DISSOCIATION OF GRANULATION AND EPITHELIZATION IN WOUNDS OF RABBITS BY MEANS OF TOPICALLY APPLIED HYDROCORTISONE*

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The belief that physiologically the epidermis and dermis are intimately related is supported by the concomitant changes which take place in both of these layers during the phases of hair growth and their various responses to injury (1). In experiments where an area of skin has been surgically removed, the process of healing has been found to begin with the growth of granulation tissue, over which the epithelium later migrates (2, 3).

In this experiment, evidence is presented which indicates that, under the influence of topically-applied hydrocortisone, epithelization may proceed in the absence of granulation. When this occurs, a tissue resembling mature granulation tissue subsequently develops under the new epithelium.

MATERIALS AND METHODS

Black and dark grey domestic rabbits of mixed sexes and breeds, weighing 2500–3500 grams, were used. Each animal was kept in a separate cage and fed commercial rabbit pellets supplemented with kale. All operations were performed under nembutal anesthesia and employing as aseptic a technic as possible. Each animal was given the following antibiotics: crystalline penicillin (100,000 U i.v.), aureomycin (50 mg. i.v.), streptomycin (100 mg. i.m.), and bicillin (600,000 U i.m.).

The dorsum of each animal was clipped from the mid-thoracic to the sacral region and extending 8 cm. from the midline. The skin was then depilated with a mixture of equal parts of barium sulfide and starch made to a thin paste with water. The skin was then washed with 70% alcohol. Two circular areas were outlined with India ink over the right and left lumbar areas respectively. Each area was 25 mm. in diameter and 3 cm. removed from the midline. The operative area was prepared by the application of tincture of iodine, which was then washed off with ether.

A circular region of skin 25 mm. in diameter was cut out with fine forceps and scissors by following the India ink outline. All epithelial elements were removed and an effort was made to remove the entire dermis, leaving behind the intact panniculus carnosus with its blood vessels.

Contraction of wounds was hindered by specially-constructed stainless steel splints‡, shown in Figs. 1–6. As seen in these figures, 12 vertically placed wires (18 gauge surgical steel) lie against the wound edge and hold the wound open. Above the wound, each wire bends horizontally and joins a stainless steel ring as shown in Figs. 1–6. Beneath the wound, in the subcutaneous tissue, each wire again bends horizontally and outward forming a U-shape in which the skin edge rests. Actually, each pair of wires constitutes a single unit and is made out of one piece of wire. The wire starts at the ring, enters the wound, makes a loop in the subcutaneous tissue (see Fig. 2), and comes out to rejoin the ring, thus forming a single prong. Six small cuts were made through the panniculus carnosus at the edge of the wound so that the six prongs could be inserted under the whole thickness of the skin in the subcutaneous space.

A saline suspension of hydrocortisone acetate was applied to the wound on the right side. This consisted of 0.25 cc. which contained 6.25 mg. of hydrocortisone acetate. The wound on the left was treated with 0.25 cc. of the suspending

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‡ The stainless steel splints were originally designed and used by Breedis for studying regeneration of skin. They will be more fully described elsewhere.

solutions.* Each wound was covered by a sterile disc of filter paper over which was placed a sterile gauze pad impregnated with vaseline in order to prevent drying and evaporation. A cover glass was then fastened on the stainless steel splint, thus forming a protective chamber.

The chambers were opened every other day and the healing wounds observed. At these times the right wound received another 0.25 cc. of hydrocortisone and the left wound 0.25 cc. of the suspending solution alone. Both wounds were redressed with fresh filter-paper discs and vaseline gauze. The wounds were changed and treated in this manner until they were completely epithelized. Photographic records were also made during the process of healing.

Animals were sacrificed with nembutal at various intervals up to 58 days after operation. A region of skin 3–4 cm. square was removed, full thickness, with the chamber included in the center. The skin was pinned on cork boards and fixed in Bouin's fixative for 24 hours. Sections were then cut perpendicular to the skin surface, including old and new skin. Paraffin sections were prepared and stained with hematoxylin and eosin for microscopic examination.

OBSERVATIONS AND EXPERIMENTAL RESULTS

The results will be discussed under the headings of gross and microscopic observations.

All animals included in this study met the following criteria: (1) They remained healthy throughout the experiment. (2) Their chambers remained in place throughout the experiment, thus hindering contraction of the wound. (3) Drying of their wounds was prevented until complete epithelization had taken place.

