REACTION OF REGENERATING EPITHELIAL CELLS IN THE SKIN TO HORMONES

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The reparative capacities of the epidermis have repeatedly attracted the attention of experimentors [1, 3, 11]. The study of hormonal influences on the epithelialization of injured surfaces has not been made. Various authors indicate in a general way the dependence of epithelialization upon one or another hormonal influence [5, 6, 12]. In this regard, data concerning the regard, data concerning the reaction of epithelial cells in different layers of regenerating epidermis have not been obtained.

We set out to follow the effects of hormones on cells in the regenerating epithelium of the skin, which goes through different phases of specific differentiation. Two groups of hormones were used. One group, conditionally called adrenal cortical hormones (cortisone, hydrocortisone, prednisolone) and ACTH sharply stimulate differentiation. The other group, including desoxycorticosterone acetate (DOCA), somatotrophin and thyroid hormone, mainly activate processes of proliferation in young tissue.

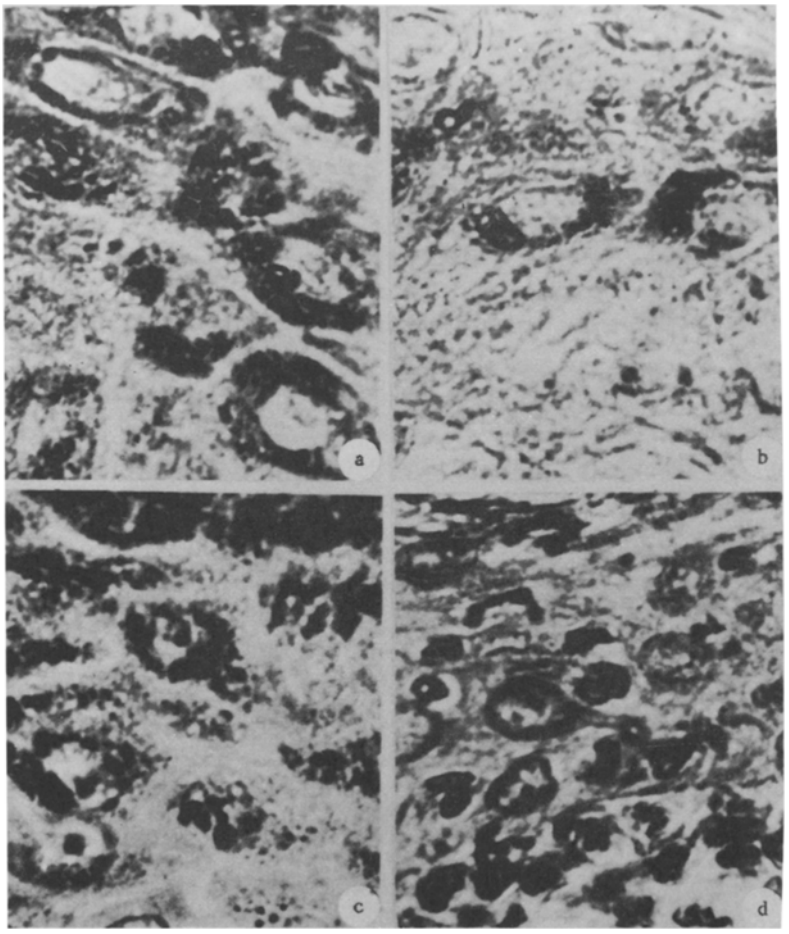
METHODS

The experiments were performed on 163 white rats weighing 120-180 g. All animals were divided among 8 groups. The first group served as controls. The second received cortisone (5 mg); the third—hydrocortisone (3 mg); the fourth—prednisolone (3 mg); the fifth—DOCA (3 mg); the sixth—ACTH (5 units); the seventh—somatotrophin (2 mg) and the eighth—thyroidin (5 mg). The hormonal preparations for the experimental animals were administered from 3 days before the injury [4]. Ten days after the operation the animals were sacrificed; a small piece of young tissue adjoining the region of the wound was removed from the edge of the wound.

