A Study of Skin Carcinogenesis in the Mouse with Single Applications of 9,10-Dimethyl-1,2-benzanthracene at Different Dosages*

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SUMMARY

Groups of female Swiss mice were treated with single applications of either 20, 50, 100, 200, 250, 500, or 1000 µg of 9,10-dimethyl-1,2-benzanthracene in acetone on the skin of the interscapular area. Dose of 20 and 50 µg did not give rise to epidermal carcinogenesis; a small number of benign papillomas was induced with a dose of 100 µg. Overt epidermal carcinogenesis, involving the induction of both benign and malignant tumors in a high proportion of animals, was detected at the 200-µg dose level; above this dose no additional effect was noted. Many of the tumors induced eventually regressed, although a large number of those remaining became malignant. The results are discussed.

Several investigators have reported that large, single doses of polycyclic hydrocarbon carcinogens will induce tumors in the skin of mice (1, 6-8, 12, 14, 15, 18). No data have been provided in these studies that determine the dose level at which such compounds become effective, although it is well known that small doses alone are without apparent effect. At these lower dosage levels, however, even though no tumors may occur without further treatment, subsequent application of promoting agents such as croton oil (5, 16) or Tween 60 (20) will result in the occurrence of many tumors.

In the present study, an effort has been made to determine the dosage level at which a single application of a polycyclic hydrocarbon, 9,10-dimethyl-1,2-benzanthracene becomes fully effective as carcinogen to the skin of the mouse. The life history of all tumors induced has been carefully recorded.

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MATERIALS AND METHODS

Female Swiss mice, originally obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and bred randomly in this laboratory since 1951, were used. The animals were housed in plastic cages, on wood shavings, were fed Rockland mouse diet in pellets, and received water ad libitum. The mice were 8-9 weeks old and in the resting phase of the hair cycle. The skin of the interscapular area was clipped free of hair with an electric clipper several days prior to the application. The carcinogen used was 9,12-dimethyl-1,2-benzanthracene (DMBA) from Eastman Organic Chemicals, purified by chromatography on fluorisil. It was dissolved in reagent grade, redistilled acetone. Solutions of 0.04, 0.1, 0.2, 0.4, 0.5, or 1 per cent were prepared a few minutes before the applications. They were delivered evenly to an area of shaved skin of 1.5 X 1.5 cm. in the interscapular region, the upper edge being approximately 8 mm. caudal to a line connecting the base of the ears. All the solutions were applied with a 50-µl calibrated micropipette; in addition, the 1 per cent solution was also delivered with a 100-µl micropipette. Thus, the different groups of animals received, respectively, 20, 50, 100, 200, 250, 500, and 1000 µg of DMBA as a single application. One group received two
doses of 100 µg. 1 week apart. The animals were checked 2 or 3 times weekly for the first weeks after the application, and then once a week. All the lesions were charted on graph paper, and only those sharply defined, raised, and larger than 2 mm. were considered as tumors. In the present tabulation all the tumors present for over 1 week were considered. The weight of the mice was recorded every 2–3 weeks on a cage basis. The animals were kept until death or were killed if in poor condition. The survivors were sacrificed after the 60th week following the application. A complete pathological study was performed routinely, except in the case of a few animals lost through cannibalism or because of advanced decomposition.

**RESULTS**

The results are presented in detail in Table 1. After treatment with 20 or 50 µg., a total of four tumors were observed in 82 mice; all these, however, were outside the treated area and occurred late. With 100 µg., six mice (11 per cent) developed nine tumors, and of these two were outside the treated area. None of the epidermal tumors became malignant, although two sarcomas were recorded. With a dose of 200 µg. a marked increase in response was observed; 62 per cent of the

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>LIFE HISTORY OF TUMORS AFTER TREATMENT WITH 9,10-DIMETHYL-1,2-BENZANTHRACENE</th>
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</thead>
<tbody>
<tr>
<td><strong>TREATMENT WITH DMBA</strong></td>
<td><strong>No. MICE</strong></td>
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<tr>
<td>-----------</td>
<td>---------</td>
</tr>
<tr>
<td>20 µg., once</td>
<td>60 ♀</td>
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<tr>
<td>50 µg., once</td>
<td>22 ♀</td>
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<td>100 µg., once</td>
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<td>200 µg., once</td>
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<td>250 µg., once</td>
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<tr>
<td>500 µg., once</td>
<td>20 ♀</td>
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<td>1000 µg., once</td>
<td>21 ♀</td>
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<tr>
<td>100 µg., twice 1 week apart</td>
<td>60 ♀</td>
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</table>