I. *Gross Observations*

The times of granulation and epithelization of control and hydrocortisone-treated wounds are shown in Table 1.

A. *Control wounds.* These are the wounds which received the suspending solution without the hydrocortisone. By the sixth day, these wounds became hyperemic and inflamed, producing a moderate amount of serous exudate. On the seventh to ninth days, there was evidence of granulation tissue about the edge of the wound and over the blood vessels of the panniculus carnosus. At about the twelfth day, the whole area of the wound was completely covered by a rich growth of granulation tissue covering the blood vessels of the panniculus carnosus, as shown in Fig. 3. The difference in appearance between Fig. 3 (13 days) and Fig. 1 (4 days) is due to this new granulation tissue. By the thirteenth day, whitish projections of epithelium were seen at the margin of the wound (Fig. 3). The migrating epithelium grew towards the center of the wound from all directions. The entire wound was completely epithelized by the twenty-first day (Fig. 5).

B. *Wounds treated with hydrocortisone.* These wounds remained remarkably free of any inflammation. The absence of granulation tissue was striking. The blood vessels of the panniculus carnosus remained in clear view. In fact, the wounds appeared very much like they did on the day of operation until the eighteenth day (Figs. 2 & 4). At this time, tongues of epithelium began growing from the margin of the wound. The epithelium appeared to grow on top of the panniculus carnosus and its blood vessels in the absence of granulation tissue. This is especially well shown in Fig. 6. The entire wound was completely epithelized at about the twenty-eighth day. Its gross appearance was then almost identical with that of the completely epithelized control wound, of twenty-one days, which is shown in Fig. 5.

II. *Microcopic Observations*

A. *Control Wounds.* By the seventh day, the epidermis adjacent to the edge of the wound had increased in thickness from two to ten cell layers and showed increased mitotic activity. The cells in the uppermost layer contained many basophilic granules. Slight hyperkeratosis was also noted. In addition, there was some disorganization of the hair follicles in the area adjacent to the wound. The wound epithelium had just begun to migrate as a one-cell layer. In the base of the wound, which consisted of a very thin layer of the lower-most dermis, there was proliferation of mesenchymal cells and capillaries with moderate numbers of inflammatory cells. A thin fibrinous layer containing cell debris and polymorphonuclear leukocytes covered the wound.

By the fourteenth day, the epidermis adjacent to the wound edge continued to be hyperplastic.

* The hydrocortisone acetate and the suspending solution were both furnished, in separate vials, through the courtesy of Merck and Company, Inc.

TABLE 1

*Times of granulation and epithelization of hydrocortisone-treated and control wounds, as observed grossly**

Rabbit No.	Time of Granulation (days)				Time of Epithelization (days)				Total Period of Observation (days)
	Onset		Complete		Onset		Complete		
	Exp.†	Cont.‡	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	
SH 102	—	7	—	12	—	10	—	17	17
SH 103	—	7	—	11	—	11	—	—	14
SH 104	—	7	—	11	17	14	26	21	31
SH 105	—	7	—	9	18	11	25	21	58
SH 106	—	7	—	11	18	11	—	19	20
SH 108	—	8	—	13	18	15	—	21	23
SH 109	—	7	—	10	17	12	—	19	22
SH 111	—	7	—	11	16	11	27	20	49
SH 112	—	9	—	—	—	—	—	—	12
SH 113	—	6	—	11	16	13	—	20	20
SH 114	—	9	—	11	18	13	27	22	42
SH 116	—	9	—	11	18	13	—	18	18
SH 117	—	8	—	11	—	11	—	—	15
SH 118	—	8	—	11	—	—	—	—	13
SH 119	—	7	—	10	17	12	26	19	34
SH 120	—	7	—	10	—	12	—	—	14
SH 121	—	7	—	10	—	—	—	—	12
S 101	—	6	—	10	20	12	30	20	141
S 103	—	6	—	10	17	12	25	20	154
S 104	—	8	—	13	15	13	27	20	34
S 106	—	7	—	12	—	15	—	—	15
SH 201	—	9	—	11	19	15	—	21	21
SH 202	—	7	—	9	19	13	28	19	34
SH 203	—	7	—	11	—	14	—	—	17
SH 204	—	6	—	8	—	—	—	—	10
SH 205	—	7	—	9	15	11	—	17	19
SH 206	—	6	—	8	—	—	—	—	11
SH 208	—	8	—	—	—	14	—	—	16

* Under the microscope, in stained sections, "onset of epithelization" was seen to have occurred as much as 6 days before it was observed grossly.