The material was fixed in Bouin's, Shabadash's and Carnoy's fluids, embedded in paraffin or celloidin-paraffin. Sections were stained with hematoxylin and eosin, picrofuchsin and azan (Heidenhain). In addition, sections were studied with several histochemical methods. The PAS reaction for polysaccharides, determination of acid mucopolysaccharides (method of Hale), RNA reaction (Brachet), SH-groups (method of Barrnett and Seligman using 2,2' dihydroxy-6,6' dinaphthyl disulfide).

RESULTS

Within 10 days after infliction of the wound the epithelial wedge in control animals consisted of 7-9 layers of cells. The structure of the regenerated part and the adjoining portion of the old epidermis were significantly distinguishable from the structure of the skin under normal conditions. Differentiation in many layers decreased from the moment that epithelial regeneration started; basal elements altered their inherent prismatic form, became spread out and thus increased the area covered. Cells of the middle layers were rather large in size with round, clear nuclei often containing one to two nucleoli. The stratum granulosum of the regenerating epidermis was poorly demarcated; granules were scattered and sparse and were not seen in every cell. The stratum corneum had not yet appeared. The basal surface of the epithelial wedge more often was relatively flat. However, the growing epidermis often forms protuberances of varying size and contour which take root in the underlying tissue. The active cells, as a rule, are arranged in broader sheets in the regenerating area. These cells undergo mitosis not only in the basal, but also in several higher layers.



Histological structure of regenerating epithelium under the action of different hormones. a) Control; b) cortisone, c) prednisolone, d) hydrocortisone. a,b—Periodic acid-Schiff reaction; c,d—hematoxylin-eosin. Magnification 1800 ×.

A border zone of old epithelium, adjoining the defect, was markedly hypertrophied—the thickness $1^1/_2$-2 times that in more remote areas. In this zone the cells of the stratum granulosum contain many keratohyalin granules. The uninjured epidermis consists of 5 or 6 layers of which 3, as a rule, contain keratohyalin granules.

The layer of young epithelium in the control animals was rich in neutral polysaccharides. A more intense staining in the Schiff reaction was seen in the stratum spinosum and stratum granulosum. Single small granules of glycogen, merging together, fill the entire cytoplasm (see Fig. a). Cells of the basal layer of the regenerating area and those throughout the entire epidermis in uninjured portions of the skin—it is important to note this—do not stain with the Schiff reagent. After control treatment of sections with amylase, the cells of the middle layers of regenerating epithelium do not react to the stain. This permits us to propose that the polysaccharide present in young epithelial cells is glycogen. The presence of glycogen in regenerating epithelium has been noted by other authors, also [2, 7].

On staining the sections with the Hale method, the stratum granulosum both in uninjured skin and in the border of the injured area shows granules which stain a deep green color.

The distribution of ribonucleoprotein (RNP) in uninjured and new areas of the epidermis is under the same regulation. Most of the RNP is contained in the intensely active cells of the basal layer. In cells of this and the stratum spinosum the RNP comprises small pink granules which fill the entire cytoplasm but which become more diffuse on staining of the superficial layers.

On evaluation of SH-groups the cells of all layers are observed to stain equally. The cell nuclei are especially clear and only the nucleoli have an intense pink color. The cells of the regenerating epithelium are more weakly stained than cells from uninjured epidermis. The most intense diffuse staining is characteristic for cells of the stratum corneum.

Under the effect of cortisone the epithelialization of skin wounds undergoes significant changes. Parts of the epithelium adjoining the injured area are only slightly hypertrophied. A thinning out of the uninjured epidermis and epithelial regenerate appears. The uninjured epidermis consists of 4-5 layers, distinctly demarcated into basal layer, stratum spinosum, and 2 or 3 layers of cells which stain most intensely. A thin, close-packed wedge of regenerating epithelium forms 3-5 layers of closely packed cells. The latter have elongate shapes. Cortisone produces a marked speeding up of the cornification of epithelial cells. In the superficial layers the young tissue very early develops keratohyalin granules.

