HUMAN WOUND REPAIR

I. Epidermal Regeneration

GEORGE ODLAND and RUSSELL ROSS

From the Departments of Medicine, Biological Structure, and Pathology, University of Washington, Seattle, Washington 98105

ABSTRACT

A series of linearly incised superficial skin wounds was made on the forearms of young adult male volunteers. Wounds were sampled at several intervals between 3 hr and 21 days after wounding, for study by light and electron microscopy. The light microscopic observations show that regeneration of epidermis in human wounds conforms chronologically to that reported for the epidermis in superficial wound repair in laboratory animals. It is further shown that "ruffling" of cell membranes characterizes the cells of the migrating epidermis in early wound healing. This study reveals that the basement lamina and hemidesmosomes are established by epidermis in contact with the fibrin net at the base of early wounds. Epidermal cells in the wound environment are shown to be phagocytic. Analysis of the submicroscopic cytology of differentiating and maturing regenerated epidermis reveals that, in the sequence of events, the formation of filaments, basal lamina, and desmosomes is followed chronologically by evolution of keratohyalin granules and, subsequently, by keratinization of the surface epidermal elements. The entire sequence of migration, differentiation, and ultimate keratinization in the superficial wounds studied requires 3–5 days for completion.

INTRODUCTION

Recent advances in the knowledge of biochemistry of healing skin wounds have been attended by informative analysis of the submicroscopic structure of regenerating connective tissue components but not of the ultrastructure of regenerating mammalian epidermis (1). In man, the absence of heavy pelage and, therefore, a major hair follicle source for epithelial regeneration renders epidermal repair somewhat distinctive from that of hairy mammals. To our knowledge, the ultrastructure of regenerating connective tissue components has not been analyzed in man. In order to fill some of these gaps in information, but more importantly to relate the concomitant processes of connective tissue and epithelial repair, we have undertaken this study of repair of incised superficial skin wounds in man. This paper concerns itself with the fine structure of the regeneration of human epidermis.

MATERIAL AND METHODS

Several series of superficial linear incisions were made in the skin of the flexor aspect of the forearms of four young adult male volunteers. Incisions were made parallel to the long axis of the forearm. Each wound was 8–10 mm in length and about 0.5 mm in depth. In no instance did the incision extend through the full thickness of the dermal connective tissue. In a given subject, wounds were made at different times such that all wounds representing different periods of repair could be sampled by biopsy at the same time and processed simultaneously. In each series of wounds in one subject, individual wounds

were spaced at least 3 cm apart. In several different series, the wounds were evaluated at time intervals of 3 and 12 hr, and 1, 2, 3, 5, 7, 14, and 21 days after wounding. With a high speed drill fitted with a skin biopsy punch of 2½ mm diameter, two discs of skin were removed from each wound and fixed in 1% osmium tetroxide buffered in *s*-collidine (pH 7.4) at 4°C for 2 hr. Some of the wounds were post-fixed in neutral buffered formalin. Glutaraldehyde fixation was not employed because the original blocks fixed in this agent did not appear to be well preserved. The tissues were embedded in Epon 812. The blocks were remounted on aluminum chucks in order to orient them to provide full-thickness sections of the wound cut perpendicular to the length of the original incision. Sections 1–1½ μ in thickness were made from all blocks for light microscopy. They were stained by the method of Huber with basic fuchsin and methylene blue (2). Thin sections prepared for electron microscopy were cut at thicknesses between 600 and 1,000 A and were stained with uranyl acetate solution followed by lead citrate (3). Specimens were examined in either a Siemens Elmiskop II, an RCA EMU 3-G, or an AEI-EM6B electron microscope.

OBSERVATIONS

The regeneration of epidermis in mammals has been described conventionally by histologists to consist of three phases: mitosis, migration, and differentiation. No attempt was made to evaluate mitotic activity.

