THE EFFECT OF HYDROCORTISONE ON THE RESPONSE OF FETAL RAT SKIN IN CULTURE TO ULTRAVIOLET IRRADIATION

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Cortisone and hydrocortisone are known to have a beneficial effect on various diseases and injuries that cause cellular breakdown in the skin. The cutaneous lesions of pemphigus, various chronic dermatoses (Kanof, 1961), ultraviolet (U.V.) (Järvinen, 1951) and x-radiation (Mellett, Walter, and Houghton, 1961) as well as those of systemic lupus erythematosus (Soffer, 1961) respond well to cortisone treatment. The reason for this is not known; the hormone might act through the vascular system, through a systemic response or directly on the injured tissue.

Other work being done in this laboratory prompted the idea that some of the damage to skin exposed to U.V. radiation might be caused by the release of hydrolytic enzymes into the tissue from degenerating cells, and that the beneficial effect of cortisone might be due, at least in part, to an inhibition of this liberation. The results that led to this idea were briefly as follows.

The resemblance between the effects of papain protease and vitamin A on cartilage matrix in culture caused Fell and Thomas (1960) to suggest that the breakdown of intercellular material in cartilage and bone grown in the presence of excess of vitamin A, was due to an enhanced activity of hydrolytic enzymes, possibly owing to "their increased liberation through an increased permeability of the cells or their organelles." Dingle, Lucy, and Fell (1961) and Lucy, Dingle, and Fell (1961) provided evidence in support of this view, and Fell and Dingle (unpublished) demonstrated that cartilage grown in the presence of excess of vitamin A both released and synthesized much more acid protease, probably cathepsin, than controls in normal medium. Gianetto and de Duve (1955) had found that the acid hydrolases, including cathepsins, of liver cells were situated in cytoplasmic granules which they termed "lysosomes." Dingle (1961) suggested that the increased proteolytic activity of the vitamin A–treated cartilage might be due to an action of the vitamin on lysosomes in the cartilage cells, and he was able to show that vitamin A added to the large granule fraction isolated from various tissues, including cartilage, released an acid protease. Fell and Thomas (1961) found that the effect of vitamin A on bone and cartilage in culture could be largely inhibited by hydrocortisone, but nothing was known about the cellular mechanism of this inhibition.

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Since severe damage to skin frequently follows exposure to ultraviolet light in such diseases as systemic lupus erythematosus (Rothfield *et al.*, 1961, Rothfield and Weissmann, 1961), it seemed possible that U.V. light caused a breakdown of the lysosomes of the cutaneous cells similar to that produced by vitamin A in cartilage and in the isolated organelles, and that hydrocortisone might inhibit this release, possibly by stabilising the lysosomal membranes. To investigate this possibility, Weissmann and Dingle (1961) isolated the large granule fraction from rat liver and exposed it to a mixed beam of U.V. light; they found that the irradiated organelles rapidly disintegrated, liberating an acid protease. If, however, the rats were pretreated with hydrocortisone, the granules released far less protease than did those isolated from normal animals.

The experiments described in the present paper, were undertaken to see whether hydrocortisone would protect fetal rat skin grown in organ culture from the full effects of U.V. irradiation, in the same way that it partially protected embryonic cartilage and bone (Fell and Thomas, 1961) and skin (Fell, 1962) in organ culture, against the action of vitamin A. If the hormone had a beneficial effect on skin irradiated in culture, this would be clear evidence of a direct protective action of hydrocortisone on the cells, since all systemic and vascular effects are eliminated from an *in vitro* system. The results recorded below show that the hormone does exert a direct action of this type.

Material and Methods

Details of the material and methods used in each experiment are given in Table I.

Organ culture.—Hooded rats from an inbred but not pure laboratory strain were used, and fetuses were taken at various periods between the 17th and last day of gestation. Skin was stripped from the trunk and upper part of the limbs and cut into fragments 2 to 3 mm in diameter.

Cultures were grown by Shaffer's modification (1956) of the watch glass method (Fell and Robison, 1929). The culture vessel consisted of a watch glass (4 cm in diameter) enclosed in a Petri dish (8 cm in diameter) carpeted with absorbent cotton wool saturated with 10 ml of sterile distilled water to provide a moist chamber. The medium in the watch glass was a mixture of 3 parts of cock plasma and 1 part of chick embryo extract prepared by mixing equal quantities of a pulped 13 day embryo and tyrode supplemented with 1 per cent glucose. The skin explants were spread, epidermis upwards, on squares of rayon acetate cloth, each square being about 0.5 cm in width. Four explants were grown in each watch glass.

Hydrocortisone sodium succinate (solu-cortef, Upjohn Co., Kalamazoo) dissolved in sterile distilled water, was added to the plasma so as to give a concentration of 7.5 μg/ml in the final culture medium; the same quantity of distilled water was added to the plasma of the controls.

It seemed probable that the hormone would take much longer to reach the cells by diffusion from a plasma clot, than it would *in vivo* with the benefit of a blood circulation. The explants, therefore, were grown for 2 days in hydrocortisone-containing (HC) and normal medium, respectively, before being irradiated. Immediately after irradiation, they were transferred to freshly prepared HC and control media and grown for a further 1 to 8 days, with transplantation at 2 day intervals.

Irradiation.—The source of radiation was the same as that used by Weissmann and Dingle (1961) *viz.* an Hanovia Lamp, No. 11. Most of the experiments (Table I) were made with the unfiltered light, since the object of the irradiation was merely to cause a reproducible degree of necrosis in the skin, not to study the biological effects of monochromatic radiation. This

TABLE I

This table shows the design of the experiments and the number of explants histologically examined in each. The skin used for the experiments of group 1 was at a much earlier stage when explanted than that for group 2. In the 9th column 2 + 1 etc. means 2 days before and 1 day after irradiation.

Exp. No.	Group	No irradiation			Irradiation					
		Culture period	No. explants		Filter	Distance	Duration	Culture period	No. explants	
			HC	C					HC	C
		days				*in.*	*min.*	*days*		
349	1	3	1	1	None	15	5	2 + 1	1	1
		4	1	1				2 + 2	1	1
		5	1	1				2 + 3	1	1
		6	1	1				2 + 4	1	1
350	1	2	4	4	"	15	5	2 + 1	4	4
								2 + 6	8	8
351	2	2	4	4	"	15	5	2 + 1	4	4
								2 + 4	4	4
								2 + 6	12	8
354	2	2	4	4	"	9	2	2 + 1	4	4
		8	3	3				2 + 2	4	4
								2 + 4	4	4
								2 + 6	4	4
357	2	2	4	4	OX7 transmits 3100 to 3800 A	9	2½	2 + 1	4	4
								2 + 2	4	4
								2 + 4	4	4
								2 + 6	8	8
								2 + 8	4	4
366	2	2	—	8	Quartz + water	9	2	2 + 1	—	4
								2 + 2	—	4
					Quartz			2 + 1	—	4
								2 + 2	—	4
					None			2 + 1	—	4
								2 + 2	—	4

lamp[1] emits the whole mercury spectrum; the experiments reported on p. 374 indicate that the effective U.V. wavelengths lie below 3,000 A.

