of continuous treatment, the skin showed increased desquamation, some superficial erosions, and focal areas of epidermal thickening. At the end of the experiment, the females had developed a total of nine papillomas; the males had 23 epidermal tumors and two skin fibrosarcomas. In addition, one sarcoma of the retroperitoneal soft tissue and one hemangioma of the spleen were seen in the female group.

Croton oil and Tween 60.—Four groups of ten hamsters, five females and five males each, were given twice-weekly treatment of 6 drops of croton oil as 0.5 per cent solution in acetone or Tween 60, undiluted. Two groups received such treatment for 38 weeks following a single application of 300 µg. of DMBA as 0.1 per cent solution in acetone, given 3 weeks before. Two other groups received the croton oil or Tween 60 applications for 41 weeks with-
out the initial DMBA treatment. A fifth group of ten animals was given a single application of 300 \( \mu g \) of DMBA and no further treatment. In the groups given croton oil or Tween 60 after a single application of DMBA the animals died earlier, and the survivors were killed at the 36th week; in the other groups the observation of the survivors was prolonged up to the 70th week. In the groups treated with croton oil or Tween 60 alone, no melanotic tumors or any other types of tumor developed; in the groups given DMBA followed by croton oil or Tween 60, melanotic tumors developed, with no significant quantitative or qualitative variations as compared with hamsters treated with DMBA alone. Other tumors occurring were one squamous-cell carcinoma of the skin in a female hamster treated with DMBA alone, one hemangioma of the spleen in a male which received croton oil following the single application of DMBA, and two papillomas of the forestomach in a male given DMBA alone.

**DISCUSSION**

The effects of a series of carcinogenic chemicals applied to the skin of the hamster have been reported. Only one of them, DMBA, consistently gave rise to melanotic tumors and, in addition, to numerous minute melanotic lesions. The diagnosis of neoplasia in these experiments is, of necessity, an arbitrary matter; we have set ourselves a standard, calling grossly nodular lesions of 2 mm. or more in diameter “melanotic tumors,” and using the term “melanotic lesion” to describe the smaller nodules or the flat melanotic spots. Many melanotic tumors, one with metastases, were observed in the DMBA-treated animals; among the other carcinogens studied, only MC was associated with a single, small melanotic tumor. Multiple melanotic lesions were observed in the MC-, 2-A-, and, to a lesser extent, BP-treated hamsters. It seems clear that some chemical specificity has determined this result, and it is of interest to note that the only melanotic tumors previously reported in studies with pure chemicals followed treatment with DMBA (1) or the closely related 5,9,10-trimethyl-1,2-benzanthracene (2).

A single application of DMBA was an adequate dose; 800 \( \mu g \) in mineral oil, applied to the entire back, or 50 \( \mu g \) in acetone, applied to a smaller area, gave rise to melanotic tumors. An increase in the dosage to three applications produced somewhat more melanotic tumors and additionally an appreciable number of squamous-cell and adnexal tumors. In our original study (3) it was found that, whereas a single application of the carcinogen gave rise to melanotic tumors, repeated applications given weekly produced only the squamous-cell tumors. It is clear that a definite relationship exists between dosage and response, since the melanotic tumors require extremely small quantities of carcinogen to ensure their emergence, whereas the squamous-cell tumors require relatively large doses and more prolonged stimulation.

Of the other carcinogens tested, only MC and 2-A gave rise to squamous-cell and adnexal tumors. Surprisingly, with BP neither epidermal nor melanotic tumors were observed.

The series of events leading to the development of melanotic tumors is reminiscent of the many instances of progression reported in the literature (5, 6, 11, 15). In this case the early changes, marked as they are with an increase in pigment, are particularly outstanding. They consist of small accumulations of melanocytes; in all probability, this phenomenon is analogous to the epidermal hyperplasia preceding papilloma formation. It is probable that the same significance and can occur with and without subsequent tumor formation. It does, however, become a matter of great difficulty to know when a pigmented spot has been transformed from a hyperplastic reaction into a neoplasm. It would seem clear that criteria other than morphological are needed to clarify this matter.

In common with other workers (4, 8) we have observed malignant melanomas in hamsters without any relationship to treatment. It seems strange that we have not observed such tumors following treatment with these carcinogens but rather a melanotic tumor of cellular blue nevus type that is distinctly different both histologically and biologically. One would not be surprised at the emergence of large numbers of these tumors following the application of a small dose of the carcinogen were this a common tumor in the untreated animal of this species. It is our impression that these melanotic tumors are distinctly uncommon in untreated animals, and the current investigation supports this conclusion in that they did not occur in the majority of carcinogen-treated groups.

In the instance of the studies with Tween 60 and croton oil following a single application of DMBA, no observable effect that could not be attributed to the carcinogen alone was noted. One may conclude from this that neither of these mouse promoting agents have any such effect in the hamster when squamous-cell tumors were considered. It is, of course, possible that, had a considerably lower dose of the DMBA been employed, it might have been possible to demonstrate a promoting effect on melanotic tumors. This would
appear to be a particularly difficult experiment to conduct, since a dose of DMBA as low as 50 µg. was an effective carcinogen for these tumors.

REFERENCES
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