* TBA = new tumor-bearing animals. Tumors = new tumors appearing in each period; in parentheses, the tumors outside the treated area. Regr. = tumors regressing in each period. D/T = dying animals/tumors present at death; in parentheses, the number of animals dying with no tumors.
† In addition, three basal-cell carcinomas.
animals developed tumors, 26 per cent of these progressing to carcinoma. An almost identical response was observed when the 200-μg. dose was administered as two 100-μg. doses separated by an interval of 1 week. With the higher doses of 250, 500, and 1000 μg., no further increase in response was encountered.

Although the majority of tumors appeared on the treated area, some arose either on the abdomen, or on the face, head, and lower dorsal area. Considering all the experiments together, a total of nineteen tumors (10 per cent) appeared outside of the interscapular area. All of them arose at a late stage of the experiments, their average latent period being 49 weeks. Only 3 of them regressed.

The tumors arising on the treated area had an average latent period of 10.5 weeks. No significant variations among the groups were observed. The first tumor appeared 4 weeks after the treatment.

A large number of the induced tumors, namely, 82 (i.e., 45 per cent) of a total of 188 regressed. Five regressions occurred through the merging of a papilloma into an adjacent malignant tumor. The average time of disappearance for the regressing tumors was the 20th week following the application, with variations ranging between the 16th and the 35th week, according to the groups. Thus, the average life span of the regressing tumors was 10 weeks. Occasionally, the regression of a tumor occurred suddenly, but in most cases, it took several weeks during which a progressive flattening of the lesion was observed.

Many of the tumors on the treated area arose from lesions appearing shortly after the application of DMBA. These lesions consisted of epilation, edema, and congestion of the dermis, thickening and hyperkeratosis of the epidermis, and, in some cases, superficial erosion or deep ulceration. This damaging effect of DMBA was not evident in the groups receiving 20 or 50 μg.; it was mild in the animals given 100 μg. and increased in severity at the higher doses. It attained its maximum at the 3d–4th week after the treatment; at that time ulcers were present in 25 per cent of the animals given 100 μg. and in 75 per cent of those receiving 200 μg. or more of DMBA. Later the ulcers reduced in size in most animals and disappeared completely or were the site of papillomas. In 41 instances the ulceration persisted past the 10th week of the experiment. These persisting ulcers were observed in one animal (1.8 per cent) at the level of 100 μg., in eight in both the experiments in which 200 μg. were given (16 and 18 per cent, respectively), in twelve (40 per cent) following an application of 250 μg., and in six instances in both the 500- and 1000-μg. experimental groups (30 and 29 per cent, respectively). Eight ulcers persisted without modifications until the death of the animals; nineteen healed, eleven without tumor formation, seven with papillomatous growth, one with an intradermal hemangioma; fourteen persisted and progressed to malignancy, five being squamous-cell carcinomas and nine sarcomas.

Thirty-eight squamous-cell carcinomas were seen in the treated area, and four outside; as already mentioned, five of the former arose from ulcers present for many weeks; the others arose from pre-existing papillomas. The average time of gross diagnosis of carcinoma was the 31st week, although the first carcinoma was grossly recognized as early as 7 weeks after the treatment. In five instances there were metastases to the regional lymph nodes or to the lungs.

Thirty sarcomas were seen, all of them in the treated area. Their incidence appeared to be higher in the groups receiving 250 μg. or more of DMBA than in the groups receiving 200 μg. Twenty-one sarcomas appeared grossly to arise from a pre-existing papilloma, nine from a pre-existing ulcer. The average time in which this transformation occurred and its pattern were the same as for the carcinomas, and only histological examination permitted the diagnosis of this type of malignancy. Histologically, they were mostly poorly differentiated and highly invasive fibrosarcomas. In four instances a coexistence of a squamous-cell carcinoma and a fibrosarcoma was seen in the same lesion. In three cases the carcinoma was superficial and the sarcoma below with some intermingling of the two tissues at the borderline; in all, a papilloma had been present for many weeks before undergoing malignant transformation. In the fourth instance, a confluence between two separated tumors had occurred 2 weeks prior to death, and the carcinoma and the sarcoma were side-by-side. In addition, three basal-cell carcinomas with adenalex differentiation and two benign hemangiomas of the dermis were observed in the treated area.

