Repression of Methylcholanthrene-induced Epidermal Hyperplasia by Hydrocortisone*

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SUMMARY

Hydrocortisone, administered topically as the free alcohol, was found to repress completely the early epidermal hyperplasia of CAF1 mice caused by a single application of 20-methylcholanthrene. The effects were measured by epidermal and mitotic cell counts.

The present study was undertaken in the light of interest engendered by conflicting reports on the effect of glucocorticoids upon the development of skin tumors in mice treated with polycyclic hydrocarbon carcinogens (2, 4, 7-9, 13, 14, 17, 20, 21). Some investigators also found that systemically administered cortisone did not influence the increased mitotic rate (12) or epidermal hyperplasia (9, 17) initiated by methylcholanthrene. The histological quantitation of the epidermal mitotic rate and epidermal hyperplasia of mice which have been subjected to topical treatment with similar agents is presented here, because our findings are at variance with the results reported earlier by Green and Savigear (12), Piccagli et al. (17), and Gilman et al. (9).

MATERIALS AND METHODS

All mice used were hybrid CAF1 males, 2-3 months of age, housed in air-conditioned quarters. Animals were caged in groups of ten except for weight-loss controls, which required individual housing. Rockland mouse diet and water were given freely, except where otherwise noted. Application of carcinogen and hydrocortisone was accomplished by administering the materials dropwise from a calibrated glass pipet onto the midline of the animal’s back, which had been carefully clipped free of its hair with scissors between the shoulders and the midpoint of the back.

The following treatment groups were used: (a) 20-methylcholanthrene (MCA) preceded and followed by hydrocortisone, eighteen mice; (b) MCA alone, 27 mice; (c) MCA preceded and followed by acetone, 27 mice; (d) hydrocortisone† alone, eighteen mice; (e) acetone alone, eighteen mice.

The MCA was prepared as a 0.15 per cent solution in acetone. Each animal received 3 drops, containing a total of 0.1 mg. of the carcinogen, in a straight line over the midline area mentioned above. MCA was applied only once, at the beginning of the study period, in those mice which were given the carcinogen.

Hydrocortisone, when used in conjunction with MCA, was applied 1 day prior to the carcinogen and daily thereafter. On the day when both hydrocortisone and MCA were given, hydrocortisone was administered 15-30 minutes before the carcinogen. Five drops were applied, delivering a total of 1.0 mg. of hydrocortisone as the free alcohol form dissolved in acetone. The dose of acetone alone was always 5 drops, approximately 0.1 ml.

Weight-loss control animals were treated with MCA, then placed on a quantitatively restricted diet to produce a loss in weight similar in amount and rate to those mice treated by MCA plus hydrocortisone.

Animals were sacrificed by cervical dislocation on the days indicated by points on Graphs 1, 2, 3, and 4, between 11:00 A.M. and 2:30 P.M. The skin was removed from the area previously designated. It was spread out and allowed to adhere to a piece of stiff paper on which the midline of the skin was made to coincide with a pencil mark, the latter being used as a reference point. The specimen was

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then fixed in Zenker's formalin. After fixation the tissue was cut through the midline mark, embedded in paraffin, and sectioned at 6 μ, with both halves used. All tissue sections were stained by hematoxylin and eosin.

Cell counts were made by the method of Davibhadhana (6), with a net reticule measuring 7.6 μ under oil immersion objective lens with 10X oculars. The tissue section having the greatest degree of hyperplasia was selected from the two to four slides available from each sample, and on the selected slide the area which would yield the highest epidermal cell count was selected for quantitation. From the midpoint of the hypercellular focus, five reticule-widths of epidermal cells were counted in each direction. Counts from the epidermis of two mice were averaged for any given day of a given treatment group, with the exception of MCA and MCA plus acetone groups, in which cases three mice were used. The samples used for weight loss controls consisted of three mice at 7 days post-treatment, two mice at 10 days and one mouse at 14 days.

In this work no effort was made to correlate time of treatment or sampling with any particular stage of the hair cycle.

RESULTS

The influence of MCA alone or MCA followed by daily paintings of acetone (vehicle) produces a fairly marked hyperplastic reaction in the basal-cell layer (Chart 1) and an outstanding one in the spinous and granular cell layers of the epidermis (Chart 2). Chart 3 summarizes the hyperplastic response of all layers combined. The increase in cell count begins about the 3d day following MCA painting and appears to be correlated with the sudden increase in mitotic figures (Chart 4). Indeed, this explosion of mitotic activity explains the simultaneous and precipitous increase in both basal and differentiating (spinous plus granular)
cell layers. Most of the mitoses are present in the basal cells. The significance of the two peaks of mitotic activity in the MCA-treated groups is not apparent to us and may represent sampling variation.

