The Effect of Cortisone and Hair Cycle on the Incidence of Chemically Induced Epidermal Tumors in Mice*

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In previous communications we reported that under certain experimental conditions systemic cortisone administration accelerated the occurrence of chemically induced epidermal tumors in mice (15-17, 20, 26). The results were confirmed by Spain, Molomut, and Novikoff (25). We postulated that this effect is caused by an inhibitory influence of the steroid on hair follicular growth (15-17, 24). This assumption was based (a) on our own observation that subcutaneous cortisone injections inhibit hair follicular growth and thus protract hair follicular rest in mice (which was in line with similar observations made by Baker et al. in rats [2, 3, 11, 27, 28]) and (b) on the observation made almost simultaneously by Scandinavian investigators (1, 5) and by Cowdry's group (18) that chemically induced epidermal tumors more readily develop when exposure to the carcinogen occurs during the resting phase of the hair cycle than when it occurs during the growing phase.

Two large-scale experiments conducted a few years ago to prove the validity of our hypothesis failed to yield any conclusive results, because of shortcomings in the procedure.

A new hair cycle was initiated artificially in these experiments by plucking of the fur. In both experiments one group of the animals was subjected to a single exposure to the carcinogen (dimethylbenzanthracene) during the growing phase—anagen—of the hair cycle, whereas a second group was subjected to the same exposure during the resting phase—telogen—of the cycle. About half of each group of mice received cortisone injections at the time of the carcinogen exposure, while the other half (controls) did not receive any cortisone. According to our theory, a higher tumor incidence was expected to occur in the animals given injections of cortisone during the growing phase of the pelage than in the corresponding control mice, whereas no such difference was expected in the animals given injections of cortisone during the resting phase.

The technical shortcomings responsible for the lack of distinctive differences in the tumor incidence throughout these experiments were as follows:

a) In the first experiment dimethylbenzanthracene was employed in only 0.1 per cent concentration (in benzene), which subsequently necessitated frequent paintings with croton oil for a long period of time. The ensuing synergistic effect, as well as the late and protracted appearance of tumors obscured the outcome (Rusch et al. [7, 22, 23]).

b) Hair plucking as such might inadvertently have interfered with the purpose of our experiment, inasmuch as the carcinogen solution may have had better access to the deeper sites of the hair follicle when it was applied shortly after hair plucking, i.e., during follicular growth, than when it was applied a month after the plucking, i.e., during follicular rest.

c) The fur surrounding the clipped or plucked areas of carcinogen application allowed the carcinogen solution to collect along the hairy borders of the areas. As a result, the tumors appeared almost exclusively along the hairy border zones.

To avoid these shortcomings, an experiment was devised in which the carcinogen was employed during “natural” anagen or telogen, respectively. For this purpose we adopted K. Borum's technic of dyeing the fur, aimed at recognizing the phase of the hair cycle at any given time1 (6).

It is the object of this presentation to report on the results obtained in this more recent experiment.

1 It had, moreover, become apparent that, for accurate information regarding the precise age of the animals, we could not depend on any supplier from the outside, so that breeding and rearing of the mice in our own premises were imperative.
MATERIALS AND METHODS

Hair dye.—The dye employed by us was "Roux," a commercial para-phenylenediamine preparation, of the darkest shade available. Prior to use, equal parts of the dye and 30 vol. per cent hydrogen peroxide were mixed. This mixture was spread all over the back of the mouse by means of a cotton applicator (except that head, tail, and extremities were spared).

When the fur of a 4-week-old albino mouse is dyed black in this manner, growth of the second hair generation is recognized upon ruffling of the fur (e.g., in the interscapular area) by the appearance of white, undyed hair just above the skin surface. According to Borum, this is due to occur during the 5th week of life. As soon as the new generation of hair has reached its final length, black and white hairs form an evenly greyish "pepper and salt" coat all over the back. This finding indicates the end of the second growing phase, hence the start of the second resting phase of the hair cycle in this region.

Carcinogen.—A 0.5 per cent w/v solution of 9,10-dimethyl-1,2-benzanthracene (DMBA) in benzene was employed. A fresh solution was prepared every 2 weeks; the same batch of the substance was used throughout, and weighing was carried out on an analytical (semi-micro) balance. The solution was kept in a brown flask and stored in a dark cabinet.