The incidence of tumors in other organs did not exceed that seen in untreated controls. The incidence of malignant lymphomas in the groups in which the diagnosis was histologically confirmed in all cases was as follows: 50 μg. of DMBA: 5/32 (13 per cent); 100 μg.: 7/55 (13 per cent); 500 μg.: 7/50 (14 per cent); 1000 μg. : twice 6/60 (10 per cent), with a total of 23 malignant lymphomas in 187 animals (12 per cent). This incidence is not significantly higher than that observed in untreated female controls kept in this laboratory until over 60 weeks of age.
DISCUSSION

The principal finding in this investigation is that a critical dose level exists at which single application of carcinogen becomes fully effective. Using single applications of DMBA, doses of 20 or 50 µg gave rise to no tumors in the treated area; a small number of skin tumors remote from the site of application, after a long latent period and considered insignificant, were observed. At a level of 100 µg., a low incidence of tumors occurred; none of the tumors appearing at this dose progressed to carcinoma. At a dosage of 200 µg., most of the animals developed tumors, many of which became malignant. Doubling the dose from 100 to 200 µg. resulted in an increase of from 5 to 7 times in tumor incidence. Increasing the single dose above 200 µg. to 250, 500, or 1000 µg. did not lead to a further increase in tumor response. In the instance of the higher doses increased local damage and conceivably the systemic toxicity of DMBA (32) may have obscured or altered the results.

Secondly, it has been observed that many of the tumors induced will regress, but that, nevertheless, a high incidence of malignant transformations occurred. When tumors are induced with a single application of a low dose of a carcinogen followed by croton oil (21) or Tween 60 (10), many of the tumors regress, and few become malignant. The tumors resulting from a single large dose of carcinogen thus resemble those induced by the initiation-promotion procedure in one respect but not in another. This finding is in confirmation of the observations on regression by Mider and Morton (15) and by Law (14) and on the high incidence of malignancy by Bielschowsky and Bullough (6).

Lastly, the general condition of the skin was carefully studied. It was noted that gross ulceration occurred mostly at levels of 200 µg. of DMBA and above. However, at these higher levels gross damage by no means occurred in all the animals and could not be related consistently to the occurrence of skin tumors. Efforts were made to quantify the damage, but this was found to be impossible in any meaningful manner.

If carcinogenesis is conceived to be the result of a single continuous process, then the results of this study must be considered to be simply a matter of dose-response. However, many investigations of skin carcinogenesis have provided evidence that at least a two-stage mechanism is involved. If the present findings are described in terms of the two-stage hypothesis of initiation and promotion of carcinogenesis, then it could be said that the property of promotion is acquired by the carcinogen at levels above 100 µg. Trauma as a tumor-promoting factor has been amply discussed (9, 15). It is generally proved that wound healing can exert a promoting effect in skin carcinogenesis, although this is most effective in the rabbit (11) and only minimal in the mouse (9, 18, 17). However, from other studies with promoting agents in skin carcinogenesis well reviewed by Salaman (19), it seems clear that a specific alteration is involved. Hypothetical propositions as to the mechanism involved have been advanced (4), but direct experimental evidence is lacking. Although it would seem unlikely that wound healing and a simple reparative response would be adequate to explain the results of this study, it does appear as though the degree of damage associated with the higher doses of carcinogen may have played a role in the development of tumors.

REFERENCES

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Announcements

VIII INTERNATIONAL CANCER CONGRESS

The tentative date for the VIII International Cancer Congress is July 23-28, 1962. At a meeting of the National Organizing Committee it was also agreed that the working languages of the Congress will be English, Russian, and French, and that the proceedings of the Congress will be published in these three languages.

Further notices will be published as received, so that applications for reports may be submitted during 1961. The deadline for applications and abstracts of papers is December 1, 1961.

Anyone wishing additional information relating to the Congress should write to the General Secretary of the National Organizing Committee of the U.S.S.R.—Prof. L. Shabad, Academy of Medical Sciences of the U.S.S.R., 14 Solyanka, Moscow.

Erratum

In the paper by Montague Lane, "The Effectiveness of Cyclophosphamide (Cytoxan) against Well Established Transplanted Rodent Tumors," published in the September, 1960, issue of Cancer Research, the following change should be made in Table 4 on page 1271: the units under dose should be given as mg/kg.
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