Cortisone and Carcinogenesis in Mouse Skin

I. Effect of Cortisone during Multiple Paintings with Methylcholanthrene*

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It has been demonstrated that cortisone may enhance the development of metastases in both transplanted and spontaneous experimental tumors (2, 5, 7, 8). Whether or not this effect is the result of alterations in the primary tumor, the tumor bed, the sites of metastases, or is systemic is not clear. Furthermore, contradictory results have been reported concerning the effects of cortisone on the action of carcinogens in mice. Both a lowered incidence of tumor production (4, 10) and an increased incidence (11) have been reported. However, the experimental procedures have not been identical. There have been variations in the strain of mice, the dose of cortisone and duration of its administration, the number of times the carcinogen was painted, and the nature of the carcinogen used. Conflicting findings have also been reported concerning the role of the pituitary gland in experimental skin carcinogenesis (1, 9).

To gain further information about the role of the pituitary-adrenal axis, and particularly about cortisone, relative to experimental tumor production and metastases, and in an attempt to reconcile some of the conflicting observations, a series of experiments utilizing cortisone and methylcholanthrene have been instituted. In these studies cortisone is being administered during the early phases of methylcholanthrene painting, during the entire latent period, continuously throughout the course of tumor growth, or only after the appearance of papillomas. The number of paintings is also being varied. This report concerns the first of these completed studies.

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MATERIALS AND METHODS

One hundred and one BALB/C mice, 6-8 weeks of age, were divided into three groups, as follows: I, 30 animals (fifteen male, fifteen female); II, 39 animals (nineteen male, twenty female); III, 32 animals (eighteen male, fourteen female). All animals were painted twice weekly for 6 weeks with 3-methylcholanthrene (Eastman Kodak Co.), 0.6 per cent weight/volume in benzene, applied with a No. 3 camel's hair brush to the shaved skin of the back.

Six days earlier, all animals had received one intraperitoneal injection of penicillin (400 units in saline). Group I had no other treatment and served as the control for the cortisone-treated groups II and III. These latter two groups received daily subcutaneous injections of cortisone (cortone acetate, Merck) 0.2 mg/mouse, for 5 days prior to instituting methylcholanthrene paintings and for 10 days thereafter. Injections were then given 3 times weekly. Group II was kept on cortisone for the duration of the experiment (40 weeks). In Group III cortisone treatment was stopped after twenty injections, in the 4th week of methylcholanthrene painting (eight paintings) when first signs of papillomas had appeared in seven out of 33 (30 per cent) of the animals.

RESULTS

All 30 animals of Group I are included in the calculations of tumor incidence, whereas early deaths, probably as a consequence of cortisone treatment, made necessary the exclusion of ten animals of Group II and of nine of Group III from the papilloma percentages and of two additional animals of Group II from the carcinoma percentages.

The time of appearance of carcinoma was judged by gross external appearance, when the
base of the papilloma had expanded with white tissue. Microscopic examination of 36 random samples of such “white bases” showed 27, or 75 per cent, to be carcinomas; the remaining 25 per cent were apparently still papillomas. In many instances the body of the papillomas expanded considerably but remained constricted at the base. These were judged to be papillomas, and microscopic examination of thirteen random samples showed this to be true of ten, or approximately 80 per cent, with the remainder being carcinomas.

The marked stimulation of papilloma formation by cortisone is apparent from the charts. As Chart 1 shows, at the 10th week only 12 per cent of the animals in the control group had tumors, whereas over 60 per cent of Group II and over 70 per cent of Group III had developed tumors. When the experiment was terminated (40th week), only two animals (7 per cent) in Group II and three (13 per cent) in Group III were tumor-free, whereas this was true of eleven (57 per cent) of the animals in the control group.