In no animal was the single application of carcinogen followed by any subsequent application of the solution or of another agent such as croton oil.

Cortisone.—A 2.5 per cent suspension of cortisone acetate in physiologic saline solution was diluted with saline solution to a 0.2 per cent suspension. This suspension was injected subcutaneously into the inguinal region 5 times weekly for 2 consecutive weeks. The injections were started 1 week prior to the exposure to the carcinogen. The single dose of cortisone acetate was 0.3–0.5 mg., depending on the animal's weight.

Mice.—The mice were bred in our own laboratory; they were from the Swiss albino strain supplied by Blue Spruce and Carworth Farms. Each new litter was divided into four groups, depending on the number of mice available. The number of litters available varied from two per week to several per day.

New litters were obtained for the experiment over a period of 20 months.

DESIGN OF EXPERIMENTS

Series I.—
Group A: Subjected to a single DMBA application (0.05 ml.; 0.5 per cent in benzene) at the time when second hair growth was first observed.
Group B: Subjected to single DMBA application and subcutaneous cortisone injections at the time when second hair growth was due.

Series II.—
Group A: Subjected to single DMBA application during the period of second hair follicular rest.
Group B: Subjected to single DMBA application and subcutaneous cortisone injections during the period of second hair follicular rest.

TABLE 1
NUMBER AND PERCENTAGE OF TUMOR-BEARING MICE

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>FEMALES</th>
<th></th>
<th>MALES</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. mice*</td>
<td>No. Tumor-bearers†</td>
<td>Per cent</td>
<td>No. mice*</td>
</tr>
<tr>
<td>Series I (growing phase)</td>
<td>A (Controls)</td>
<td>141</td>
<td>9 (2)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>B (Cortisone)</td>
<td>165</td>
<td>65 (14)</td>
<td>39</td>
</tr>
<tr>
<td>Series II (resting phase)</td>
<td>A (Controls)</td>
<td>186</td>
<td>145 (33)</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>B (Cortisone)</td>
<td>159</td>
<td>108 (24)</td>
<td>68</td>
</tr>
</tbody>
</table>

* No. of animals alive on day of appearance of first tumor, i.e., 18 days after DMBA application.
† Numbers in parentheses indicate number of tumor-bearers with at least one tumor showing the features of malignancy.

In general the destructive alterations, including ulceration, scarring, and disturbed regrowth of hair, were much more severe in the two groups subjected to the carcinogen application during the resting phase of the hair cycle (Groups IIA and IIB) than in those receiving the carcinogen application in the phase of hair growth (IA and IB). However, regrowth of hair, as well as improvement of the destructive changes of the skin produced by the carcinogen, were distinctly slower and less complete in the group (IB) which was exposed to DMBA during the phase of hair growth and given injections of cortisone than in the corresponding control group (IA) which was not given injections of cortisone.
The weight of the animals was followed up carefully; a transitory arrest in growth and in gain of weight was noticeable in many of the cortisone-injected mice during the period of steroid administration. The weight, however, again rapidly reached that of the control animals upon cessation of the injections, which in general had been tolerated remarkably well, especially regarding the danger of possible complication by infectious diseases of the intestinal tract, etc.

Tumors appeared from the 3d through the 10th week—though preponderantly prior to the 7th week following the DMBA application. The observation period was terminated 3 months after the exposure to DMBA.

Roughly, one-half the number of tumor-bearers in Groups IA, IB, and IIB, and two-thirds of the tumor-bearers in Groups IIA showed multiple tumors.

Most of the early tumors persisted; grossly, they first appeared as minute papillomas, and the microscopic features of one of these—seen first 22 days after exposure to the carcinogen in a mouse which died 2 weeks later—is shown in Figures 1 and 2. Approximately 25 per cent of the female and 12 per cent of the male tumor-bearers (see Table 1) showed malignant tumors, preponderantly squamous-cell carcinomas, at the end of the first 3 months of observation (see Figs. 3 and 4).

