

# GROWTH INHIBITION IN THE SKIN FOLLOWING DIRECT APPLICATION OF ADRENAL COR- TICAL PREPARATIONS <sup>1</sup>

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## ELEVEN FIGURES

Examination of the skin of rodents following adrenalectomy has shown that the adrenal glands exert a regulatory influence over the growth of hair. Butcher and Richards ('39), Butcher ('41) and Ralli and Graef ('43) observed accelerated growth of hair after adrenal ablation which began as early as two to three days following the operation. Stimulation of hyperplasia of the hair follicles and bulbs by adrenalectomy was sufficiently strong to overcome the atrophy of the growing portion of hair which had been induced by under-feeding (Butcher and Richards, '39) or by prior feeding of a diet deficient in the filtrate factors of the vitamin B complex (Ralli and Graef, '45). Conversely, the latter authors found that adrenal cortical extract and, particularly, desoxycorticosterone acetate reduced the hyperplasia of hair follicles and bulbs which followed adrenalectomy. Thus, it appears that the adrenal cortex normally acts directly or indirectly as an inhibitor of hair growth.

Likewise, it has been shown that parenteral administration of a hormone of the anterior hypophysis (adrenocorticotropin), may inhibit growth of hair, epidermis and, to a lesser degree, of sebaceous glands (Baker, Ingle, Li and Evans, this journal). In that report other evidence was summarized

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which suggests that adrenocorticotropin prepared by the method of Li, Evans and Simpson ('43) causes the accelerated release by the adrenal cortex of the 11-oxysteroids<sup>2</sup> (glucocorticogenic). Presumably, the growth inhibition which follows the injection of adrenocorticotropin is effected ultimately by these hormones. At least two basic questions are raised by this thesis. First, is this effect mediated by any other endocrine gland such as the hypophysis? Second, since the 11-oxysteroids are known to catabolize protein to form carbohydrate in the liver, is the liver an essential link in the growth-inhibiting action of the 11-oxysteroids?

In order to answer these questions, experiments have been carried out in which cortical steroids were applied directly to the end-organ (skin) and the growth of epithelial structures within it was studied. It was discovered that one 11-oxysteroid (11-dehydro 17-hydroxycorticosterone) and an aqueous adrenal extract stopped the re-growth of hair (Whitaker and Baker, '48). The present study deals primarily with the microscopic picture found in the skin of these rats and furnishes additional evidence that the 11-oxysteroids are responsible for the growth inhibition induced by adrenocorticotropin.

#### MATERIALS AND METHODS

The site chosen for study was the dorsal half of the neck since this area is relatively inaccessible to licking and scratching. The area studied was clipped weekly and photographic or sketched records made of the extent and location of hair growth during the preceding week. The hormones were applied just caudal to the right ear. Adult male rats (Long-Evans and Wistar strains) were divided into three groups and treated daily: (1) 13 rats received 0.1 cm<sup>3</sup> of a 1 mg per cubic centimeter solution of 11-dehydro 17-hydroxycorticos-

<sup>2</sup>By "11-oxysteroids" is meant those compounds isolated from the adrenal cortex which are oxygenated in the C-11 position and which, in general, affect protein and carbohydrate metabolism. These include corticosterone, 11-dehydrocorticosterone, 17-hydroxycorticosterone, and 11-dehydro 17-hydroxycorticosterone. In contrast, certain other compounds have no oxygen at the C-11 position, such as desoxycorticosterone, and these exert their main effect on electrolyte metabolism.

terone in 25% alcohol, (2) 8 rats received 0.1 cm<sup>3</sup> of 25% alcohol and (3) 5 rats, 0.1 cm<sup>3</sup> of aqueous cortical extract.<sup>3</sup> Eleven-dehydro 17-hydroxycorticosterone is an "11-oxysteroid" and the cortical extract used contains low concentrations of this and other cortical steroids oxygenated at the C-11 position. Each animal was kept in an individual cage. The studies were carried out from January 24, 1948 to March 22, 1948 and during this period the varying hair growth patterns in the alcohol controls (group 2) and in groups 1 and 3 for the first three to 4 weeks of treatment were, with the exception of minor variations, bilaterally symmetrical. Therefore, the left side of the neck served as the animal's own control as well as group 2 serving as a control for groups 1 and 3.