[1] The authors are deeply indebted to Professor C. B. Allsopp and Mr. K. Twinn, Radiological Technician to Guy's Hospital Medical School, London, for testing the lamp and filters used in these experiments; they also received much valuable advice and information from Professor Allsopp.

For irradiation, the cultures were rapidly transferred from the incubator to a hot plate which maintained the culture dish at 37.5°C. For Experiments 349, 350, 351, the lamp was arranged so that the tube was 15 in. from the explants; as soon as the culture vessel was in position, the lid was lifted from the Petri dish and the skin exposed for 5 minutes. In order to shorten the time of exposure to the air, in Experiments 354, 357, 366, the distance between the lamp and the explants was reduced to 9 in. and the period of irradiation to 2 minutes. (Experiments 354, 366) or 2½ minutes. (Experiment 357).

Histology.—The explants, while still adherent to the rayon cloth, were fixed for about 30 minutes in Zenker's fluid containing 3 per cent glacial acetic acid, followed by an hour in Zenker's fluid without acetic acid. After being washed in tap water and dehydrated in ethanol in the usual way, the explants were transferred to 3 baths of acetone to remove the rayon, cleared in cedar wood oil and embedded in paraffin wax. Serial sections were cut and stained with azan, Delafield's hematoxylin and chromatrope or with the periodic acid–Schiff reaction followed by Mayer's acid hemalum.

RESULTS

Skin at Explantation

The skins used in these experiments may be roughly divided into two groups, according to their stage of differentiation at explanation.

Group 1.—(Table I, Experiments 349, 350.) The epidermis (Fig. 1) consisted of a columnar stratum (s.) germinativum and a single layer of intermediate cells, covered by a layer of greatly flattened periderm. A few very early primary hair follicles had appeared as rounded epidermal buds projecting into the dermis, each associated with a small basal condensation of mesenchyme.

The dermis, consisting of a stellate reticulum with a rather sparse network of intercellular fibres, was bounded on its lower surface by a sheet of early myoblasts in various stages of differentiation which represented the panniculus (p.) carnosus. The panniculus was absent from the posterior region of the skin.

Group 2.—(Experiments 351, 354, 357, 366.) The skins of this group were taken from fetuses at or near term, and varied slightly in their degree of differentiation.

In Experiment 351, the epidermis comprised an s. germinativum, 2 to 3 layers of s. spinosum, 4 to 5 layers of s. granulosum, an s. lucidum and a thin s. corneum covered by very attenuated periderm. There were many hair follicles, the largest of these penetrated deeply into the dermis, had a well formed dermal papilla at the base, and contained a hair cone. The dermis was fairly dense, and the p. carnosus had recognisable muscle fibres.

The skin of Experiment 354 (Fig. 15) was rather more advanced than that of Experiment 351, as indicated by a slightly thicker s. corneum and better keratinised hair cones.

The most highly differentiated skin was that of Experiments 357 and 366 which was taken from fetuses that were on the point of birth. The s. spinosum had only 1 to 2 layers, the s. granulosum had 4 to 5, and the s. corneum was thicker than in the other experiments. Hairs were present in the largest follicles, and the dermis was denser and more fibrous.

The Effect of Hydrocortisone on the Differentiation of Unirradiated Skin in Culture

Hydrocortisone accelerated the differentiation of the epidermis as compared with that of controls in normal medium. This was shown best by Experiment

349 (Table I), in which unirradiated explants were fixed at intervals of up to 6 days' growth (Figs. 1–5), but, as will be seen in the next section, the same phenomenon was observed in the other experiments (Figs. 16, 17) in all of which a set of explants were fixed after 2 days' cultivation with and without hydrocortisone, to show the histological structure at the time of irradiation.

As mentioned above, the skin used for Experiment 349 was at an early stage of histogenesis and belonged to group 1. After 3 days' cultivation (Fig. 2), the originally two-layered epidermis had become much thicker in the controls in normal medium. Above the s. germinativum, was an s. spinosum, 4 to 5 layers in thickness, then 2 to 3 layers of s. granulosum and a thin, incomplete s. corneum; the cells of the s. granulosum contained very few keratohyaline granules. The hair follicles had elongated and formed dermal papillae. In the sister explant grown for 3 days in HC medium (Fig. 3), there were only 2 to 3 layers of s. spinosum, but 4 to 5 layers of s. granulosum the cells of which were stuffed with keratohyaline granules; there was also a fairly thick s. corneum. The hair follicles were usually smaller than those of the control.

During the 4th, 5th, and 6th days (Fig. 4), the control epidermis remained abnormally deep, and the s. corneum became thicker. The dermis diminished in amount, however, apparently by the emigration of cells into the medium and dissolution of intercellular material. The explants treated with hydrocortisone (Fig. 5) behaved very differently. As in normal embryonic development, the epidermis became thinner and the s. spinosum was reduced to 1 to 2 layers, as against 5 to 6 spinous layers in the controls. In both HC-treated and control explants there were 2 to 3 layers of s. granulosum, but the s. corneum was thicker in the former series. The epidermal cells of the explants exposed to the hormone, were considerably smaller and more densely staining than those of the controls. It was interesting that hydrocortisone prevented the partial dissolution of the dermis that took place in normal medium.

Skin explants of group 2 behaved in a similar way to those of Group 1, except that at 2 days the s. corneum, which was very well developed, appeared equally thick in both series (Figs. 16, 17). The histology of the 2-day explants of Group 2 will be described in more detail in the next section. Three pairs of unirradiated explants (Table I, Experiment 354) were fixed after 8 days in culture; those treated with hydrocortisone presented a striking contrast to their controls in normal medium. In the latter, the dermis had almost disappeared, even the intercellular fibres having been digested away, so that the very thick and grossly abnormal epidermis largely rested directly on the rayon fabric. The dermis of the HC-treated skin, on the other hand, was well preserved and populated with viable fibroblasts; the epidermis, with tiny hair follicles, showed the characteristic thinning produced by hydrocortisone, but few degenerate cells.

The Effect of Irradiation with the Unfiltered Beam on Normal and HC-Treated Explants (Table I)

Group 1.—(Table I, Experiments 349, 350.)

a) Before irradiation: Four pairs of explants (Experiment 350), were fixed after 2 days' cultivation, to show the structure of the skin grown in normal and in HC medium, immediately before irradiation. In the controls (Fig. 6) the epidermis was abnormally thick and consisted of an s. germinativum, 5 to 6 spinous layers and 1 to 2 layers that corresponded to the s. granulosum but which contained very few and small keratohyaline granules; there was no s. corneum and the periderm, though present, was largely degenerate and seldom formed the keratinous inclusions that it produces in normal development. The hair follicles had elongated. The dermis had increased in density, and showed a ramifying system of endothelial channels. Mitosis was abundant throughout the explant and there were few degenerate cells.

The sister explants in HC medium (Fig. 7), had all formed a single layer of s. corneum covered by a thin, keratinised periderm; the cells of the s. granulosum (3 to 4 layers) contained many more and larger keratohyaline granules than those of the controls. The s. spinosum (3 to 4 layers) was rather thinner than that of the explants in normal medium. Mitosis was plentiful.

(b) After irradiation: The explants were irradiated for 5 minutes at a distance of 15 in. from the tube; irradiation for 2 minutes at 9 in. was found to be fatal to these thin explants.

