

THE EFFECT OF HYDROCORTISONE ON SKIN IN ORGAN CULTURES

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Organ cultures of adult human skin, in which fragments of skin retain their characteristic structure and function are useful in studying substances thought to influence growth *in vivo*. In this paper the effect of two glucocorticosteroids, hydrocortisone hemisuccinate and prednisolone 21 phosphate on the growth of human epidermal cells in organ culture is described. Hydrocortisone did modulate the behavior of cultured skin in that both epidermal cell migration and epidermal DNA synthesis were inhibited, but neither steroid induced any unique morphological pattern of growth.

Materials and Methods

Slices of normal human adult skin approximately 0.4 mm. in thickness were removed from fresh surgical specimens with a Castroviejo keratome. Explants of 3 mm² were placed dermis side down on sterile lens paper, the edges of which had been waxed and the paper floated on the surface of liquid culture media. The culture medium was a mixture of equal parts of pooled normal human serum and tissue culture

medium 199 containing neomycin 100 µg per ml. The cultures were incubated at 32°-36°C. in an atmosphere of moist air. Hydrocortisone hemisuccinate¹ or prednisolone 21 phosphate² was added at the outset to various cultures in concentrations ranging from 0.04 mg. per ml. to 2.0 mg. per ml. The cultures were incubated for periods of up to 15 days with a change of medium on alternate days. Tritiated thymidine was added for the final three hours (1 µc per ml. specific activity 6.7 curies per millimole)³ after which the explants were fixed in Helley's solution, histological sections prepared and stained with haematoxylin and eosin and the PAS stain. Autoradiographs made with stripping film⁴ were stained with haematoxylin after three week's exposure.

Results

A total of 112 explants were cultured in three separate experiments with hydrocortisone hemisuccinate in concentrations of 0.2, 0.5, 1.0 or 2.0 mg. per ml. Fifty-six explants were cultured with prednisolone

¹ Solucortef[®], the Upjohn Co.

² Hydeltasol[®], Merck, Sharp & Dohme.

³ New England Nuclear Corp.

⁴ Kodak AR10.

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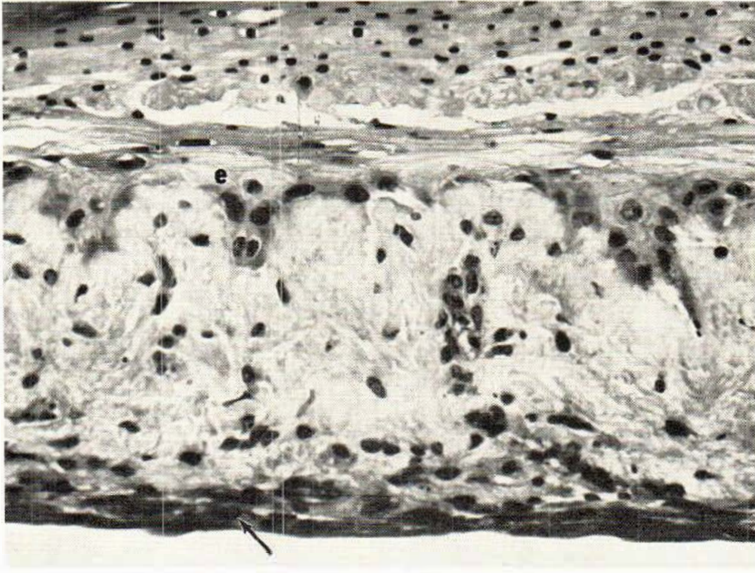


Fig. 1. Control explant of normal adult skin grown for seven days. Surviving epidermal cells in the deeper layers of the surface epidermis (e) are irregular in shape and are poorly oriented towards each other. The exposed surface of the dermis is covered with a stratified layer of cells (shown by the arrow) which have migrated from the cut edge of the surface epidermis.