Animals treated daily with hydrocortisone alone show basal and differentiating cell counts that are below those for controls which were given daily treatments of acetone alone. Those treated once with MCA and daily with hydrocortisone have basal and differentiating cell counts which roughly parallel those in the group which received hydrocortisone alone throughout, but somewhat exceed the latter from the 6th day onward (Chart 2). The hyperplasia-inducing action of the carcinogen appears to be completely suppressed by the hydrocortisone; moreover, mitotic figures are relatively rare.

The weight-loss control animals, which were treated with MCA alone, yield epidermal counts which are nearly identical to those in mice treated once with MCA and thenceforth with daily applications of acetone. The mice in these samples lost, on an average, 14, 21, and 31 per cent of their original weight by 7, 10, and 14 days, respectively. The corresponding losses among the MCA-hydrocortisone treated mice average 13, 13, and 17 per cent, respectively.

In examining these skin samples for information on the state of the hair cycle, we note that in the MCA plus hydrocortisone and the hydrocortisone alone group all but one animal in each group are in the resting phase. In the two exceptions, the mice had received the hydrocortisone for only 2–3 days prior to the time of sacrifice. However, the treatment groups of MCA alone, MCA plus acetone, and acetone alone are found to contain a number of animals in both resting and growth phases, the majority being in the resting phase.

**DISCUSSION**

The work of Green and Savigear (12) and, later, that of others (9, 17), showed that cortisone was relatively ineffective in suppressing both mitosis and hyperplasia induced by a strong carcinogen applied to the epidermis of mice. In our own study, the data indicate that topical application of large quantities of hydrocortisone was completely effective in suppressing the hyperplasia-inducing effect of 20-methylcholanthrene on mouse epidermis.

The discrepancy of observations noted here may be attributed possibly to differences in the method of investigation. In the first place, Green and Savigear and others utilized repeated applications of large doses of the carcinogen, whereas in the present study a single dose of a relatively concentrated solution was used. Secondly, the method of administration of cortisone was different in that the previous workers used a systemic route, whereas in our study topical applications were utilized. It is probable that the local concentration of the glucocorticoid, under the conditions of our experiment, was considerably greater than that produced by the systemic route. The local tissue concentration of the agents used is probably critical to the final establishment or repression of carcinogen-induced epidermal hyperplasia. It is evident that early hyperplasia ordinarily induced by a single dose of 0.1 mg. of 20-methylcholanthrene applied topically can be completely controlled by daily applications of 1 mg. of hydrocortisone (free alcohol in acetone) administered by the same route and over the time period studied here.

It seems likely, then, that the glucocorticoids exert a restraining influence upon the hyperplasia induced by topically applied carcinogens and that this may be involved in the inhibition of tumor formation found by many authors (2, 4, 7–9). Several workers (13, 14, 17, 20, 21), however, have more recently found that the glucocorticoids may increase the number of tumors formed per animal as well as the number of animals bearing them. The explanation for the latter effect appears to lie in the capacity of the glucocorticoids to delay the development of the growth stage of the hair follicle (15). Our findings that hydrocortisone-treated animals were, in nearly all instances, in the resting phase of the hair cycle seem to support the conclusion of Herrmann et al. (15) that cortisone...
administration produces an inhibitory effect on hair follicular proliferation resulting in protraction of the phase of follicular rest. Mice treated with the carcinogen, while the hair follicles are in the resting stage, have been found to be much more susceptible to the development of tumors (1, 3, 16). Thus, the activity of cortisone may influence the epidermal growth in two conflicting ways: one, by allaying the early hyperplasia so often associated with the application of an effective carcinogen; the other, by favoring the resting phase of follicular development, a phase more susceptible to the tumorigenic action of the carcinogen.

Bullough (5) has shown that biological stress may inhibit mitosis in the mouse epidermis but suggested that the phenomenon might be due to the action of adrenal glucocorticoids. Since our MCA-hydrocortisone-treated mice did lose weight over a period of days, some degree of biological stress was undoubtedly a contributing factor. If nutritional factors leading to weight loss were responsible for mitotic inhibition they could represent a complicating variable in our results. Hence, the study was controlled by using mice receiving only MCA and restricting their food intake to produce the amount of weight loss which was at least equivalent to and occurring over the same time period as that of the MCA-hydrocortisone-treated group. The loss of weight due to restricted food intake does not seem to be a major factor in restraining epidermal hyperplasia during treatment with MCA alone in our study. A more direct mode of action for hydrocortisone (5, 11), or through its more general anti-inflammatory effect (18, 19) by preventing the inflammatory reaction produced by the carcinogen, are two possible explanations of the data observed in the present study.

REFERENCES


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