One day after exposure (Figs. 8, 12), the epidermis of the controls grown in normal medium was very necrotic; degeneration was greatest in the s. germinativum, then in the s. spinosum, and least in the s. granulosum. The degree of disintegration varied in different explants and in different areas of the same explant. 4 of the 5 controls contained areas in which the whole epithelium below the upper layers of the s. granulosum had disintegrated into cellular debris above the raw surface of the dermis. Apparently viable epithelium persisted at the margin of the explant, where the epidermis often formed a thickened ring, and in the hair follicles. The dermis also was very necrotic (Fig. 12); the intercellular material had a ragged, disorderly appearance, and except in the deepest parts, most of the cells were pycnotic. The p. carnosus was less affected. No mitosis was found.

The 5 HC-treated explants (Figs. 9, 13) presented a very different histological picture from that of the controls. There was far less degeneration and no areas of complete epidermal disintegration were seen. The dermis also was less affected (Fig. 13); it contained a greater proportion of apparently viable fibroblasts and the intercellular material looked almost normal. In general the structure of the skin was much less disturbed than in that grown in normal medium. There was no mitosis.

The first signs of repair were seen on the 2nd day after irradiation (Experi-

ment 349). In both the control and HC-treated skin fixed at this stage, most of the epidermis was completely necrotic, but epithelium was growing out of some of the surviving hair follicles and was also spreading inwards from the less damaged marginal ring described above; a few basal cells also survived and had expanded on the surface of the dermis. While there was more regenerated epithelium in the control, the general picture of repair was far more orderly in the HC-treated explant. In the control the regenerating epithelium was much more variable in thickness, structure, and degree of differentiation, the dermis contained many more dead cells, and the intercellular fibres were sparser than in the presence of the hormone. Mitosis had reappeared in both explants.

A pair of explants (Experiment 349) fixed 3 days after irradiation showed an advance in epithelialisation and in the repopulation of the dermis with fibroblasts. As in the previous pair, the main difference between the two lay in the much greater regularity of the healing process in the HC-treated skin.

There was a much more striking difference between 2 explants grown with and without the hormone, respectively, and fixed 4 days after irradiation (Experiment 349). In the control the old epidermis had become lifted from the dermis to form a fluid-filled blister above the still incompletely covered surface of the dermis; as before, the new epithelium was very variable in thickness and degree of differentiation. The dermis, though now containing many actively dividing fibroblasts, still showed traces of its previous disorganisation. In the HC-treated skin, on the other hand, the dermis was completely covered by a well differentiated, regular keratinising epidermis, above which were the necrotic remains of the dead epithelium—almost the only remaining sign of the original injury; the dermis was richly populated with healthy fibroblasts between which were regularly arranged fibres.

Eight pairs of explants (Experiment 350) were examined 6 days after irradiation. Of the controls, 2 were completely epithelialised with a thick, well differentiated epidermis, in 5 epithelialisation was incomplete and very irregular (Fig. 10), and in one there was no epithelial regeneration. As in the explant fixed 4 days after exposure, the old s. corneum with some adherent dead granulosum cells, was lifted above the denuded surface of the dermis to form a blister filled with fluid and fine cellular debris. The dermis was repopulated by invading fibroblasts from the deeper regions, which were migrating among the pale corpses of the original cells; they were often accompanied by branching endothelial channels. Intercellular fibres, however, were sparse. In some explants the panniculus was regenerating and young myoblasts were seen.

Again, the 8 explants grown in HC-medium differed sharply from their controls. Epithelialisation was almost or quite complete in all, and the new, usually keratinised epidermis was better differentiated and much more regular than in the controls. In regions where the dermis was not yet fully covered

(Figs. 11, 14), the necrotic remains of the old epidermis remained stuck to the connective tissue instead of forming a vesicle above it. It was interesting that the dead cells of the old epithelium stained more intensely, and were much less disintegrated than those of the controls. The dermis (Fig. 14) was similar to that of the skin in normal medium but more fibrous.

Skin of Group 2. (Experiments 351, 354.)

(*a*) *Before irradiation:* Eight pairs of explants were examined after 2 days' cultivation. The epidermis of the controls grown in normal medium (Fig. 16) consisted of s. germinativum, 3 to 4 (Experiment 351) or 2 to 3 (Experiment 354) layers of s. spinosum, 2 to 3 layers of s. granulosum, an s. lucidum and a thick s. corneum in which about 7 layers could usually be distinguished. The hair follicles had changed little as compared with those of the original skin, but there was some necrosis in the deeper parts of the larger follicles. There were also many degenerate cells in the dermis of the thickest explants; this was probably due to imperfect nutrition and oxygenation of the cells which were set in a fairly dense fibrous stroma and covered by a heavily keratinised epidermis. In explants from parts of the body where the skin is thinner, there was very little necrosis.

The epidermis of the HC-treated explants (Fig. 17) was much thinner; in addition to the s. germinativum, there were 1 to 2 layers of s. spinosum, 2 to 3 layers of s. granulosum and a multilayered s. corneum about equal in thickness to that of the controls. The epidermal cells were smaller and more densely staining than those of the controls. The hair follicles and dermis were similar to those of the skin in normal medium.

(*b*) *After irradiation:* In Experiment 351, the skin was irradiated for 5 minutes at a distance of 15 in. from the lamp, *i.e.* it received the same dose as the explants of group 1; in Experiment 354 it was exposed for 2 minutes at 9 in., which proved to be a rather more drastic treatment.

Of the controls of Experiment 351 one of those fixed 1 day after irradiation showed little degeneration in the epidermis which, however, had lost much of its staining capacity. The remaining 3 (from the same watch glass as the first), were severely damaged; the s. germinativum was very necrotic and over large areas the s. spinosum was separated from the dermis by a space filled with dead cells. Many of the hair follicles and most of the dermal cells were completely degenerate. Apparently viable epithelial cells were present in some of the follicles, at the margin of the explant where the epidermis dipped over the cut edge of the dermis, and here and there on the surface of the dermis. In all 4 controls of Experiment 354, fixed 1 day after irradiation, the s. germinativum was completely necrotic, and the spinous layer was separated from the dermis by a space filled with debris. Cells that looked viable persisted only in the depths of the follicles and, in 2 explants, at the extreme periphery. Few fibroblasts survived in the dermis, but the p. carnosus was less affected.

The 8 HC-treated explants from the two experiments, examined 1 day after irradiation, were very different from their controls. Except in a few small areas, the epidermis remained firmly stuck to the dermis, many of the basal cells appeared viable and the follicles were less degenerate. More of the dermal fibroblasts survived, the intercellular material was denser than in the controls and the fibres had a more orderly arrangement, running roughly parallel with the surface.

Two days after irradiation (Experiment 354) the first signs of recovery could just be distinguished. In the 4 controls (Figs. 18, 24), the epidermis had almost completely disintegrated and the s. corneum and outermost layer of the s. granulosum were raised from the denuded dermis. Hardly any living epidermal cells remained; a few survived deep in the follicles and there were occasional small patches of very thin, expanded cells on the surface of the dermis. Nearly all the dermal fibroblasts were dead. No mitosis was seen.