† Exp. = Treated with hydrocortisone in a suspending medium.

‡ Cont. = Treated with the suspending medium alone.

The epithelium at the edge continued to grow into the wound, which was now filled in with granulation tissue. This granulation tissue consisted primarily of mesenchymal cells and a rich network of capillaries (Fig. 7). The older granulation tissue, that immediately above the panniculus carnosus, appeared to consist chiefly of new fibroblasts placed parallel to the wound surface.

By the twentieth day, the hyperplastic epidermis adjacent to the edge of the wound had decreased in thickness. The wound had become completely epithelized. Under this epithelium, or new epidermis, a thick layer of stratified fibrous connective tissue extended down to the panniculus carnosus (see Fig. 9).

By the fifty-eighth day, the epidermis adjacent to the wound edge had returned to normal. The fibrotic layer immediately under the epidermis appeared much less cullular, showing fewer fibroblasts. This modified layer contained the bases of hair follicles newly formed from the wound epithelium. Beneath the hair follicles, the dense fibrotic layer persisted (see Fig. 11).

B. *Hydrocortisone-treated wounds.* The epidermis adjacent to the edge of the wound showed the same changes as did the control. By the seventh day, the wound epithelium began to migrate over the edge of the wound. The wound showed little exudate and few inflammatory cells in the small amount of dermis covering the

panniculus carnosus. This lowermost part of the dermis, which could not be removed due to technical difficulties, formed the base of all wounds. It consisted mostly of collagen fibers and a few fibroblasts. Prior to epithelization, it failed to give rise to granuation tissue or to a fibroblastic layer.

About the fourteenth day, the edge of the wound epithelium had grown into the wound proper. The epithelium continued to grow over the very thin layer of lowermost dermis in close proximity to the panniculus carnosus and its blood vessels (Fig. 8). This was one of the main differences between the control and the hydrocortisone-treated wounds. As seen in Figs. 8 and 10, the epithelium appeared to grow over the panniculus carnosus and its vessels without the interposing rich substrate of granulation tissue that was seen in control wounds (Fig. 7). However, as the epithelium grew towards the center of the wound, a stratified fibroblastic layer appeared under the epithelium beginning at the edge of the wound (Fig. 8).

The wound was epithelized in about 28 days. The fibroblastic layer continued to thicken and eventually extended under the whole length of the new epithelium (Fig. 9). The further healing and follicle formation in these wounds was similar to that of the controls. In fact, their microscopic appearance by the fifty-eighth day was almost identical (see Fig. 11). The significant difference in their healing was that the control wounds granulated before epithelization, whereas the treated wounds did not granulate at all; treated wounds developed the fibroblastic layer without granulation after epithelization was complete.

DISCUSSION

The healing of cutaneous wounds by secondary intention has been studied extensively. The formation of granulation tissue and the subsequent epithelization have been reported by many investigators. Thus Forbus (3) and Anderson (4) maintain that the wound must first fill in with granulation tissue before the epithelium can grow and cover the wound. Furthermore, Bishop (5) stated that "the degree of maturation of the granulation tissue appears to determine the time of covering by epithelium."

The anti-inflammatory action of the adrenocortical steroids has been under investigation since Hench *et al.* reported the antirheumatic activity of cortisone (6). Systemic administration of cortisone was found to inhibit granulation of wounds in the rabbit (7). Similar observations in mice were reported by Spain (8). ACTH was also shown to delay wound healing with inhibition of granulation tissue in man (9).

In 1951, Baker (10) reported atrophy of the epidermis, hair follicles, and sebaceous glands in rats after local applications of hydrocortisone. The anti-inflammatory action of hydrocortisone and its ability to suppress neovascularization are well known (11, 12). In the present experiments, wounds in rabbits treated locally with hydrocortisone failed to form granulation tissue. The epithelium grew, in the absence of granulation tissue, and completely epithelized the wounds with a lag of about six to eight days as compared to the controls. As the epithelium grew, a new connective tissue developed under the epithelium beginning at the edge of the wound. The phrase "new connective tissue" is used in order to differentiate this fibroblastic layer from that which develops after maturation of granulation tissue. An explanation for the subsequent formation of this new connective tissue may be the relative inability of the aqueous suspension of hydrocortisone to penetrate the new epidermis and reach the dermis in sufficient concentration to inhibit fibroblastic proliferation.