Skin biopsies of the right and left sides of the neck in all animals were made at varying intervals from 21 to 47 days after the beginning of treatment. Skin was taken from the same relative areas on both sides. Some of the biopsies were made with a no. 6 Keyes skin punch while in other cases, a strip of skin 3 mm wide extending from the dorsal mid-line to the lateral line of the neck was excised from both sides. This report is based on 14 paired biopsies from group 1, 8 from group 2 and 6 from group 3.

The skin samples were fixed in either Zenker-formol, Zenker-base, Carnoy's or Bouin's fluid and stained with eosin and methylene blue (disodium phosphate-citric acid and sodium acetate-acetic acid buffers), hematoxylin and eosin, the Weigert procedure for elastic fibers, and the Masson technique.

#### OBSERVATIONS

As observed macroscopically, percutaneous application of 11-dehydro 17-hydroxycorticosterone stopped re-growth of hair and formation of associated pigment in the skin of the area treated as early as three weeks after the initiation of

<sup>3</sup> We wish to express our appreciation to Dr. J. J. Pfiffner of Parke, Davis and Company, for supplying us with aqueous adrenal extract (Eschatin).

treatment. These processes were held in abeyance for the duration of the experiment. There was no disturbance in hair growth on the left side (non-treated area) in such animals, thus creating an asymmetry in the growth pattern. On the basis of the evidence at hand the 4 Wistar albino rats treated with 11-dehydro 17-hydroxycorticosterone did not show as complete an inhibition of hair growth at three weeks as did all of the 9 Long-Evans black-hooded rats. However, by the 4th or 5th week inhibition was equally as marked.

In group 2, treatment with the solvent, 25% alcohol, caused no significant disturbance in the growth pattern.

The cortical extract induced inhibition of hair growth which at 6 weeks was as extensive as that induced by 11-dehydro 17-hydroxycorticosterone at three weeks (fig. 11).

The microscopic picture of the skin was similar after treatment with 11-dehydro 17-hydroxycorticosterone and the aqueous extract except that in the latter instance a longer period of treatment was required to elicit the effects. In all cases changes observed in the treated areas are considered in relationship to the left or normal non-treated side of the neck.

*Hair.* It should be borne in mind that normal hair growth in the rat is characterized by alternate cycles of activity and inactivity. Furthermore, at any one time an inactive area may be adjacent to one of actively growing hair.

The hair follicles of the treated areas showed a picture of growth stasis (figs. 1 and 2, 3 and 4). In general, they extended down to the middle of the dermis and rarely did a follicle reach the panniculus adiposus. If it did, the follicle was small and inactive and in no case were growing hair bulbs observed. Epithelial buds were present but many of them seemed to be abnormal as compared with those in the areas of skin of the left side in which hair was not growing. In the former, the cells were smaller and their nuclei more compactly arranged (figs. 9 and 10). Sometimes the nuclei were of irregular shape and even pycnotic. Occasional evidence was obtained of actual atrophy of the inactive hair follicles as shown by loss of normal size and pycnosis of the

cells of the epithelial and connective tissue sheaths. In fact, it is probable that an actual reduction in number of follicles was elicited in some cases. This was most evident in biopsies removed after the longest period of treatment with 11-dehydro 17-hydroxycorticosterone (47 days) and the extract (55 days).

*Epidermis.* On the left (control) side, the epidermis was quite variable in its surface contour and thickness. Some of these irregularities probably resulted from contracture of the sample after excision. For the most part, three distinct regions could be delineated, namely, the stratum germinativum, granulosum and corneum. In all cases but one the treated area of the right side showed a definite thinning of the epidermis. The exceptional case had been treated for only 21 days but a slight reduction in thickness of the epidermis was indicated nonetheless. Such atrophic epidermis was characterized by a reduction in the number of cell layers in the stratum germinativum and diminution in size and flattening of the constituent cells (figs. 5, 6, 7, and 8). Sometimes, they were aggregated into clumps separated by stretches of extremely thin epidermis. The stratum granulosum, which on the left side was fairly prominent, was reduced greatly and frequently obliterated. In general, the stratum corneum was thinner after treatment but the extent of this change was difficult to evaluate.

