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Carcinogenesis in the Mouse’s Skin by the Infrequent Application at Long Intervals of Methylcholanthrene*

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This paper is a contribution to a group investigation on the early stages of experimental carcinogenesis organized by Dr. E. V. Cowdry. From a histological study of the early changes produced by a strong carcinogenic stimulus as represented by a 0.6 per cent solution of methylcholanthrene in benzene applied to a large area of skin 3 times a week, the conclusion was reached that the action of the carcinogen is not a direct stimulating effect inducing epithelial proliferation. Its direct effect is, on the contrary, a short toxic one lasting for several days, injuring the epithelial cells and inhibiting their mitotic activity. The subsequent epithelial proliferation which conveys the impression of a protective response can be accounted for adequately by assuming as a working hypothesis the formation in the skin of substances stimulating the cells to multiply over a prolonged period. For convenience of reference we shall call these hypothetical substances “proliferin.”

This conclusion was based partly on the finding that a single application of methylcholanthrene is capable of inducing in a small percentage of a group of mice an active epithelial proliferation which persists for many weeks producing a massive hyperplasia, while it fails to do so in other animals of the same strain subjected for a period of 1 or 2 months to frequently repeated applications of the carcinogen (Figs. 1 and 2). The detailed evidence on which these conclusions are based is now being prepared for publication.

If the carcinogen had a direct stimulating effect on the epithelium, as is generally believed, then the degree of the epithelial hyperplasia should be a direct function of the amount of the carcinogen applied. This is obviously not the case when a single application of the carcinogen can produce in some animals a high degree of hyperplasia while in others frequently repeated applications fail to elicit a hyperplasia. But this phenomenon can be accounted for on the alternative conception mentioned above. In that case the applications of a strong carcinogen repeated frequently at short intervals of time, as used in the conventional technic of experimental carcinogenesis, may produce an inhibition sufficiently effective to overcome the proliferative stimulus due to the hypothetical proliferin. While the ultimate proof of this conception lies in the identification of that substance, it is possible to test experimentally the validity of this view by spacing the applications of the carcinogen at longer intervals. This would allow the stimulating effect of proliferin, which from our observations persists over a long period when once formed, to continue for a longer period without being counteracted by the toxic inhibitory effect of the carcinogen, which is more transient.

MATERIALS AND METHODS

We carried out, therefore, 3 series of experiments on mice of the Swiss strain, using a method of application which in previous experiments with frequently repeated applications had been found to give a near optimum effect; namely, a 0.6 per cent solution of methylcholanthrene applied to a large area of skin by a single brush stroke from the nape of the neck for a distance of about 1.5 cm. By applying 10 similar brush strokes to a piece of weighed filter paper it was found that the average amount of methylcholanthrene applied to the skin at each brush stroke is 0.1 mgm. Using less absorbent typing paper the amount delivered at each brush stroke was 0.07 mgm. In the standard technic this treatment is applied 3 times a
week for 14 weeks; i.e., 42 applications, when all painting is stopped. By this standard method 100 per cent of the mice develop skin cancer, the first malignant tumor appearing in the 6th week; i.e., after 15 applications; the last in the 24th week.

In order to test the results of prolonging the intervals of application, the carcinogen was applied once every 2 weeks to a series of 10 mice (Series I) of which one died early in the experiment. In another series of 12 mice it was applied once every 3 weeks (Series II). The results being successful, a third experiment was begun with 50 mice which received one application once a month (Series III). Although this third experimental series is still in progress, the results obtained after 7 applications are sufficiently striking to make possible a comparison with the standard technic. For convenience of reference, the method of infrequent painting at long intervals just outlined will be called the protracted technic.

RESULTS

The results are given in Table I. There is obviously an essential difference between the standard and the protracted method of application in the selection of the factors by which the carcinogenic effect of the hydrocarbon is determined. In the standard method it is measured by the ratio of the number of cancerous mice, expressed in percentages, to the time necessary to induce cancer, reckoned from the first exposure to the carcinogen; i.e., the latent period. In the protracted method this time factor of exposure to the carcinogen is disregarded and the carcinogenic effect is determined by the ratio of the number of cancerous mice to the effective carcinogenic dose of the carcinogen applied, as shown in Table II. This is the usual method to determine a direct biological effect of a chemical substance.

Table II shows that in all the three series treated by the protracted method, the carcinogenic potency of the hydrocarbon is higher, or, if expressed in terms of its effective dose, the effective dose is smaller than in the standard method. Thus, in Series I the dose necessary to induce cancer in all the animals, 1.4 mgm. in 14 applications, is equal to that applied in a month's painting by the standard method (3 times weekly). In Series II, it is equal to the dose applied in 3 weeks' painting by the standard method. In Series III, which

DESCRIPTION OF FIGURES 1 AND 2

Fig. 1.—Slight epidermal proliferation of mouse skin painted thrice weekly for 52 days (17 paintings) with 0.6 per cent methylcholanthrene in benzene. Axial section. Mag. X 12.

Fig. 2.—Marked epidermal proliferation in mouse skin 28 days after a single application of the same carcinogen. Axial section. Mag. X 12.
at the time of writing has only reached the stage of 7 monthly applications, 50 per cent of the mice have developed cancer in response to a dose of carcinogen equal to that applied in only 2 weeks' painting. No such results have been obtained by us after a corresponding number of applications by the standard method.

