Epidermal Carcinogenesis in the Mouse Induced by One, Two, or Three Applications of 9,10-Dimethyl-1,2-benzanthracene Followed by Repeated Applications of Croton Oil. A New Hypothesis of the Mechanism of Carcinogenesis*

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(Introduction, The Chicago Medical School, Chicago 8, Ill.)

INTRODUCTION

Treating the skin of the mouse with a dose of 3,4-benzpyrene so small that, of itself, it is unable to make more than an occasional tumor appear, has been found to influence the skin so that subsequent applications of the noncarcinogenic substance croton oil are enabled to induce many tumors in the prepared area (1). Other carcinogens of the benzanthracene series act in the same way, and even a single application has been found adequate to prepare the skin (2, 8). These experiments provide the basis for the hypothesis that carcinogenesis in the skin of the mouse can be divided into two stages. In the first, the carcinogen prepares the skin so that, in the second, the noncarcinogenic agent croton oil, further applications of a carcinogen, or other adequate stimuli may make tumors manifest (3).

Some of the factors affecting the first stage of this process—the preparation of the skin by the carcinogen—have been investigated. When tumors are induced in the skin of the mouse by a single application of a carcinogen, followed by repeated applications of croton oil, there is a latent period during which no tumors are seen; then, a time during which more papillomas appear; and, finally, a period during which few if any new tumors arise (3). The number of tumors that croton oil can manifest in the prepared skin is thus limited. Once treated with carcinogen, the skin remains prepared indefinitely; for, whether the first application of croton oil be made a few days, or as long as 48 weeks after the application of carcinogen, the number of tumors made manifest by the oil is the same (5). The number of tumors induced depends on the nature and concentration of the carcinogen used for the preparatory application (3, 4). If different carcinogens are used for this initial application, croton oil manifests different numbers of tumors. If different concentrations of the same carcinogen are used to prepare the skin, the number of tumors croton oil can manifest varies with the concentration of carcinogen (4). It has also been noted that the average latent period of tumor induction remains constant whatever carcinogen is used for the preliminary application, and whatever its concentration is, provided the latent period be measured not from the time the carcinogen was applied but from the first application of croton oil (3).

When tumors are induced in the skin of the mouse by a single application of carcinogen followed by repeated applications of croton oil, they are at first all benign papillomas. As the experiment continues, many will remain benign papillomas, but some will regress and disappear, and a few will become malignant (10).

This paper reports two experiments: a pilot experiment in which one, two, or three applications of a 1.0 per cent solution of 9,10-dimethyl-1,2-benzanthracene was applied to the skin of mice and followed by repeated applications of croton oil, and a larger-scale experiment in which 0.2 per cent 9,10-dimethyl-1,2-benzanthracene was used in the same manner. The numbers of tumors manifest in the various groups were compared.

METHODS AND RESULTS

The mice in the pilot experiment were C3H males bred and maintained in this laboratory, and those in the main experiment were Swiss females bred at the Roscoe B. Jackson Memorial Labora-

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tory, Bar Harbor, Maine. All the mice were approximately 3 months old at the beginning of the experiments.

The carcinogen was either a 1.0 per cent solution or a 0.2 per cent solution of 9,10-dimethyl-1,2-benzanthracene (Eastman Kodak) in mineral oil (Superla 34, light mineral oil, Standard Oil of Indiana); the croton oil was a 5 per cent solution of Oleum crotonis B.P. (Boots) in the same mineral oil. An area 1.5 cm. square in the interscapular region was kept free of hair by clipping with scissors, and the solutions applied to this area with an eye dropper. Each dose of carcinogen was 1 drop and each dose of croton oil 2 drops.

The tumors were charted weekly for the first 30 weeks and thereafter every 2 weeks. Those tumors which regressed before they had been present 4 weeks have been disregarded. All others, whether persisting throughout the experiment or not, have been counted as tumors.

**EXPERIMENT 1**

Thirty male C3H mice were used in each of the three groups:

*Group 1.*—The mice were given a single application of 1.0 per cent 9,10-dimethyl-1,2-benzanthracene, and 5 weeks later croton oil was begun and was continued twice a week for 35 weeks.

**TABLE 1**

<table>
<thead>
<tr>
<th>Survivors at time of first tumor</th>
<th>Av. latent period (weeks)</th>
<th>Tumor-bearing mice</th>
<th>Total no. tumors/tumor-bearing mice</th>
<th>Av. tumors/mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1†</td>
<td>23</td>
<td>15</td>
<td>27</td>
<td>2.0</td>
</tr>
<tr>
<td>2†</td>
<td>22</td>
<td>15</td>
<td>22</td>
<td>2.5</td>
</tr>
<tr>
<td>3§</td>
<td>18</td>
<td>15</td>
<td>24</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* C3H male mice.

† A single application of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene followed by croton oil.

‡ Two applications of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene followed by croton oil.

§ Three applications of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene followed by croton oil.

* Group 2.—The mice received two applications of 1.0 per cent 9,10-dimethyl-1,2-benzanthracene with an interval of 1 week between applications. Five weeks after the first application, croton oil was begun and was continued twice a week, as in Group 1.

* Group 3.—The mice were given three applications of 1.0 per cent 9,10-dimethyl-1,2-benzanthracene at weekly intervals. Five weeks after the first application, croton oil was begun and was continued twice a week as in Groups 1 and 2.