21 phosphate at concentrations 0.04, 0.2 and 0.4 mg. per ml. Together with the simultaneous control cultures a total of 173 normal control explants were available for histological comparison (1). Three quarters of such normal control organ cultures showed evidence of growth during 15 days in vitro, in one of three main growth patterns. In the most common pattern, shown by 36 per cent of control explants, epidermal cell migration occurred from the cut edges of the explant around the free surface of the dermis, so as to enclose it completely, this process being termed epiboly. In the second pattern, shown by 26 per cent of the controls, epidermal migration was slight or absent and the epidermis became irregularly thickened with projections downwards into the dermis. Basal layer cells were often labeled with tritiated thymidine and showed mitotic figures. In the least common growth pattern, shown by 10 per cent of the controls neither epidermal cell migration nor epidermal thickening occurred. Instead the viable epidermis remained as a thin layer of large spindle-shaped cells which, although irregular in shape and poorly oriented to-

wards each other, were able to incorporate tritiated thymidine and divide.

Hydrocortisone produced concentration dependent effects on both epidermal cell morphology and on the overall growth patterns of the explants. With from .5 to 1 mg. per ml. the following differences were recognized in cultures of up to one week's duration. The stratified squamous architecture of the epidermis was less disorganized in the presence of hydrocortisone than in corresponding controls: the basal cells were small and arranged in a more orderly manner to their neighbors (Figs. 1 and 2). There was inhibition of the uptake of tritiated thymidine by basal layer cells and in most of the hydrocortisone treated explants basal cell mitoses were not seen. However, in two out of three explants cultured with 1 mg. hydrocortisone per ml. for five days up to two per cent of the basal layer cells showed mitotic figures. Yet even in these explants, in which mitotic activity was present, the basal layer cells did not incorporate tritiated thymidine.

Although each of the three growth patterns seen in the controls did occur, epidermal cell migration was diminished and

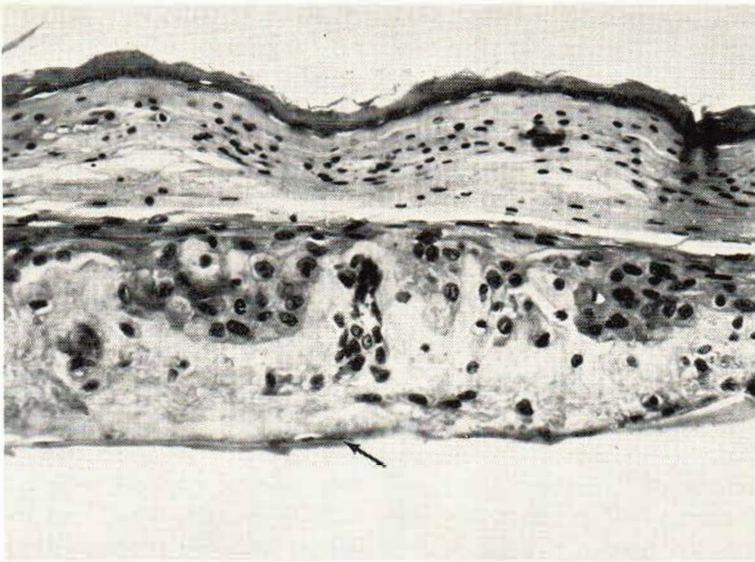


Fig. 2. Explant of skin from the same specimen as in Fig. 1, grown with 1 mg. hydrocortisone hemisuccinate per ml. for seven days. Surviving epidermal cells in the deeper layers of the surface epidermis (e) are smaller and arranged in a more orderly manner to each other than in the corresponding control explant. Migration of epidermal cells around the exposed surface of the dermis shown by the arrow, has occurred to only a slight extent.

only 13 per cent of the growing hydrocortisone explants showed epiboly compared to 50 per cent of the controls.

By the tenth day of culture explants grown with 1 mg. of hydrocortisone per ml. were degenerate while these with .5 mg. per ml. resembled their corresponding controls, having large germinative cells irregularly arranged. This may represent an escape from the restraining influence of this concentration of hydrocortisone after one week of culture. At low concentration, 0.2 mg. per ml., hydrocortisone had no effect on the behavior of the explants. At high concentration, 2 mg. per ml., the explants were well preserved during the first two days, resembling uncultured skin. However, no epidermal migration occurred and all explants cultured for more than two days with this concentration degenerated.