In the corresponding 4 HC-treated explants (Figs. 19, 25), breakdown of the epidermal cells was much less advanced and instead of being lifted from the dermis the necrotic epithelium remained adherent. Especially near the margin of the explant, a few surviving basal cells had spread out on the surface of the dermis which contained more living fibroblasts than in the controls.

Of the 8 controls (Experiments 351, 354) fixed 4 days after irradiation, one (Experiment. 351) was completely epithelialised by a very thin epidermis which showed very early keratinisation. In the remaining 7 (Figs. 20, 26), large areas of dermis were still denuded, but in all there were patches of extremely flattened epidermal cells some of which were migrating from the margin of the explant and others crawling out of the hair follicles (Fig. 30). A few mitoses had now appeared. The old epidermis either formed a fluid-filled blister above the largely bare surface of the dermis, or was partially detached. The dermis was very necrotic.

Healing was far more advanced at this stage in the 8 HC-treated explants (Figs. 21, 27) which presented a striking contrast to their controls. Six were fully and two nearly epithelialised. The cells of the regenerating epidermis were smaller, denser, and more actively dividing, and differentiation was more advanced than in the controls. The old epidermis was sometimes partly detached from the regenerating epithelium beneath. More dermal cells survived.

Six days after exposure (Experiments 351, 354) all but one of the 8 controls were completely epithelialised (Figs. 22, 28), but in the same explant the new epidermis might vary from a well developed keratinising epithelium in one region to a single layer of flattened cells in another; mitosis was now copious.

Epidermal regeneration was complete in the 12 explants grown in the presence of hydrocortisone for 6 days after irradiation (Figs. 23, 29), and as usual the histological picture was more uniform than in the controls. The new epidermis had now acquired the same type of structure as that described above in unirradiated skin grown for several days in the presence of the hormone. It was

shallow, of uniform thickness throughout the explant and consisted of an s. germinativum, 1 to 2 layers of s. spinosum, 1 to 2 layers of s. granulosum and an s. corneum; the cells of all the strata were very flat, small, and densely staining, and there was now less mitosis than in the regenerating epithelium of the controls. The deeper part of the dermis was quite well populated by fibroblasts, but there were few cells in the subepidermal region.

Four pairs of explants (Experiment 354) were fixed 8 days after irradiation. The new epidermis of the controls was better differentiated and keratinisation was more advanced in both sets, but otherwise there was little change.

Experiments to Determine the Efficacy of the Various Wavelengths in the Mixed Beam

The following experiments were made to ascertain which of the wavelengths emitted by the lamp used in these investigations were mainly responsible for the necrosis produced in the skin explants exposed to the unfiltered radiation.

The effect of U.V. radiation of wavelengths above 3000A.—(Table I, Experiment 357.)

The explants were grown for 2 days with or without hydrocortisone, before being irradiated at a distance of 9 in. for 2½ min. with the Hanovia lamp to which an 0X7 filter (Chance Bros.) had been fitted. Spectroscopic tests made in Professor Allsopp's laboratory by Mr. Twinn showed that this filter transmitted nearly 100 per cent of radiation at wavelengths of between 3100 and 3800 A but almost completely excluded emission below 3100 A. Tests with a thermocouple[2] recorded no rise in temperature over a 5 min. interval, indicating that the filter also cut out the infrared rays.

(*a*) *Before irradiation:* The epidermis of the 4 controls grown in normal medium comprised a basal layer, 2 to 3 layers of s. spinosum, 2 to 3 layers of s. granulosum and a thick s. corneum. The deeper parts of many of the larger hair follicles were degenerating. The dermis was more fibrous than at explantation; some of the fibroblasts were dead. The epidermis of the 4 HC-treated explants showed the same thinning and increased staining capacity observed in the previous series; the s. corneum was of about the same thickness as that of the controls. The follicles and dermis were similar to those of the skin in normal medium.

(*b*) *After irradiation:* The irradiation had no detectable effect on the skin. The 4 control explants fixed 1 day after irradiation differed little from those fixed immediately before exposure. The corresponding 4 explants grown in HC-medium, however, appeared healthier than those fixed before irradiation, as indicated by a decline in the number of degenerate cells; this suggested that the hormone had aided recovery from the traumatic effects of explantation.

[2] We are indebted to Dr. S. Fitton Jackson and Mr. S. Pink for making the thermocouple readings.

During further cultivation, the skin in both series behaved exactly like unirradiated tissue. Thus in the controls the epidermis remained thick while the dermis diminished in amount. In the HC-medium, the epidermis became thinner, but the structure of the dermis was well preserved.

The Effect of Radiation from which Infrared Rays Were Excluded with a Water Filter.—(Table I, Experiment 366.)

The Hanovia lamp used in these experiments emits some infrared radiation, and it seemed possible that this might enhance the destructive action of the U.V. light on the skin explants. An experiment was therefore made to see whether elimination of the infrared waves would diminish the effect of the irradiation on the tissue.

To prepare a suitable filter, a brass tube of 27 mm internal diameter and 28 mm in height was painted a dull black on its inner surface, sealed to a thin quartz plate, and filled with glass distilled water. This eliminates the infrared but fully transmits the U.V. radiation; that it excluded all heat effects was shown by measurements with a thermocouple which recorded a negligible rise in temperature in a culture watch glass placed beneath the filter and irradiated for 2 minutes at 9 in.

Explants grown for 2 days in normal medium were irradiated at a distance of 9 in. for 2 minutes under the following conditions: (1) with the filter empty, (2) with the filter filled with glass-distilled water, (3) with no filter. Immediately after exposure the explants were transplanted to fresh (normal) medium and fixed 1 or 2 days later.

(*a*) *Before irradiation:* The histological structure of the 8 explants fixed after 2 days, was essentially the same as in the 2-day controls of the previous experiment (357) but 3 of the explants showed rather more cell degeneration.

(*b*) *After irradiation:* The irradiated explants underwent similar degenerative changes to those described above in the irradiated controls of group 2 (Table I, Experiments 351, 354) fixed 1 and 2 days after exposure. There was no obvious difference in the degree of necrosis produced in the 3 groups.

This result indicated that the infrared rays did not add appreciably to the degeneration produced by the U.V. radiation.

Conclusion.—Since radiation of between 3100 and 3800 A was ineffective (Experiment 357), and infrared rays had little or no additive effect, it is concluded that the effective wavelengths were below 3000 A.

DISCUSSION

The results described above show that the first effect of hydrocortisone on normal fetal skin in culture is to accelerate differentiation; this is followed by a partial atrophy of the epidermis which becomes thinner than in the controls, while the cells diminish in size but increase in staining capacity. Very little necrosis appears in either the epidermis or dermis of the hormone-treated skin,

and both are well preserved at a stage when in the control cultures the dermis has almost disappeared and the epidermis become grossly abnormal. This observation confirms the recent work of Gillette, Findley, and Conway (1962) on adult mouse skin in organ culture; they found that after 3 weeks in culture cortisone-treated explants were less necrotic than the controls; when grafted back onto the donor aninals, 83 per cent of the cortisone-treated but none of the control explants were successfully established.