Light Microscopy

MIGRATION OF EPIDERMIS: Examination of histological preparations reveals that the earliest detectable change in the epidermis is a thickening of the epidermis remaining at the wound margin adjacent to the early clot. This thickening appears to result from an increase in the volume of the epidermal cells adjacent to the wound. At 12 hr, there has been no apparent movement of epithelium into the wound. At 24 hr, the cut edge of the epidermis apposed to the clot shows distinctive cytoplasmic processes that project toward the clot (Fig. 1). Suprabasal cellular elements of the thickened epidermis at the wound edge show degenerative characteristics of decreased cell volume and darkening of the cytoplasm (Fig. 1). These degenerated cells appear to maintain contact with viable-appearing elements of the epithelium. In the interval between 24 and 48 hr the epidermis has commenced migrating from the incised edge toward the center of the wound. In these small superficial wounds, migration usually appears to proceed symmetrically from both sides toward the center of the wound; hence, in profile, the regenerating epidermis has the appearance of a pair of wedges directed towards each other at the middle of the wound (Fig. 2, insert). In some instances, migration from one side of the wound commences in advance of that from the other side so that, at the time of sampling, there appears to be migration from only one side of the wound. At the leading edge of the moving epithelial sheet which migrates across the wound surface, cells have a flat (squamous) contour when seen in cross-section. Toward the margin of the wound, away from the leading edge, stratification of the epithelial elements is more apparent (inserts, Figs. 1 and 2). The advancing epidermis appears to follow a plane defined by a fibrin net which in turn is enclosed by serous exudate containing inflammatory cells. This plane lies deep to the wound crust.

DIFFERENTIATION OF EPIDERMIS: Epithelization of these small wounds is invariably complete by 3 days and, often, by 2 days after wounding. After wound closure, the newly regenerated epidermis becomes highly stratified and thicker than the normal surrounding epidermis (Fig. 3). Epidermal thickness decreases to nearly normal by the 5th–7th day after wounding.

On the 2nd–3rd day the cytoplasm of the regenerated epidermal cells in the upper layers, near the center of the wound, has evolved keratohyalin granules; this evidence of cellular differentiation and subsequent keratinization appears first at the periphery of the regenerating epithelium near the original wound margin, and does not appear in the center of the wound until after the epithelial sheet has covered the entire wound and has become stratified (Fig. 3).

Electron Microscopy

MIGRATION OF EPIDERMIS: Electron microscopic analysis of the wound at the time of early epidermal cell migration reveals a substantial increase in the diameter of cells at the advancing margin. Ribosomes are relatively prominent, but intracellular filaments are sparse. Extensive pseudopodial projections of cytoplasm at the cortex of such cells have a pale appearance in comparison to the cytoplasm of unaffected cells located peripheral to the wound margin. The cytoplasmic projections contain no organelles