In entire agreement with our hypothesis, Table 1 shows that in the animals subjected to the carcinogen and to cortisone administration at a time when hair was due to grow (IB), the tumor incidence was increased to a highly significant degree over the incidence in the controls not given injections of cortisone (IA). In a number of the cortisone-animals (IB), i.e., in approximately one-fourth to one-third of the series, the inhibition of hair growth was somewhat imperfect; but even in these animals, the tumor occurrence was augmented, though—among the females—to a lesser degree than when the inhibition of regrowth was more pronounced.

The mice subjected to cortisone injections during hair follicular rest certainly did not show any increase in the tumor incidence (IIB); in fact, a diminished incidence was apparent in the females of this group (see Table 1).

DISCUSSION

The fact that DMBA produced more damage to the skin of the "cortisone mice" exposed to DMBA during the growth period of the hair cycle (Group IB) than to the skin of the control mice (IA) is in precise agreement with our earlier observations (16, 20); and the stronger destructive alterations effected by the carcinogen during the phase of hair follicular rest (Group IIA) are in line with the findings of Montagna's group (9, 19, 21).

The low tumor incidence observed in the control animals of Series I which were painted with DMBA during hair follicular growth conforms with the reports of Borum (5, 6), although the Scandinavian author did not obtain any tumor response, whereas we did observe tumors in a few instances.

The striking increase in the incidence of tumors in the animals in which normal hair growth was impaired by cortisone (Group IB) proves the validity of our assumption that cortisone administration is capable of augmenting the tumor occurrence by inhibition of hair follicular growth.

Statistical follow-up of our results revealed that the reduction in tumor occurrence observed in the female mice which had received cortisone during hair follicular rest was limited to a period of time when there was a distinct rise in the incidence among the female controls not given injections of cortisone. This reduction presumably is comparable with the observation made by a number of previous investigators that the development of experimental tumors was inhibited by cortisone employed while neoplastic growth was on its way (Boutwell and Rusch [8], Englebreth-Holm and Asboe-Hansen [10], Zachariae and Asboe-Hansen [29, 30], Baserga and Shubik [4], Ghadially and Green [12], Gillman, Penn, Bronks, and Roux [13], and Gillman, Hathorn, and Penn [14]).

Our biostatistician has furnished the additional information that in our control groups (A groups) the incidence of DMBA-induced tumors was significantly lower in the female mice born during spring and summer than in the females born during fall and winter, whereas the reverse held true for the male group in which the animals born in spring and summer showed a significantly higher tumor incidence than those born in the other two seasons. At this time we are not inclined to place more emphasis on this (coincidental) observation.

It is felt that a more careful study of the variables would be warranted, which possibly produce different results during the two seasonal periods and/or in the two sexes.

SUMMARY

1. The skin of one series of Swiss albino mice was exposed to a single application of 9,10-dimethyl-1,2-benzanthracene (DMBA) at the time of natural growth of their second hair generation, while another series was exposed during the corresponding resting phase of the hair cycle.

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Fig. 1.—Section (H. & E.) from tumor first noted 22 days after exposure to DMBA. Verrucous papilloma. X150.5.

Fig. 2.—Portion of section shown in Figure 1, at higher magnification (X304). Verrucous growth, without any atypical or infiltrating proliferation.

Fig. 3.—Section (H. & E.) from tumor obtained 59 days after exposure to DMBA. Squamous-cell carcinoma, infiltrating cutis throughout. X55.6.

Fig. 4.—Portion of section shown in Figure 3, at higher magnification (X415). Strands of infiltrating and entirely undifferentiated cells, together with keratinous pearls and wisps.
About half the number of animals in both series received subcutaneous cortisone injections.

2. A much higher tumor incidence was obtained in the mice exposed to the carcinogen during hair follicular growth than in those exposed during hair follicular growth.

3. A significant increase in the tumor incidence occurred in the mice exposed to DMBA at the time when hair growth was due, but inhibited by cortisone.

4. No increase in the tumor incidence occurred in the mice given injections of cortisone and exposed to DMBA during hair follicular rest.

5. The results afford evidence that cortisone is able to increase the incidence of chemically induced epidermal tumors by interfering with hair follicular growth.

REFERENCES


The Effect of Cortisone and Hair Cycle on the Incidence of Chemically Induced Epidermal Tumors in Mice

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