When fetal rat skin grown in normal medium is exposed to U.V. radiation, the epidermal cells and especially those of the s. germinativum are rapidly broken down, and 2 days after irradiation the s. corneum with adherent cellular debris becomes either completely detached from the almost denuded dermis, or raised to form a fluid-filled blister; the resemblance between this lesion and those seen in systemic lupus erythematosus (Rothfield and Weissmann, 1961) would warrant further study. In the dermis also there is extensive necrosis, and some digestion of the interfibrillar material with consequent disorganisation of the fibres.

The work of Beloff and Peters (1945), summarised by Peters (1945), has an important bearing on the results obtained with U.V. radiation. These workers showed that normal mammalian skin contains a proteinase which is released in thermal burns. Peters (1945) writes: "the fact that the proteinase must be liberated has led us to advance the hypothesis that the loosening of the tissue essential for the blister-formation is due to the enzyme's uncontrolled activity on its way from the tissue cell to the circulation."

Proteolytic activity in human epidermis has been demonstrated by Burbach (1957) who also showed that a solution of crystalline trypsin injected intracutaneously into a human subject, produced a subepidermal vesicle. The hypothesis of Beloff and Peters would readily explain the blister formation and partial digestion of dermal intercellular material seen in our irradiated control explants. Although we have no direct evidence that a protease is liberated by U.V. irradiation in the skin cultures, the fact that U.V. rays release a protease from the large granule fraction isolated from rat liver (Weissmann and Dingle, 1961) suggests the possibility that this may also happen in the irradiated explants.

The presence of hydrocortisone in the medium, influences the response of the skin to U.V. irradiation in the following ways: it (1) reduces and retards the breakdown of cells, (2) prevents vesication, (3) preserves the organisation of the dermal intercellular material, (4) hastens epithelialisation, and (5) accelerates the differentiation of the regenerating epidermis. Effects (2), (3), and (4) are probably secondary to (1). Thus it might be expected that less disintegration of cells would be correlated with the release of smaller quantities of hydrolytic enzymes and toxic breakdown products into the tissue. On Beloff and Peters's hypothesis, a diminished liberation of protease would account for the

absence of blister formation. It is probable also that many cells survive in the HC-treated skin that would be poisoned in the irradiated controls by the abundant breakdown products; this increased survival of cells would facilitate healing because more viable cells would be available to carry out this process. The better preservation of the interfibrillar material of the dermis is probably another indication of less free enzymic activity in the presence of the hormone and indirectly may assist epithelialisation by providing the regenerating epidermis with a more favourable substrate on which to spread.

The mechanism whereby hydrocortisone inhibits cellular disintegration in the skin is not known. The fact that pretreatment of rats with this hormone inhibits the subsequent release, by U.V. radiation, of protease from the large granule fraction of the liver raises the possibility already mentioned, that hydrocortisone may stabilise the membrane systems of the cell; this hypothesis receives further support from the finding (de Duve, Wattiaux, and Wibo, 1961) that hydrocortisone inhibits the thermal release of phosphatase from isolated lysosomes. If this hypothesis proves to be well founded, it may also explain, at least in part, the beneficial effect of the hormone on a wide range of skin disorders.

Why hydrocortisone should accelerate epidermal differentiation in both the embryonic and regenerating epidermis, and why it should eventually produce an atrophic type of epithelium which nevertheless is very resistant to the unfavourable conditions of prolonged cultivation, remains obscure. A similar atrophy has been described in the epidermis of cortisone-treated rats (Castor and Baker, 1950; Baxter, Schiller, Whiteside, and Straith, 1951).

In our experiments the skin was grown for 2 days with and without the hormone before being irradiated, in order to ensure that the hydrocortisone would enter the non-vascularised tissue in time to affect its response to the irradiation. Consequently, at the time of irradiation the HC-treated skin was rather more advanced in development than the corresponding controls in normal medium. The protective action of the hormone on the irradiated explants, however, was not correlated with the degree of differentiation of the tissue. For example, in Experiment 350 the skin was explanted at an early stage of development and when irradiated on the 2nd day in culture the epidermis of the controls was unkeratinised and that of the HC-treated explants showed only the very beginning of keratinisation. On the other hand, the skin used in Experiment 351 was obtained from fetuses near term, and after 2 days in culture both the HC-treated and control explants had acquired a multilayered s. corneum of about equal thickness in the two series. In spite of this big difference in the degree of differentiation of the skin in the two experiments, however, hydrocortisone had essentially the same effect on the response of the explants to the same dose of radiation.

Preliminary experiments by Glücksmann and Cherry have shown that the

beneficial action of hydrocortisone on the repair of U.V.-induced lesions in rat skin is not peculiar to organ cultures, since similar histological phenomena are observed in the skin of adult rats irradiated with the same Hanovia lamp as that used in the present study, and treated with the hormone before or shortly after exposure.

Pretreatment of rabbits with cortisone also inhibits the release of beta glucuronidase and protease from the large granule fraction of liver after such animals have been injected with bacterial endotoxins (Weissmann and Thomas, 1962). A similar protective effect of hydrocortisone against an excess of vitamin A acid *in vivo* has recently been shown to occur after hypervitaminosis was induced orally in rabbits and amphibia (Weissmann and Thomas, unpublished).

These observations suggest that the therapeutic efficacy of gluco-corticoids in skin lesions may be due, at least in part, to a stabilising action of the hormones on lysosomes. Similarly, the activation by sunlight of systemic lupus erythematosus, and the response of such flareups to adrenal steroids may indicate that certain lysosomes of patients with this disease are perhaps more 'fragile' to a variety of injurious agents than normal; such 'fragility' might be due to a heritable flaw, or be secondary to hypersensitivity phenomena, but at present this is merely a matter for conjecture.

SUMMARY

1. The effect of hydrocortisone on the development of fetal rat skin in organ culture, and on its repair after exposure to a mixed beam from a mercury lamp, are described.

2. The addition of hydrocortisone (7.5 μg/ml) to the culture medium (HC medium) caused accelerated differentiation and keratinisation of the epidermis followed by atrophic changes as *in vivo*.

3. Explants were grown for 2 days in either normal or HC medium and then irradiated with light from an Hanovia lamp.

4. Irradiation of the control explants produced severe necrosis in both epidermis and dermis and much disorganisation of the dermal intercellular material; 2 days after exposure the s. corneum with adherent cellular debris had become either completely detached from the denuded dermis, or raised to form a fluid-filled blister. Epidermal regeneration had begun by the 4th day after irradiation and was usually complete by the 6th day.

5. Hydrocortisone modified the response to irradiation as follows: (1) reduced and retarded cellular breakdown, (2) prevented vesication, (3) preserved the organisation of the dermal intercellular material, (4) hastened epithelialisation, (5) accelerated the differentiation of the new epidermis. Effects (2), (3), and (4) were probably secondary to (1).

6. Experiments with various light filters showed that the effective wavelengths for producing lesions in the skin explants were those below 3000 A.

7. It is suggested that the beneficial effect of hydrocortisone on the repair

of irradiated skin explants might be due, at least in part, to a reduced proteolytic activity in the damaged tissue through a stabilising action of the hormone on the lysosomes.

8. The relationship of these findings to clinical observations is discussed.

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EXPLANATION OF PLATES

Photography performed by Mr. M. F. Applin.

PLATE 43

Unirradiated explants (Experiment 349) from group 1. Sections stained with azan. × 215.