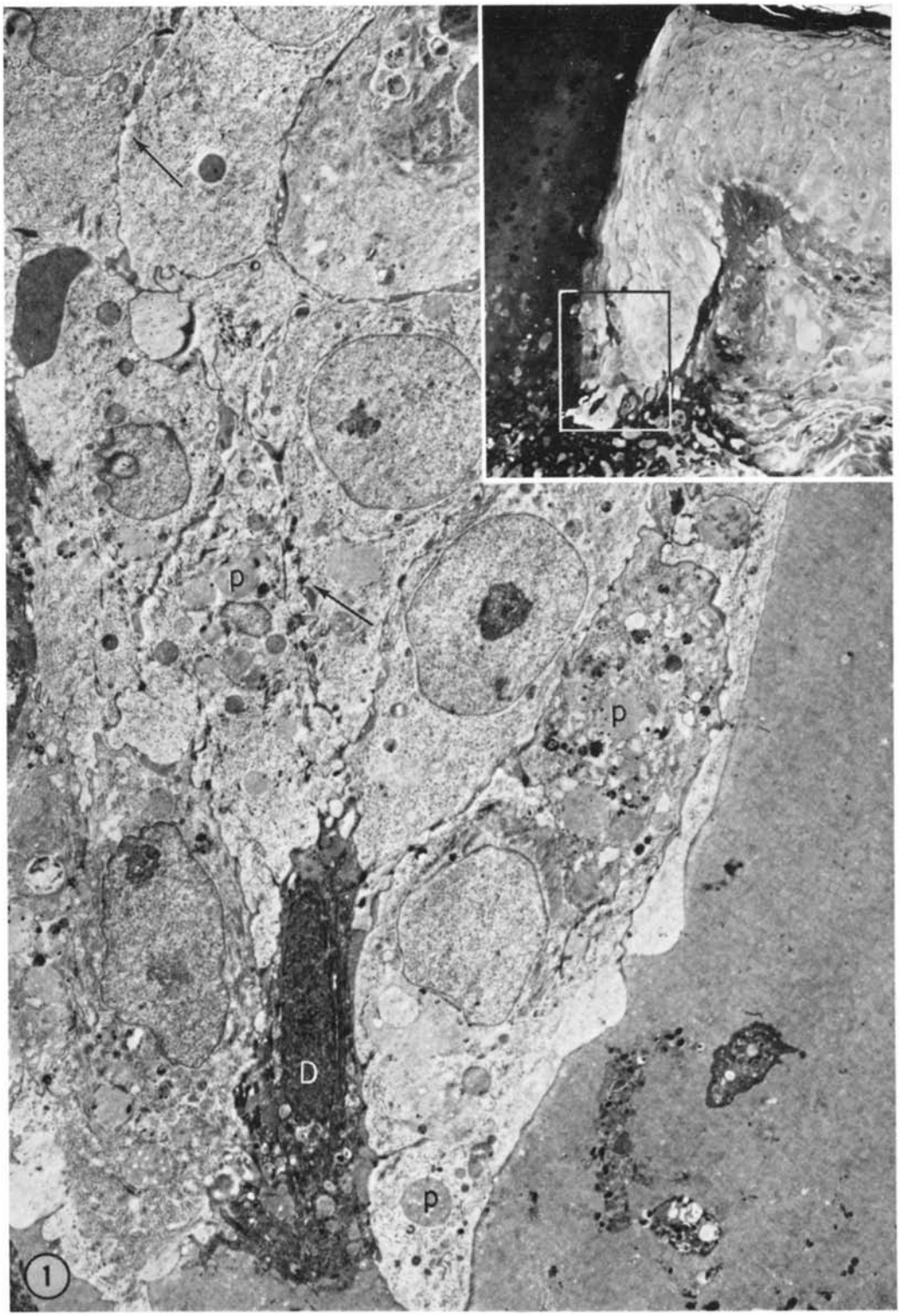

FIGURE 1 Advancing margin of epidermal sheet in 24 hr old wound. Basal surface is at left margin; serous exudate occupies lower right corner of micrograph. Note relatively pale projections of cortical cytoplasm of advancing cells, especially at their surfaces directed toward wound exudate. Numerous phagocytic inclusions (*p*) are seen in cytoplasm of migrating epidermal cells. Dark cell with pycnotic nucleus (*D*) is interpreted to be degenerated. Arrows, desmosomes. × 3,500.

Insert is a photomicrograph showing comparable region (rectangle) in mirror-image relationship to field depicted in accompanying electron micrograph. In center, note tongue of migrating epidermis extending beneath wound crust (left) from thickened epidermis of wound margin (upper right). × 190.