The formation of new hair follicles from wound epithelium has also been reported by Breedis (13) and Billingham *et al.* (14) in non-hydrocortinized wounds. Kligman (15) reported the formation of new vellus hair follicles from reconstituted human epidermis after abrasion. The thickening of the epidermis and the disorganization of hair follicles adjacent to wounds have been reported in detail by Argyris (16).

It is possible that, under certain conditions, the epidermis and the dermis may respond independently to injury. When the dermis is inhibited and granulation tissue is not formed, the epidermis proceeds on its own to cover the exposed wound. As soon as the dermis is no longer inhibited, it then, too, proliferates even though the wound has already been covered with epithelium. Excessive proliferation of this type may conceivably be the basis of keloid formation. After both epidermis and dermis have proliferated and covered the wound, there must undoubtedly be some interaction between them in order to form the hair follicles which are specialized structures of the skin.

SUMMARY

1. Topical application of hydrocortisone on the wounds of rabbits inhibited the formation of granulation tissue but did not prevent the growth of the epidermis over the wound.

2. The regenerating epithelium was capable of migrating over the base of wounds having little or no granulation tissue.

3. The lowermost part of the dermis was capable of proliferating and developing into a fibroblastic layer after it had been covered by growing epithelium.

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PLATE I

FIG. 1. Wound not treated with hydrocortisone, at 4 days. The chamber is in place; the cover glass, vaseline gauze, and filter paper have been removed. Arrow points to cut in the panniculus carnosus through which one of the 6 prongs of the chamber was inserted into the subcutaneous space. The blood vessels of the panniculus carnosus are visible. × 1.

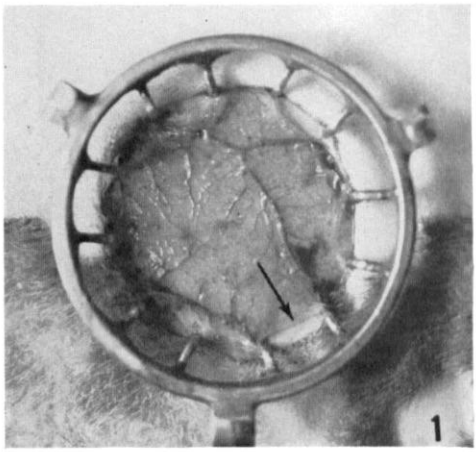
FIG. 2. Wound treated with hydrocortisone, at 4 days. The blood vessels of the panniculus carnosus are visible. The arrow points to the slightly elevated region of skin caused by the loop of wire in the subcutaneous tissue. × 1.

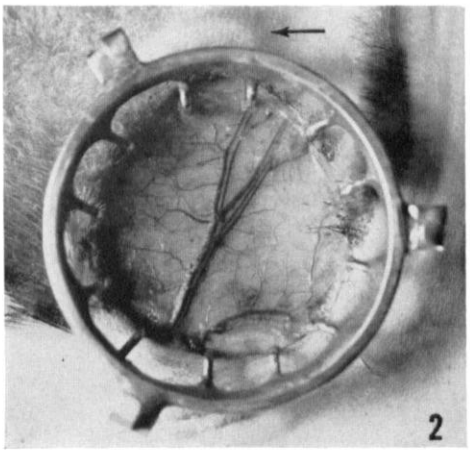
FIG. 3. Wound not treated with hydrocortisone, at 13 days. The blood vessels of the panniculus carnosus are completely covered with granulation. Arrow points to edge of migrating wound epithelium. × 1.