The results of this experiment are shown in Table 1. It can be seen that essentially the same number of mice bore tumors and the same numbers of tumors were induced irrespective of the number of applications of carcinogen administered. With a single application of carcinogen, thirteen tumor-bearing mice bore a total of 27 tumors; with two applications, fifteen mice had 23 tumors; and with three applications, thirteen mice had 24 tumors. The experiment was continued for 35 weeks from the first application of croton oil. No great differences were noted in the various latent periods.

**EXPERIMENT 2**

One-hundred female Swiss mice were used in each of the three groups:

*Group 4.*—The mice were given a single application of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene, and 5 weeks later croton oil was begun and was continued twice a week for 36 weeks.

**TABLE 2**

<table>
<thead>
<tr>
<th>Survivors at time of first tumor</th>
<th>Av. latent period (weeks)</th>
<th>Tumor-bearing mice</th>
<th>Total no. tumors/tumor-bearing mice</th>
<th>Av. tumors/mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4†</td>
<td>87</td>
<td>10.9</td>
<td>55</td>
<td>1.6</td>
</tr>
<tr>
<td>5‡</td>
<td>88</td>
<td>11.9</td>
<td>60</td>
<td>1.8</td>
</tr>
</tbody>
</table>

* Swiss female mice.

† A single application of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene followed by croton oil.

‡ Two applications of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene followed by croton oil.

§ Three applications of 0.2 per cent 9,10-dimethyl-1,2-benzanthracene followed by croton oil.

The results are shown in Table 2: the more applications of carcinogen given, the fewer the tumors produced. With one application of carcinogen, 416 papillomas were made manifest, or 7.8 per tumor-bearing mouse; with two, only 305, or 5.2 per tumor-bearing mouse; and with three, 260, or 4.3 per tumor-bearing mouse. The average latent period measured from the first application

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of croton oil was 10.9 weeks in Group 4, 11.8 weeks in Group 5, and 13.4 weeks in Group 6. Such differences in latent periods cannot be considered significant with the degree of variation seen.

It may be noted in passing that the results of this experiment only become striking when the total number of tumors is tabulated, the number of tumor-bearing animals being much the same in each group. This was also the case in previous experiments in which different concentrations of carcinogen were used (4). It would seem that in experiments of this sort it is essential to count the total number of tumors. Only in this way can quantitative comparisons be made.

DISCUSSION

One aspect of the results of these experiments is quite clear. Increased numbers of applications of a carcinogenic hydrocarbon within certain limits, when followed by applications of croton oil, will not increase the number of tumors induced. This result is seemingly at variance with the previously observed increase in the number of tumors seen with increasing concentrations of carcinogen followed by croton oil (4). In the first, and smaller, of these two experiments, similar numbers of tumors were noted in the various groups, the increases in numbers of applications having no apparent effect. However, in the larger of the two experiments, and that in which a lower concentration of carcinogen was used, a significant decrease in the number of tumors induced corresponding to increasing numbers of applications of carcinogen was observed. It should be noted that these two experiments cannot be compared in many details, as different strains of mice were used; the C3H mice appear considerably less responsive to this form of carcinogenesis than the Swiss mice.

Several explanations of the observed results seem possible within the limits of previous hypotheses of carcinogenesis. The view that skin carcinogenesis in the mouse, with a single application of a carcinogen followed by croton oil, represents the initial production of some "latent tumor cells" and the subsequent conversion of these to actual tumors by a different action has not been contradicted.

The difference between the effects of increasing concentrations or increasing numbers of applications of carcinogen requires additional explanation. In the first of these experiments it was noted that the same numbers of tumors were produced by one, two, or three applications of carcinogen followed by croton oil. In the second experiment, decreasing numbers of tumors were produced corresponding to increasing numbers of applications of carcinogen. There were several differences between the two experiments, particularly in the strains of mice, the concentrations of carcinogen used, and the relative numbers of tumors induced. It is possible that the significant decrease in the numbers of tumors noted in the second experiment, namely, 416, 305, and 260, respectively, was noted because of the large number of animals used and may have been missed in the first experiment. Nevertheless, hypothetical explanations of the observed phenomena would seem to apply equally well to the cases in which no increase was observed and the number of tumors induced remained constant, and those in which decreases were observed. Perhaps the well known necrotizing effect of the carcinogen (6, 9) or, possibly, some inhibitory action (7) accounted for these results. This does not appear a satisfactory explanation at the dosage levels used. Again, some kind of refractory state may be induced by the first application of carcinogen, making the tissue no longer susceptible to further change within the time intervals chosen. It is also possible that a combination of both necrotizing action and refractoriness accounts for the result of the second series of experiments.

However, the only conclusion that can be stated with some finality is that increases in the number of applications of a carcinogenic hydrocarbon within the present experimental limits, when followed by applications of croton oil, do not give rise to increasing numbers of tumors. This finding is a sharp contrast with that previously noted when increasing concentrations of carcinogen were followed by croton oil.

SUMMARY

1. C3H male mice received either one, two, or three skin applications of a 1.0 per cent solution of 9,10-dimethyl-1,2-benzanthracene followed by croton oil repeatedly. No differences in the tumor incidences were noted.

2. Swiss female mice received either one, two, or three skin applications of a 0.2 per cent solution of 9,10-dimethyl-1,2-benzanthracene followed by croton oil repeatedly. Decreasing numbers of tumors were recorded with increasing numbers of applications.

3. The possible significance of these findings is discussed. The secondary necrotizing action of carcinogenic hydrocarbons and the possible induction of a refractory state are considered as explanations.

ACKNOWLEDGMENTS

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