In animals which have received cortisone, the content of neutral polysaccharides is significantly reduced in cells of the regenerating epithelium. The presence of glycogen in this regenerating area is restricted to 2 or 3 layers of cells (see Fig. b). Decrease in the glycogen level in young epithelium treated with cortisone is evidently explained by the increase in cornification [8-10]. Cells of all layers in regenerating and intact areas contain little RNP and stain a pink color very weakly.

On studying preparations treated for SH-groups, the presence of an intensive pink color in the basal layer of regenerating high prismatic cells is of particular interest. In the nucleus of such cells tiny granules as well as the nucleoli were well revealed. In general, young epithelial cells in the animals receiving cortisone are characterized by more intensely staining cytoplasm and nucleus as compared to the staining of similar cells in intact mice.

Concurring in character, but still more evident disturbances in the structure of the new epithelium occur with prednisolone and especially with hydrocortisone treatment. Under such conditions, as with the cortisone effect, a more rapid cornification takes place in the young cells. The cells of regenerating epithelium contain many large granules of keratohyalin (see Fig. c). In animals which had received hydrocortisone a marked infiltration of neutrophils into the regenerating epithelium occurred. The number of migrating cells was so great that the boundaries of the cells and their basement membranes were barely apparent (see Fig. d). Under the action of prednisolone and hydorcortisone the content of polysaccharides and RNA in the new epithelial structures were significantly diminished.

The effect of glucocorticoids on epithelialization of wounds is very similar and even coincident with the effect of ACTH, which later, as it is well known, acts through the adrenals. With ACTH the epithelial layers in both intact and regenerating portions of the skin appear thinner. Accumulation of keratohyalin granules under such conditions is poorer; the amount of glycogen and RNP in the epithelial cells is decreased but not in such degree as with glucocorticoid treatment.

The epithelial cells react entirely differently when DOCA is given to the rats. DOCA, as is known, is a mineralocorticoid. Its effect on young epithelium is to cause intensive proliferation; the epithelial wedge becomes much longer and higher. It comprises 10-12 layers of cells at the very edge of the uninjured zone; this number reaches 20. DOCA stimulates the synthesis and accumulation of RNP and glycogen in the new epithelial cells. The content of SH-groups is only slightly altered from the control.

The addition of somatotrophin leads to a splendid growth of epithelium. The young tissue is smaller in extent than in the control and other groups. The young cells of the regenerating tissue are large, sometimes losing their polarity and regular distribution. This appears particularly in cells of the basal layer. The internal surface of the young epithelium in individual animals is uneven; small protuberances at its base indent the young connective tissue. Among the young cells mitotic figures are frequently noted. The cells are rich in glycogen and RNP. The content of SH-groups is moderate both in intact and in regenerating tissue.

In animals which received thyroid the regenerating tissue is pushed underneath a small scab by a layer of horizontally oriented fibroblasts. The epithelial wedge was solidly rooted in the underlying tissue. The cells contain much RNP and many glycogen granules appear in the epithelium in both the regenerating area and the central part of the protuberances. The glycogen granules, as in other groups, are absent from cells of the basal layer and from the uninjured epidermis.

This exposition permits us to make the following generalization. The epithelium represents a single tissue complex, but the reactions of its constituent cells as a result of various hormone treatments is far from uniform. On

application of glucocorticoids and ACTH, proliferation of the young cells is impeded and regeneration as a whole impaired; cellular differentiation is speeded in basal and middle layers; the content of glycogen and RNP diminished. On the contrary, under the action of DOCA somatotrophin, and thyroidin an intense proliferation of epithelial cells is noted and is accompanied by increased synthesis and accumulation of glycogen in the middle layers and of RNP in the stratum germinativum.

SUMMARY

As revealed with the aid of histological and histochemical methods, the properties and reactions of the cells comprising the regenerated epithelium are far from being uniform under the action of various hormones. Administration of glucocorticoids and ACTH inhibits proliferation of young cells and of the regeneration epithelium as a whole; cellular differentiation is accelerated in the basal and medium layers, the content of glycogen and of RNP decreases. Conversely, under the effect of DOCA, STH, and also of thyroidin there is an intensive cellular proliferation in the epithelial layer, accompanied by the rise of synthesis and accumulation of glycogen in the cells of the medium layers, and of the RNP—in the basal one.

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