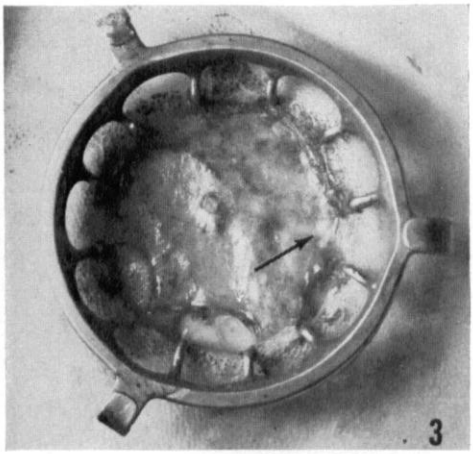
FIG. 4. Wound treated with hydrocortisone, at 13 days. The blood vessels of the panniculus carnosus are clearly visible. Note the absence of granulation tissue. Compare with Fig. 3. × 1.

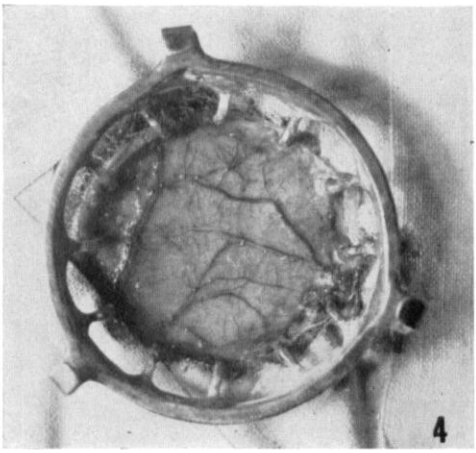
FIG. 5. Wound not treated with hydrocortisone, at 21 days. This wound is completely epithelized. × 1.

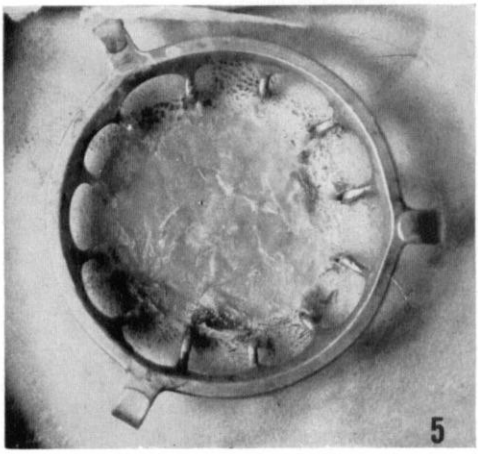
FIG. 6. Wound treated with hydrocortisone, at 21 days. Note the absence of granulation in this wound. The blood vessels of the panniculus carnosus are clearly visible. Arrow points to the edge of migrating wound epithelium. Compare with Fig. 3. × 1.











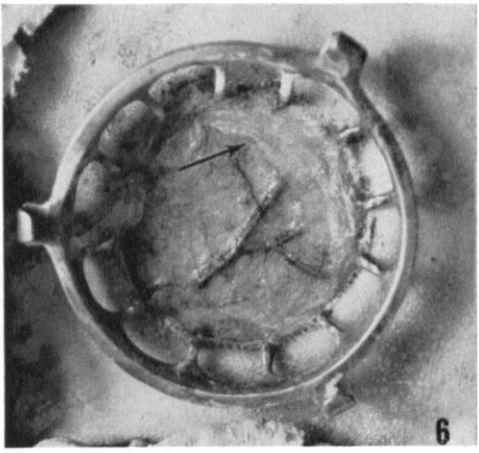


PLATE I

PLATE II

FIG. 7. Central part of wound not treated with hydrocortisone, at 14 days. The edge of the advancing epithelium has migrated to point (X). The base of the wound, above the panniculus carnosus (Z) has been filled in by a thick layer of granulation tissue. There are numerous capillaries near the surface of the wound (Y). Hematoxylin and Eosin. × 40.

FIG. 8. Central part of wound treated with hydrocortisone, at 19 days. Note the small amount of lowermost dermis (X) between the edge of the migrating wound epithelium (W) and the panniculus carnosus (Y). The new fibroblastic layer (Z) beneath the wound epithelium is shown. Hematoxylin and Eosin. × 40.

FIG. 9. Central part of wound treated with hydrocortisone, at 28 days. The new epidermis has covered the wound. A thick layer of fibroblastic tissue (X) has formed under the new epidermis. Cross sections of wounds, at 20 days, which were not treated with hydrocortisone show a similar appearance. Hematoxylin and Eosin. × 40.