*Sebaceous glands.* The sebaceous glands were exceedingly variable in number and size in the biopsies from the left side. With application of 11-dehydro 17-hydroxycorticosterone, no definite effect on the sebaceous glands could be ascertained as long as hair follicles were present. Towards the end of the experiment, if there was evidence of diminution in number of the hair follicles, then the sebaceous glands seemed to be reduced also.

*Dermis.* In general, the structure of the dermis in the treated areas was that of normal skin in which hair was not growing. The character of elastic fibers was unchanged. No clear evidence of interference with vascularity in histological preparations was apparent. The blood vessels located in the

panniculus adiposus were engorged with blood and capillaries were still present just beneath the epidermis. Mast cells appeared in considerable numbers especially in the deeper region of the dermis. Some evidence was secured of hyalinization of the collagenous fibers associated with degeneration of the connective tissue cells and pycnosis of cells in the sebaceous glands and in the sheaths of the hair follicles. The importance of this change cannot be evaluated since small patches of similar character occurred less frequently in control biopsies. However, such dermal modification seemed to increase with prolongation of the experiment, being most evident at 34 and 47 days. This suggests that if the treatment were continued beyond this period, degenerative changes in the dermis might be found to be a certain effect. The scarcity of the hormone prevented further study of this point.

The panniculus adiposus was reduced in thickness by 11-dehydro 17-hydroxycorticosterone as compared with areas of active hair growth in biopsies from the left side. Whether or not it was reduced to a significant degree beyond the state of normally inactive skin is problematical since some fat was generally present in treated areas.

The microscopic anatomy of the skin was not altered by application of 25% alcohol.

#### DISCUSSION

There is a considerable area of similarity in the effects on the skin of the parenteral injection of adrenocorticotropin (Baker, Ingle, Li and Evans, this journal) and percutaneous application of 11-dehydro 17-hydroxycorticosterone and an aqueous adrenal extract containing this and related cortical steroids. This resemblance was especially noticeable in the changes which occurred in the growth of the epidermis and hair. Likewise, there is no significant basic difference in the action of these substances as they were administered on the sebaceous glands. Thus, this information constitutes further evidence in support of the view that adrenocorticotropin prepared by the method of Li, Evans and Simpson ('43) stim-

ulates the release of at least some 11-oxysteroids by the adrenal cortex.

On the other hand, the increase in compactness of the dermis and the great reduction in the panniculus adiposus which followed the parenteral administration of adrenocorticotropin did not occur to as significant a degree after the percutaneous application of preparations containing adrenal steroids. One possible explanation for these differences may be that modification of the character of the dermis and the panniculus adiposus is dependent upon the general systemic action of the 11-oxysteroids. Thus, the injection of hypophyseal adrenocorticotropin stimulated the adrenals to produce them in quantities sufficient to exert a systemic effect while the effects of the hormone applied to the skin seemed to be limited to the area of application.

Much evidence is now available which shows that the adrenal cortex may modify growth processes to a significant degree. The relationship of adrenal cortical activity to the growth of hair was summarized previously. Ingle, Higgins and Kendall ('38) demonstrated that adrenal cortical extract (Cortin) inhibits the body growth of rats. Similar action was ascribed to corticosterone and 11-dehydro 17-hydroxycorticosterone, the latter compound being an unusually potent growth inhibitor (Wells and Kendall, '40b). Although adrenalectomy of young rats interferes with their growth (Hartman and Thorn, '30) when such rats are maintained on a salt regimen it has been possible to detect an increase in the width of the epiphyseal cartilage near the end of the second week after the operation (Wyman and Tum-Suden, '45). Wyman and Tum-Suden interpreted this effect as being due to accelerated secretion of the growth hormone by the hypophysis but, as they pointed out, it might also be explained on the basis of the release of the epiphyseal cartilage from the direct inhibitory action of adrenal steroids.