The contrast between the cortisone-treated and control groups is even more striking when the number of multiple tumors is taken into account (Chart 2). The number of total papillomas divided by the total number of mice was, at the 10th week, 0.12 in the control group, 1.17 in Group II, and 1.44 in Group III. At the 28th week, the corresponding values were 0.40, 1.73, and 2.13; at the termination of the experiment (40th week) they were 0.80, 2.66, and 2.74.

Carcinoma formation roughly paralleled papilloma formation. By the 23rd week only 17 per cent of the control group had carcinomas, as contrasted to 48 per cent for Group II and 74 per cent for Group III. At the termination of the experiment (40th week), 47 per cent of the control animals had developed carcinomas, whereas 59 per cent in Group II and 74 per cent in Group III (Chart 1) bore carcinomas.

The time interval between the appearance of a papilloma and its transformation to carcinoma was extremely variable—from 1 to 19 weeks. There was no appreciable difference in this respect between the tumors of the cortisone-treated and those of the control animals. Similarly, in another experiment, no effect on the time of appearance of carcinoma was found when individual mice were given cortisone only when a papilloma first appeared.

It can be noted from the graphs, particularly...
Chart 2, that papillomas were still appearing, in both control and cortisone-treated groups, at the termination of the experiment, 34 weeks after painting with carcinogen was stopped and more than 36 weeks after the first papillomas had developed. Regression of some of the early papillomas occurred both in control and cortisone-treated groups. This is reflected in a drop in the curve of the control group between the 7th and 10th weeks (Chart 1). Because of the rapid development of other papillomas, the curves of Groups II and III do not demonstrate the regressions which occurred.

Finally, it should be noted that no morphological differences between the tumors of control and cortisone-treated mice was revealed by microscopic examination, and also that no metastases were encountered in either the control or the cortisone-treated animals.

**DISCUSSION**

The results in this study were somewhat similar to, but more striking than, those obtained by Sulzberger and his associates (11), who studied the incidence of methylcholanthrene-induced epidermal tumors in mice under the influence of cortisone. They found 69 per cent with papillomas in the cortisone-treated mice as compared with 48 per cent in the control animals after eighteen paintings with methylcholanthrene over a 6-week period; the dose of cortisone was 0.5 mg. daily throughout the course of the experiment. This increased incidence of papilloma formation occurred despite an observed decrease in the inflammatory response at the site of methylcholanthrene painting. In the present studies the differences between the cortisone (Groups II and III) and the non-cortisone groups (Group I) was even more marked.

Our data demonstrate that the enhancement of tumor formation by cortisone occurs even if the hormone is administered only in the early phases of the carcinogenic process. There is some suggestion that carcinoma formation is somewhat greater in the animals that receive cortisone during the early phases only, as compared with the group that received it throughout the entire experimental period.

To control the possibility that the difference in numbers of papillomas observed between the cortisone and nontreated groups was due to paintings of carcinogen at different phases of hair cycle activity, the above experiment has been repeated by...
controlling the hair cycle. This was done by first plucking the hairs of all the animals and then waiting for 21 days until the hair cycle of all the animals was estimated to be in the resting stage (telogen) (3). The results of this study, while too early to be definitive, apparently confirm the present findings: fourteen of 40 animals in the cortisone-treated group and only two of twenty animals in the control group have tumors.

It is interesting to note that no evidence of metastases could be observed at autopsy. This is in contrast to the results in certain experimental situations in which cortisone has been used with transplanted and spontaneous tumors (2, 7).

SUMMARY
1. The administration of cortisone increased the incidence of skin tumors in mice that received multiple paintings with methylcholanthrene.
2. This enhancement effect occurred even when cortisone was administered only during the early phase of the carcinogenic process.
3. There was some suggestion that carcinomas were more numerous in the mice receiving cortisone during the early phases only.
4. The cortisone did not result in the formation of metastases from the methylcholanthrene-induced carcinomas.

REFERENCES
Cortisone and Carcinogenesis in Mouse Skin I. Effect of Cortisone during Multiple Paintings with Methylcholanthrene

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