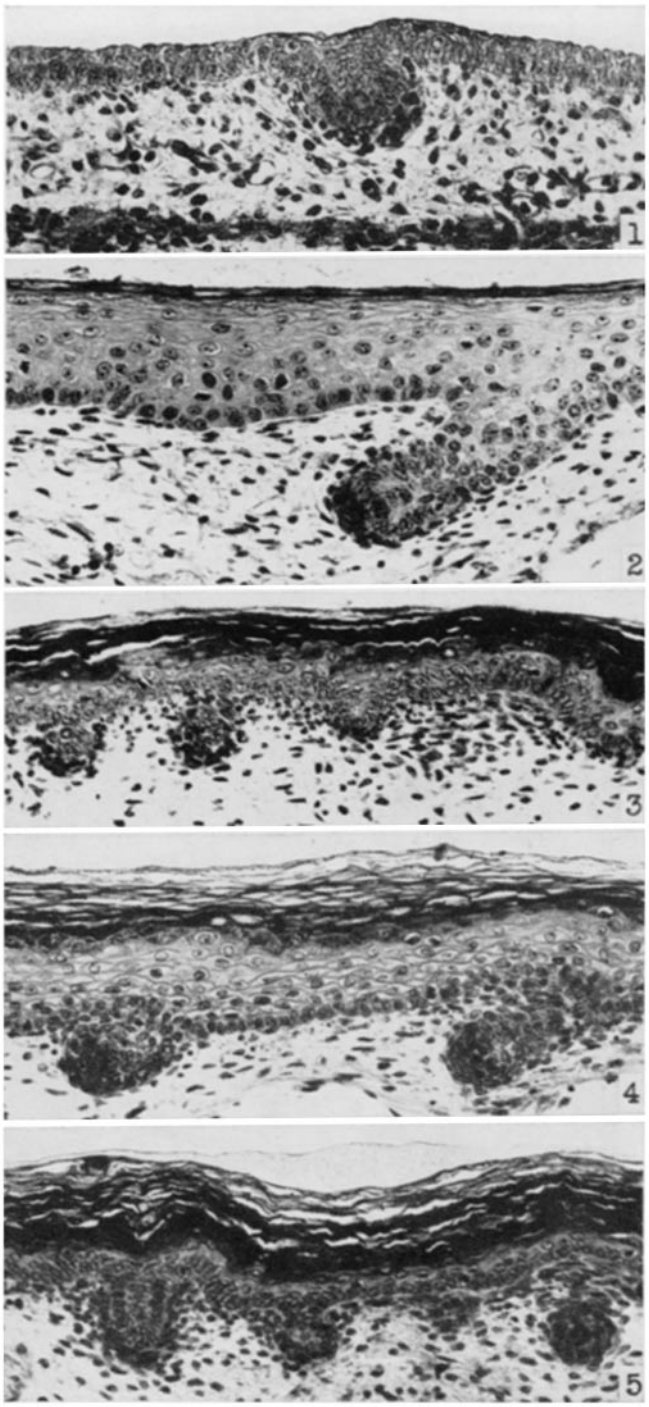
FIG. 1. Normal skin from Group 1 fetus (Experiment 349). There is a simple two layered epidermis and flattened periderm. Note early hair follicle.

FIG. 2. Control explant after 3 days in normal medium. The epidermis is much thicker than in Fig. 1 and has just begun to keratinise; the hair follicles are more advanced.

FIG. 3. Sister explant after 3 days in medium containing 7.5 μg hydrocortisone/ml (HC medium). Keratinisation is much greater than in the control, but the epidermis is thinner, the cells are smaller, and the follicles less well developed.

FIG. 4. Control explant after 6 days in normal medium. A thick s. corneum is present and the epidermis is thinner than at 3 days.

FIG. 5. Explant grown for 6 days in HC medium. The s. corneum is thicker than at 3 days, but has not increased so rapidly as that of the control in Fig. 4. The epidermis is now very thin and somewhat atrophic.



(Weissmann and Fell: Hydrocortisone and skin response to irradiation)

PLATE 44

Explants of group 1 (Experiment 350). Sections stained with azan. × 110.

FIG. 6. Control explant after 2 days in normal medium, fixed immediately before irradiation.

Keratinisation has not yet begun.

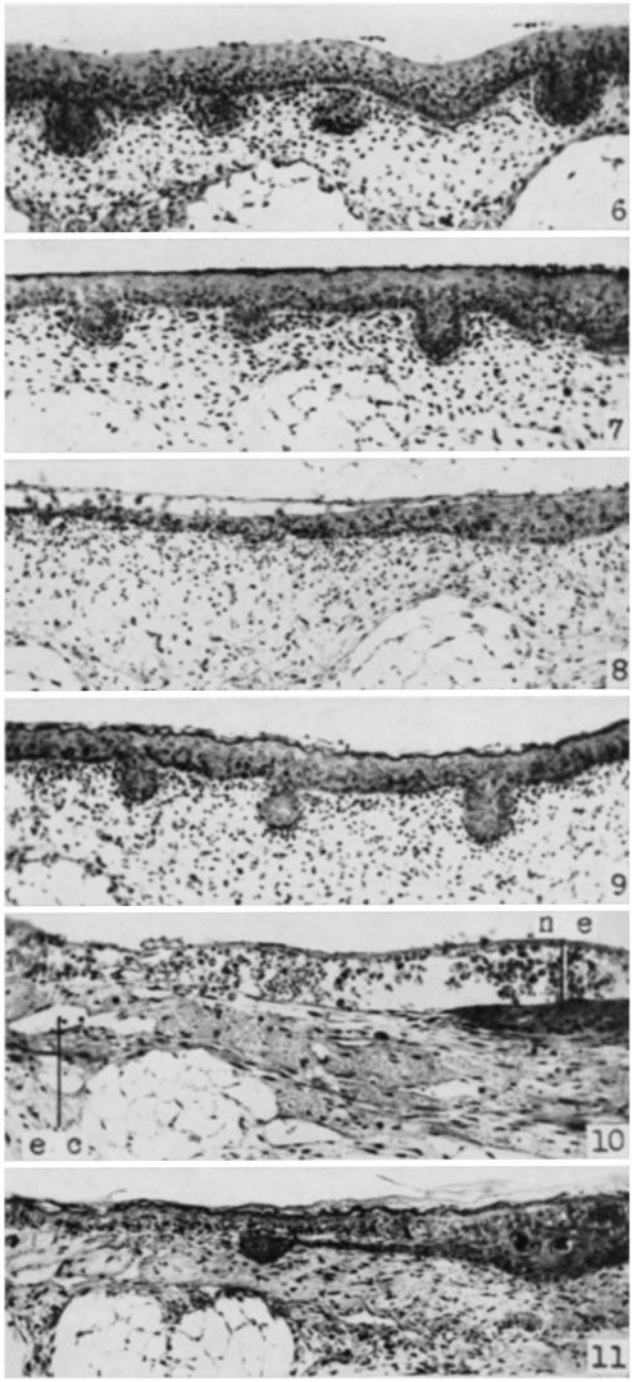
FIG. 7. Sister explant after 2 days in HC medium, immediately before irradiation. The epidermis is thinner than in Fig. 6, and the first signs of keratinisation are seen.