The experimental Series I and II were of a preliminary nature and were, therefore, carried out on a small number of animals, so that a comparison between these two series does not give differences which are statistically significant. The experiments are being repeated on a larger number of animals. However, a comparison between these two series with Series III shows that the effective carcinogenic dose of the same hydrocarbon varies with the intervals of time which elapse between successive applications. The effective dose diminishes as the interval between two successive applications is prolonged, and this is true whether one takes the minimal dose after which the first carcinoma appears or the dose inducing cancer in 50 per cent of the survivors. For the incompleted Series III with an interval of 1 month between successive applications it can be said at present that both the minimal cancer-producing dose and the dose necessary to induce cancer in 50 per cent of the animals are even smaller than in either Series I or in Series II.

Although the time of exposure to the carcinogen is disregarded in this method as a factor determining the carcinogenic potency of a hydrocarbon, the latent period, i.e., the time at which cancer appears, shows an interesting relationship. The latent period for the first carcinoma in all three series is longer than in the standard method, where with our technic it was found to lie between the 6th and 8th week. In the first two series there is a similar interval of time necessary to induce cancer in the whole group. After 27 weeks cancer had developed in all the animals of Series II and in 8 out of 9 animals in Series I. This is again longer than the length of the maximal latent period in the standard method, about 24 weeks. In the incompleted Series III the time necessary to induce cancer in all the mice will be even longer than Series I and II.

**TABLE I: DEVELOPMENT OF SKIN CANCER BY THE PROTRACTED METHOD IN RELATION TO THE DOSE OF METHYLCOLANTHRENE APPLIED**

<table>
<thead>
<tr>
<th>No. of</th>
<th>Series I</th>
<th>Series II</th>
<th>Series III</th>
</tr>
</thead>
<tbody>
<tr>
<td>of applications; also carcinoma in 0.1 mgm.</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of mice with cancer</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Latent period in weeks</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of mice with cancer</td>
<td>2</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Latent period in weeks</td>
<td>5</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>No. of mice with cancer</td>
<td>1</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td>Latent period in weeks</td>
<td>9</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Total no. of mice with cancer</td>
<td>12</td>
<td>35†</td>
<td>35†</td>
</tr>
<tr>
<td>Total negatives</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*One sarcoma included.
† Experiment not yet completed.

**TABLE II: COMPARISON OF THE TOTAL DOSAGE OF METHYLCHOLANTHRENE REQUIRED TO INDUCE CANCER BY THE STANDARD AND PROTRACTED TECHNIQUE**

<table>
<thead>
<tr>
<th>Frequency of painting</th>
<th>Methylocolanthrene applied before appearance of cancer, in mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard technic</td>
<td>In first mouse</td>
</tr>
<tr>
<td>Three times per week</td>
<td>1.5</td>
</tr>
<tr>
<td>Protracted technic</td>
<td></td>
</tr>
<tr>
<td>Once in 2 weeks</td>
<td>1.0</td>
</tr>
<tr>
<td>Once in 3 weeks</td>
<td>0.5</td>
</tr>
<tr>
<td>Once a month</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

These results are not in accordance with the accepted view that the carcinogenic hydrocarbons induce cancer in the skin by stimulating directly the mitotic activity of the epithelium. They support the conception, arrived at from a histological study of the early changes in carcinogenesis, that the carcinogenic hydrocarbons produce a transient toxic effect on the epithelium, inhibiting mitotic activity, and that the epithelial proliferation which eventually leads to cancer is due to the formation in the skin of a substance stimulating the epithelial cells to mitotic activity for a prolonged period of time. Sometimes this proliferation is so prolonged that even a single application of the carcinogen is sufficient to induce cancer. In a group of 6 Swiss mice subjected to a single application, one surviving animal developed a skin carcinoma after an interval of 6 months. A similar result has been recorded by Mider and Morton (1) working with the C57 brown strain. Another group of 14 Swiss mice received one application of methylcholanthrene 4 months before the time of writing. In this group one animal has already developed cancer (sarcoma) after 3 months, which is the shortest time recorded for a single application.
Bearing in mind that, in terms of biological time, a month of a mouse’s life corresponds to 2 years of human life, these results have an important bearing on the etiology of cancer in man by demonstrating that a continued exposure persisting over a long period of time is not essential for the development of a malignant epithelial tumor, as is generally supposed. A few isolated exposures to a strong carcinogenic stimulus separated from each other by long intervals of time can be effectively carcinogenic. At present the efficacy of the protracted method of carcinogenesis has been demonstrated only for the experimental conditions defined in this paper; namely, the application of a highly effective carcinogenic solution to a large area of skin.

**Summary and Conclusions**

Cancer has been induced in the skin of mice by a method of application in which the carcinogen acts on the cells infrequently and at long intervals. In this method the carcinogen was applied at intervals of 2 weeks, 3 weeks and of 1 month, respectively. By this technic the carcinogenic potency of an agent is measured in terms of the effective dose of the carcinogen; that is, the dose which induces cancer. In the standard method of continuous application, the carcinogenic potency is measured by the time necessary to induce cancer. Using the protracted technic, it was found that the effective carcinogenic dose is smaller than that found by the standard technic, and further that the dose becomes increasingly smaller as the interval between each successive application is prolonged.

**REFERENCES**

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