Prednisolone at low concentrations had no obvious effect on the pattern of explant growth and at high concentrations it produced irregular effects. Thus with .2 mg. per ml., although most cultures degenerated within 10 days, some grew as well as corresponding controls and were still able after

13 days to incorporate tritiated thymidine. With .4 mg. per ml. some explants behaved like control explants for up to one week while older cultures were uniformly degenerate.

Discussion

Although the morphological differences between controls and the cultures grown on media containing from .5 to 1 mg. per ml. of hydrocortisone are by no means gross they do suggest that hydrocortisone in this concentration range slows down the migration and differentiation of epidermal cells which occur in control cultures. However, in contrast to hydrocortisone, equivalent concentrations of prednisolone do not tend to preserve the general architecture of the explants, nor to retard their degeneration.

Previous studies on the effects of corticosteroids on epidermal cell growth in other species reveal differences in response between embryonic and adult skin. Fell (3) has shown that hydrocortisone accelerates the rate of differentiation of chick embryo epidermis in organ culture, whereas Gillette

and his colleagues (4) have shown that cortisone acetate will prolong in vitro survival of adult mouse skin. Glucocorticoids can also retard the growth rate of epidermal cells in vivo. When hydrocortisone is injected intradermally in adult mice the rate of epidermal cell mitosis in normal skin is reduced (2), this effect being due to an action on the cell during interphase rather than to direct interference with mitosis itself. Lahtiharju (5) reported that the systemic administration of dexamethasone to mice caused a fall in the number of epithelial cells able to incorporate tritiated thymidine in vivo in skin and stomach. Similar injections of the mineralocorticoid desoxycorticosterone had no effect. Furthermore the systemic injection of prednisolone in adult rats reduced the mitotic activity in epidermal cells of skin and epithelial cells of stomach in vivo, while the mineralocorticoid desoxycorticosterone produced an increase in mitotic activity in these epithelia after systemic administration (6). The results of the present organ culture experiments are in general agreement with published work on the effects of glucocorticoids on adult epidermal cells in vivo and in vitro.

A comparison between the concentration of hydrocortisone which was found to have an effect on the skin organ cultures, 0.5 to 1 mg per ml. and the concentrations likely to be obtained in clinical practice can be made on the basis of figures published by Schlagel and Sanborn (7). These authors found that the weight of topical preparations required for total body inunction could vary from 7.7 to 114.8 G depending on sparing or "ad lib" application. Assuming a surface area of the average adult male of 1.8 m² this gives a concentration range for topically applied one per cent hydrocortisone ointment of 4.3-64.5 µg hydrocortisone per cm². The skin explants, 3×3 mm. square, were floated on 5 ml. volumes of medium in dishes of 16 cm² area. When the concentration of hydrocortisone in the medium was .5 mg. per ml. this approximately corresponds to a concentration of 150 µg per cm² of skin. If one per cent hydrocortisone ointment is applied under

an occlusive dressing, it is possible that the resulting increase in absorption will compensate for these calculated differences in concentration.

SUMMARY

The effects of hydrocortisone hemisuccinate in the concentration range 0.2-2 mg. per ml. were studied on 112 organ cultures of normal adult skin maintained for up to 15 days. At low concentrations no effect was noted. At high concentrations all the cultures degenerated. However, with .5 to 1 mg. per ml. the following differences from control explants were seen. 1) The stratified squamous architecture of the epidermis was less disorganized. 2) The basal cells were smaller and arranged in a more orderly manner to each other; 3) DNA synthesis by basal layer cells was inhibited and 4) epidermal cell migration was diminished. The effect of prednisolone 21 phosphate in corresponding concentrations was studied on 56 explants. It did not tend to preserve the general architecture of the explants, nor retard their degeneration.

It is suggested that the concentrations of hydrocortisone found to have an effect in vitro are of the same order of magnitude as those likely to be obtained during topical clinical application of a one per cent hydrocortisone ointment under occlusion.

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