FIG. 8. Control explant fixed 1 day after U.V. irradiation. Note partial disintegration of the epidermis (see Fig. 12).

FIG. 9. Explant grown in HC medium fixed 1 day after irradiation. The epidermis is intact, though it contains many degenerate cells (see Fig. 13).

FIG. 10. Control explant fixed 6 days after irradiation. The denuded surface of the dermis is covered by a blister containing the debris of the irradiated epidermis. New epidermis (*n e*), is spreading over the dermis from the right. Note endothelial channels (*e c*).

FIG. 11. Explant grown in HC medium, fixed 6 days after irradiation. There is no blister, and the necrotic epidermis remains adherent to the dermis. New epithelium is growing beneath the dead epidermis.



(Weissmann and Fell: Hydrocortisone and skin response to irradiation)

PLATE 45

Sections stained with azan.

FIG. 12. Same control explant (group 1) as that shown in Fig. 8; note partial disintegration of epidermis (*dc*: degenerate cells) and severe disorganisation of the dermal intercellular fibres. × 300.

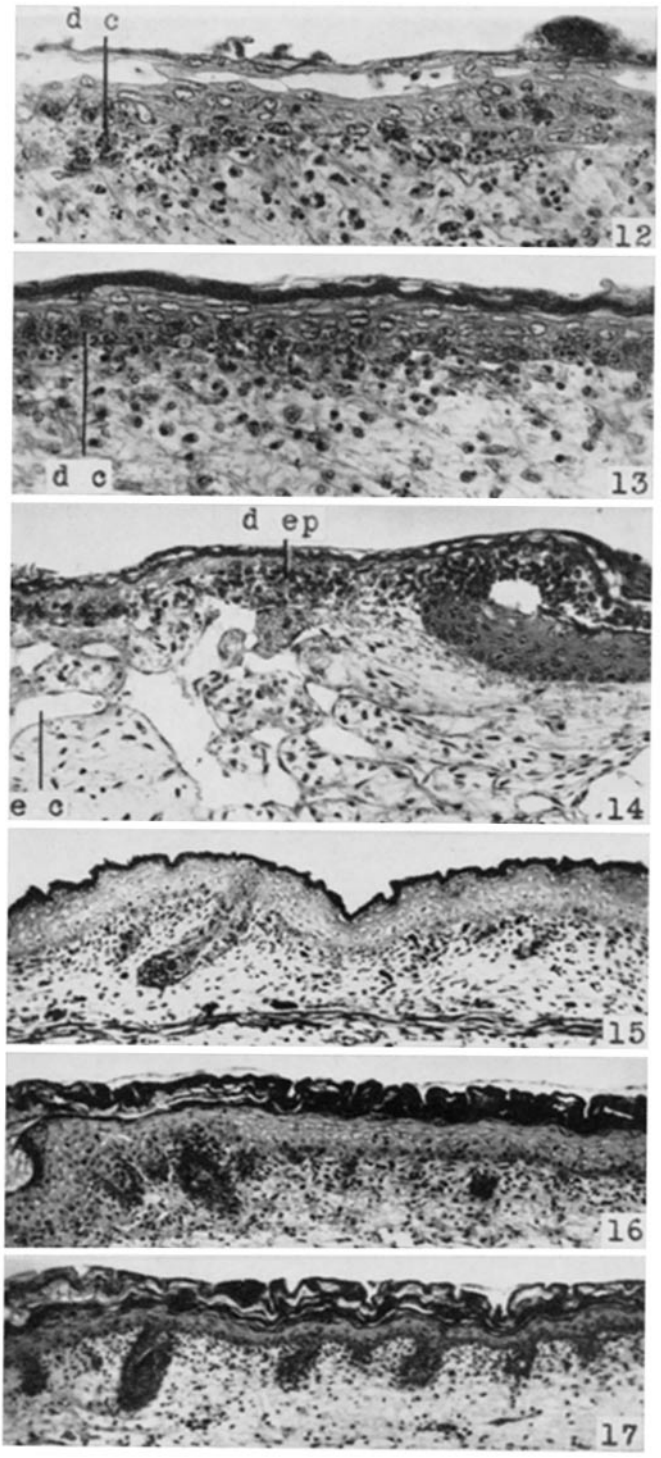
FIG. 13. Same explant (group 1) in HC medium as that shown in Fig. 9. Although there are degenerate cells (*dc*) in the epidermis, its structure remains intact. There is less disorganisation of the dermis than in Fig. 12. × 300.

FIG. 14. Explant of group 1 (Experiment 350) grown in HC medium and fixed 6 days after irradiation. Note: necrotic epidermis (*d ep*) still stuck to the dermis, regenerating epithelium growing in on the right, repopulation of the dermis with normal fibroblasts, and the branching endothelial channels. × 150.

FIG. 15. Normal skin of group 2 fetus (Experiment 354). The epidermis is thick and has begun to keratinise and hair cones are present in the larger follicles. × 105.

FIG. 16. Control explant of group 2 (Experiment 354) after 2 days in normal medium, fixed immediately before irradiation. A thick *s. corneum* has developed. × 105.

FIG. 17. Explant of Group 2 (Experiment 354) after 2 days in H.C. medium, fixed immediately before irradiation. The epidermis is much thinner than in Fig. 16. × 105.



(Weissmann and Fell: Hydrocortisone and skin response to irradiation)

PLATE 46

Irradiated explants (Experiment 354) of Group 2. Stained with azan. × 105.

FIG. 18. Control explant fixed 2 days after irradiation. The old epidermis forms a blister above the denuded dermis, and the deeper layers have disintegrated.

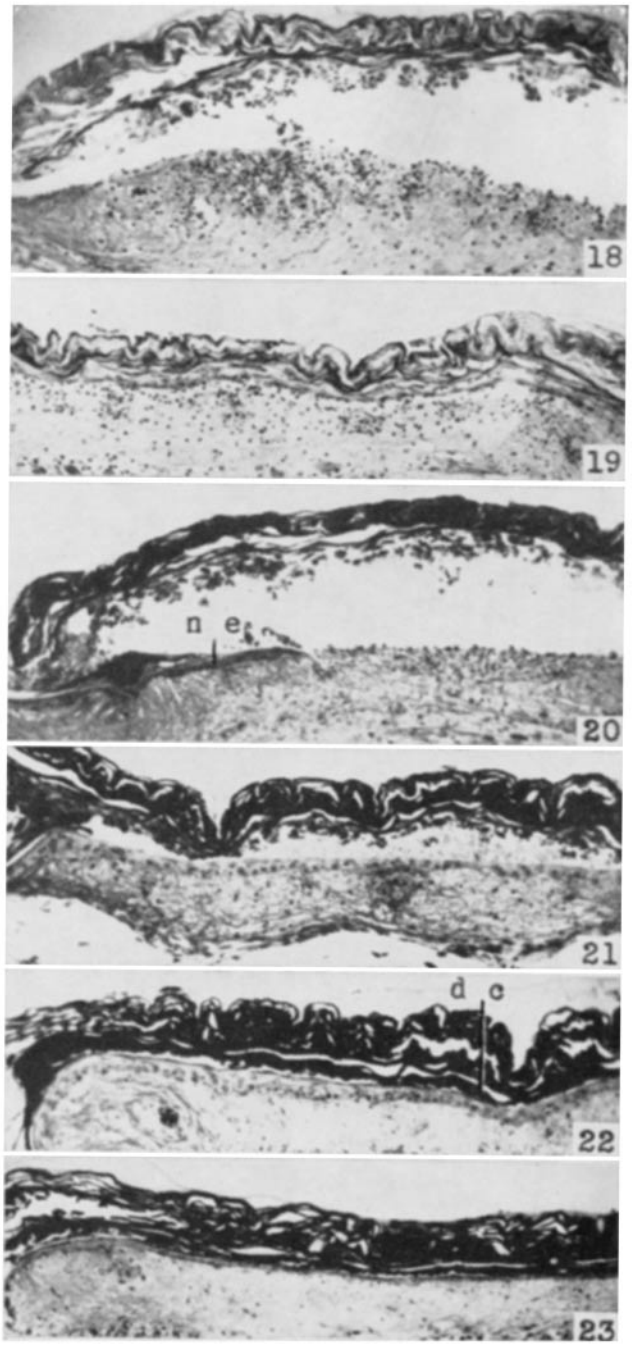
FIG. 19. Explant grown in HC medium, fixed 2 days after irradiation. The epidermis shows much less disintegration than in Fig. 18 and has not formed a blister.