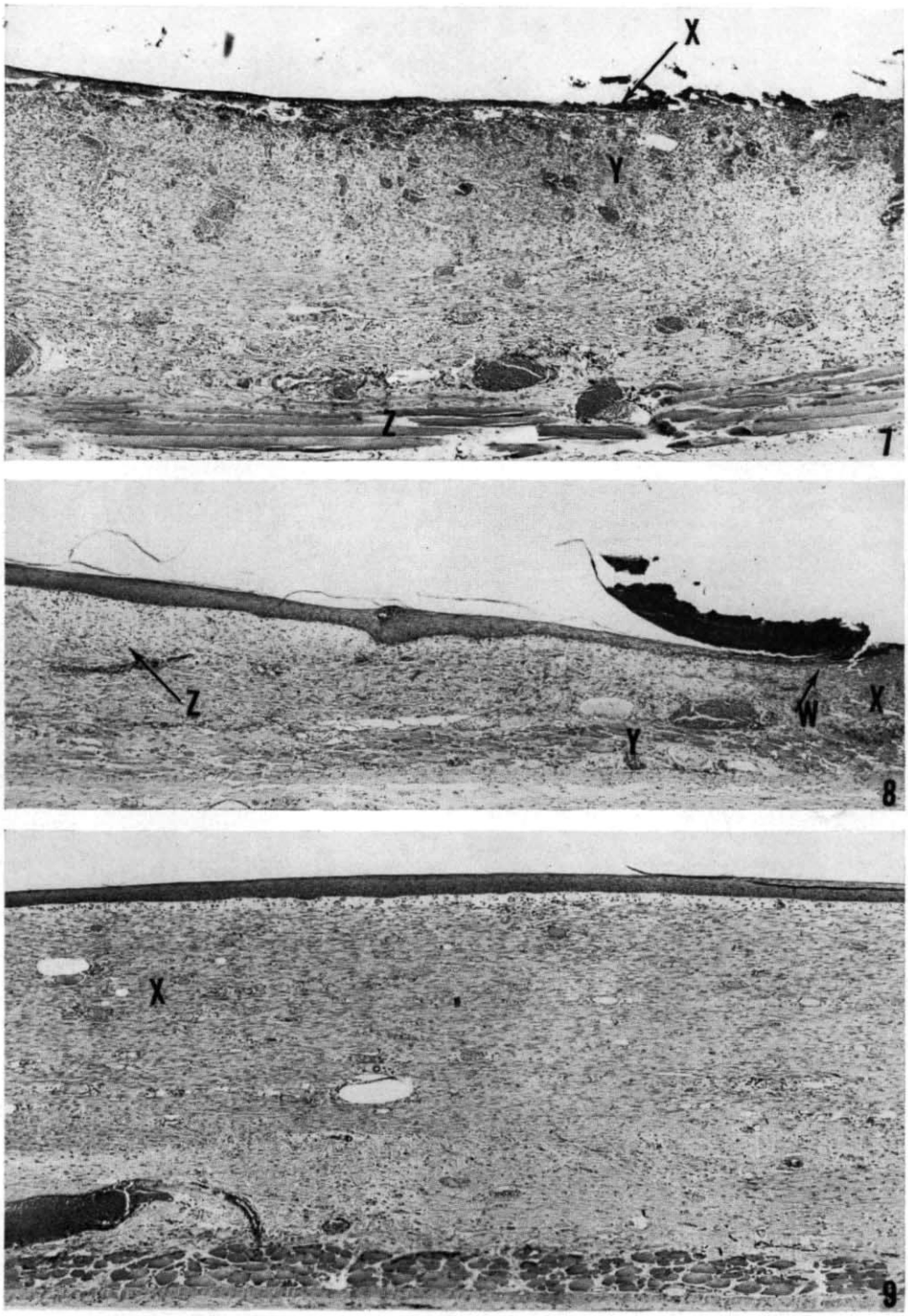


PLATE II

PLATE III

FIG. 10. Section through the advancing epithelial edge of a hydrocortisone-treated wound at 28 days. The epithelium (W) has migrated from the left to a point past the center of the photograph. The bundles of the panniculus carnosus muscles are seen at the bottom. A large blood vessel (lower left) and a smaller vessel (above X) of the panniculus carnosus are shown. The area around X consists of the lowermost dermis, note the bundles of old collagen and the absence of granulation tissue. Hematoxylin and Eosin. × 320.