In addition, adrenalectomy has been reported to cause hypertrophy of lymphoid tissue (Reinhardt and Holmes, '40) although the data of Stoerck ('44) indicate that thymic hy-

perplasia does not follow adrenalectomy. Corticosterone and concentrated extract (cortin), but not desoxycorticosterone, cause thymic involution (Wells and Kendall, '40a). It should be noted from the work of Dougherty and White ('45) that the regulation of lymphocyte dissolution by the hormones of the adrenal cortex is probably an important factor in these modifications of lymphoid tissue. However, as yet one cannot exclude the control of hyperplasia as also being of importance. Some recently reported evidence tends to detract from the theory that lymphocyte dissolution is the sole cause of lymphoid atrophy in hyperadrenocortical states. Continued treatment with adrenal cortical steroids did not accelerate antibody production which indicates a failure of these substances to maintain continuous dissolution of lymphocytes (Eisen, Mayer, Moore, Tarr and Stoerck, '47). Although Nordenson ('47) reported the blood lymphocyte picture in man to be unaltered by treatment with adrenocorticotropin, a lymphopenia was observed by Forsham et al. ('48). These conflicting findings indicate that more attention should be given to the possibility that cortical steroids cause atrophy of lymphoid tissue by inhibiting the proliferation of cells as well as by causing their breakdown.

Biochemical information is available to provide some basis for explanation of the growth inhibition caused by the C-11 oxygenated steroids. Long, Katzin and Fry ('40) found that administration of these substances causes a rise in liver glycogen and in excretion of urinary nitrogen indicating increased protein catabolism and gluconeogenesis. The amount of nitrogen excreted under the influence of adrenal cortical steroids in their experiments was so great that these workers felt that some of the catabolized protein must have been derived from tissues other than the liver. As far as the epidermis and hair growth are concerned in our experiments not only does it appear that cellular proliferation had been inhibited by the 11-oxysteroids but, also, that an actual reduction in size of the epidermal cells had occurred. The observation that epithelial atrophy may be induced by the local application

of 11-oxysteroids also suggests a stimulation of protein catabolism in tissues other than the liver. Regardless of how protein loss may be involved in the inhibition of growth our results show that *protein catabolism in the liver is not prerequisite to inhibition of growth by 11-dehydro 17-hydroxycorticosterone or cortical extract.*

Until the present time it has not been clear whether adrenal cortical steroids inhibit growth directly or by inhibition of the secretion of growth hormone by the anterior hypophysis. Thus, after observing retarded body growth when corticosterone or 11-dehydro 17-hydroxycorticosterone was administered to growing rats, Wells and Kendall ('40b, p. 327) observed that "It has been suggested that certain effects of corticosterone are referable to depression of pituitary activity and it seems probable that the inhibition of growth by this and related compounds may have a similar explanation. . . . Whether this inhibition of growth is due simply to suppression of the production of the growth factor of the pituitary or to some direct influence of the compounds on metabolic processes is not clear." Our results do not rule out the possibility that injection or oral administration of cortical compounds may suppress release of the growth hormone. Nevertheless, they do demonstrate clearly that certain cortical steroids are *capable of suppressing growth of body tissue directly rather than by first inhibiting secretion on the part of the hypophysis or any other gland.*

Finally, the capacity of cortical steroids to inhibit growth by a peripheral action has a significant bearing upon another currently-held endocrine concept. White and Dougherty ('47) have postulated on the basis of data obtained in the mouse that adrenal steroids regulate the mobilization of protein from lymphoid tissue and that the thyroid gland performs the same function in respect to the carcass. Our experiments do not indicate the precise manner in which cortical steroids inhibit growth. Nevertheless, it seems reasonable to expect that ultimately the growth of cutaneous epithelial structures is stopped either by accelerating the loss of nitrogen from

them or by inhibiting their synthesis of it. If this be true, then the belief that the adrenal cortex mobilizes protein only from lymphoid tissue is open to question. As a corollary of their thesis, White and Dougherty postulated that cortical steroids may mobilize carcass nitrogen by first stimulating the thyroid. This, likewise, could not be used to explain the results which we obtained.