FIG. 20. Control explant fixed 4 days after irradiation. New epidermis (*ne*) has begun to spread over the bare surface of the dermis above which the old epidermis forms a blister.

FIG. 21. Explant in HC medium, 4 days after irradiation. Epithelialisation is complete and the old epidermis is now detached.

FIG. 22. Control explant 6 days after irradiation; epithelialisation is complete, the blister has collapsed and the dead epidermal cells (*dc*) are compressed into a deeply staining layer on the surface of the new epidermis.

FIG. 23. Explant in HC medium, 6 days after irradiation. The new epidermis has become very thin as in the unirradiated explants in HC medium (*cf.* Fig. 5).



(Weissmann and Fell: Hydrocortisone and skin response to irradiation)

PLATE 47

Irradiated explants (Experiment 354) of group 2. Figs. 24–29: Delafield's hematoxylin and chromatrope × 510. Fig. 30: azan. × 215.

FIG. 24. Control explant 2 days after irradiation. Note disintegrated epidermis well separated from the bare surface of the dermis.

FIG. 25. Explant grown in HC medium, 2 days after irradiation. There is much less disintegration of the epidermis which is not separated from the dermis.

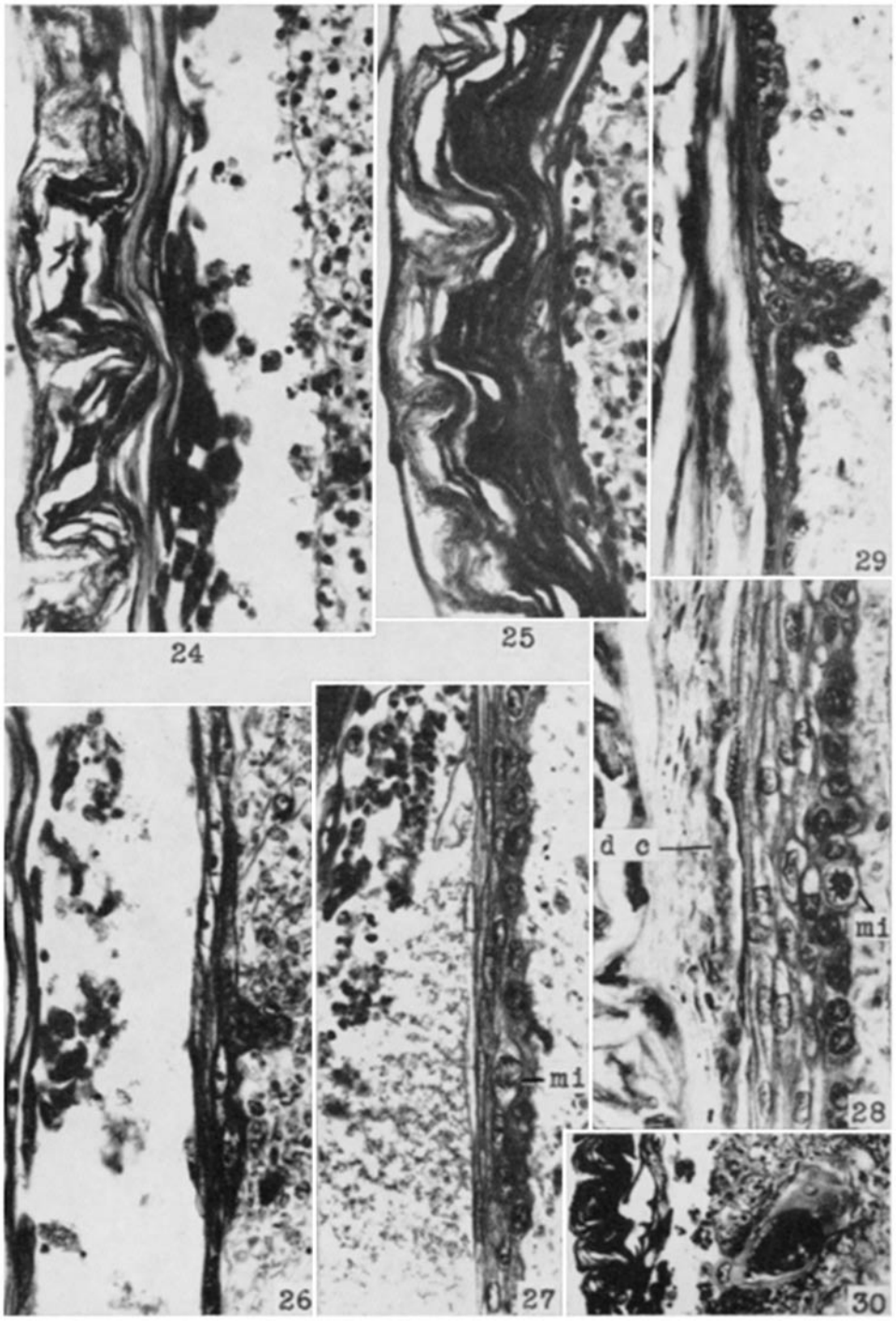
FIG. 26. Control explant 4 days after irradiation, showing new epithelium spreading on the surface of the dermis; the epithelium is flattened, shows little differentiation and few mitoses.

FIG. 27. Explant grown in HC medium, 4 days after irradiation. The new epidermis is better differentiated than in Fig. 26, and mitosis (*mi*) is plentiful.

FIG. 28. Control explant 6 days after irradiation. The new epidermis is now thick, well differentiated and contains many mitoses (*mi*). Note compact layer of degenerate cells (*dc*); the blister has collapsed.

FIG. 29. Explant grown in HC medium, 6 days after irradiation. The new epidermis is now very thin and contains fewer mitoses than the corresponding controls.

FIG. 30. In this control explant, fixed 4 days after irradiation, the only surviving epithelium was in the hair follicles. Note viable cells crawling out of the follicle onto the surface of the dermis.



(Weissmann and Fell: Hydrocortisone and skin response to irradiation)