FIG. 11. Central part of wound treated with hydrocortisone at 58 days. The new epidermis has covered the wound. Note the decreased cellularity of the upper dermis (compare with Fig. 9) and the cross sections of new follicles in this layer (Y). The persisting fibroblastic layer (Z) is clearly seen. Cross sections of wounds at 58 days which were not treated with hydrocortisone showed a similar appearance. Hematoxylin and Eosin. × 40.

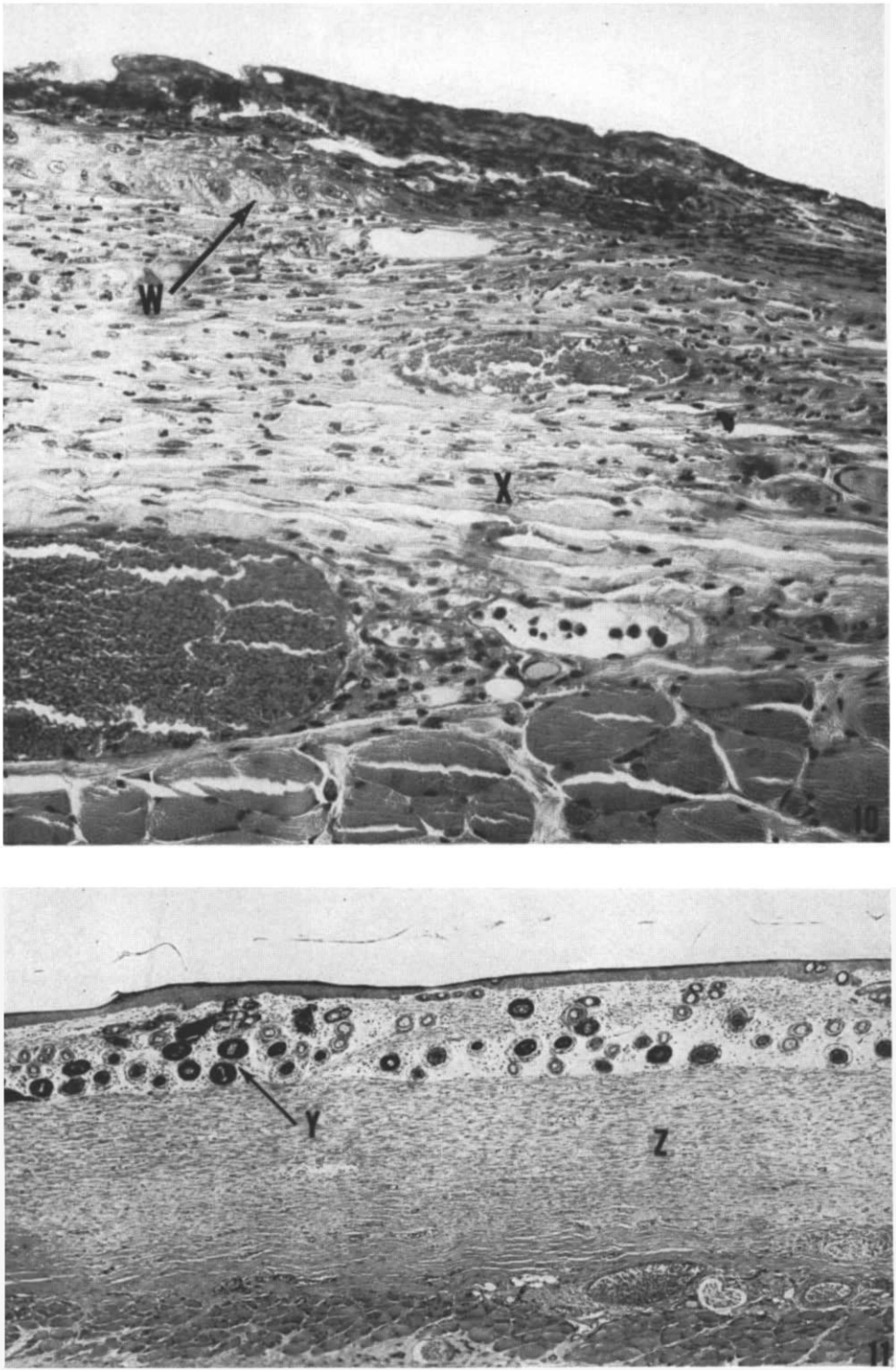


PLATE III