#### SUMMARY

The application of 11-dehydro 17-hydroxycorticosterone and aqueous adrenal extract to one side of the dorsum of the neck of rats was found to inhibit the growth of hair in the area treated. Microscopic examination of biopsy samples of the skin excised from treated and non-treated areas, showed that such treatment caused thinning of the epidermis and failure of hair follicles to develop beyond the stage of epithelial bud formation. These findings show that adrenal steroids may inhibit growth directly without the mediation of this action by any other endocrine gland or organ. Also, it is demonstrated that the catabolic action of the 11-oxysteroids on protein in the liver is not pre-requisite to their growth-inhibiting effects.

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## PLATE 1

### EXPLANATION OF FIGURES

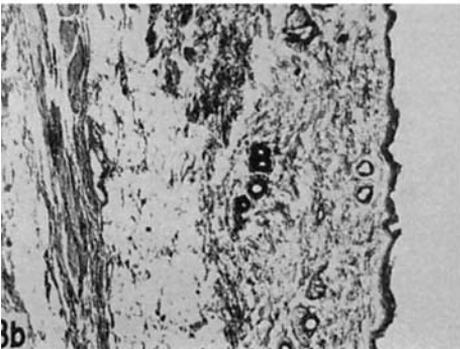
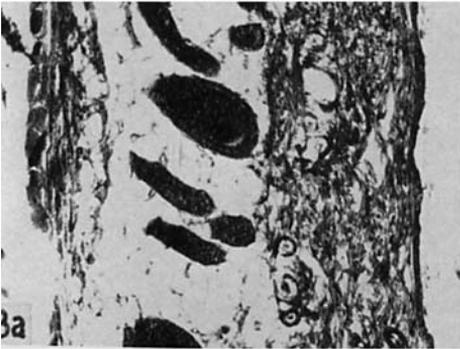
All preparations illustrated on this page were strip biopsies stained with Harris' hematoxylin and eosin.  $\times 60$ .

1 Left (non-treated) side of neck of W63. Hair is in a state of active growth and the bulbs are surrounded by a well-developed panniculus adiposus. Bouin fixation.

2 Right side of neck of W63 treated for 21 days with 11-dehydro 17-hydroxy-corticosterone. Hair growth is stopped, the panniculus adiposus is reduced although a significant amount is still present. Sebaceous glands are still large. Bouin fixation.

3 Left side of neck of BH45. 3a is of an area of active hair growth with a thick panniculus adiposus and 3b of an inactive area with a reduced but still significant panniculus adiposus. Carnoy fixation.

4 Right side of neck of BH45 treated with aqueous adrenal extract for 48 days. Hair is not growing and the panniculus adiposus is reduced. The lightly stained area below the hair follicles consists of hyalinized connective tissue. Carnoy fixation.



## PLATE 2

### EXPLANATION OF FIGURES

5 Punch biopsy, left side of neck of BH64. The epidermis is thick, the stratum germinativum consists of several layers of cells and the stratum granulosum is present. 10  $\mu$ . Bouin fixation. Hematoxylin and eosin.  $\times$  272.

6 Punch biopsy, right side of neck of BH64, treated with 11-dehydro 17-hydroxycorticosterone for 24 days. The epidermis is thinner, the cells are smaller and the stratum granulosum greatly reduced. Technique and magnification as in figure 5.

7 Strip biopsy, left side of neck of BH54. The epidermis is several cell layers thick and the stratum granulosum is present. 10  $\mu$ . Carnoy fixation. Hematoxylin and eosin.  $\times$  272.

8 Strip biopsy, right side of neck of BH45 treated with adrenal extract for 48 days. The epidermis is greatly thinned. At the right is a clumping of epidermal cells, these aggregations being observed frequently in treated areas. Technique and magnification as in figure 7.

9 Left side of neck of W63. Epithelial bud at the lower end of a hair follicle. 10  $\mu$ . Hematoxylin and eosin.  $\times$  296.

10 Right side of neck of W63 treated for 21 days with 11-dehydro 17-hydroxycorticosterone. The epithelial bud at the lower end of the hair follicle illustrates the stage in which hair growth was stopped. The cells composing it are dense and atrophic as compared with those illustrated in figure 9. Technique and magnification as in in figure 9.

11 The area of skin caudal to the right ear of this rat had been treated with adrenal extract for 48 days. Notice the asymmetry in the hair growth pattern created by the inhibition in the treated area.