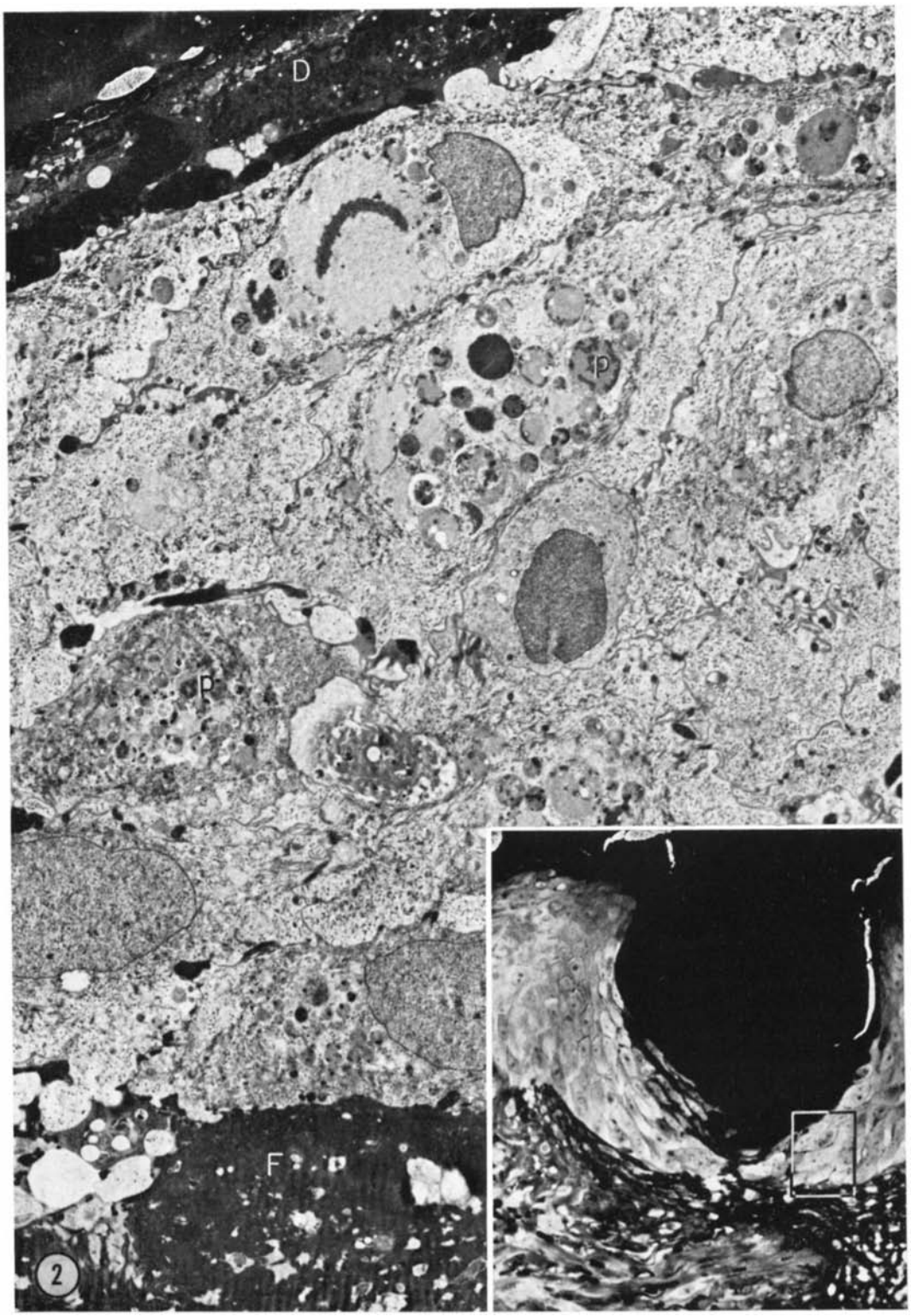

FIGURE 2 Migrating stratified epidermal sheet a few cells away from the advancing edge of a 2 day old wound. The basal surface lies upon a mat of fibrin (*F*). Pale cortical cytoplasmic processes are present within the stratified epithelial mass. Note numerous phagocytic inclusions (*p*) and degenerated cells (*D*) at the surface of the epithelium in the wound crust. × 3,500.

Insert is a photomicrograph of a 1.5 μ section from the same specimen as that shown in the electron micrograph (area of rectangle). Note that symmetrical advancing epithelial wedges have nearly met at the base of the wound. × 190.

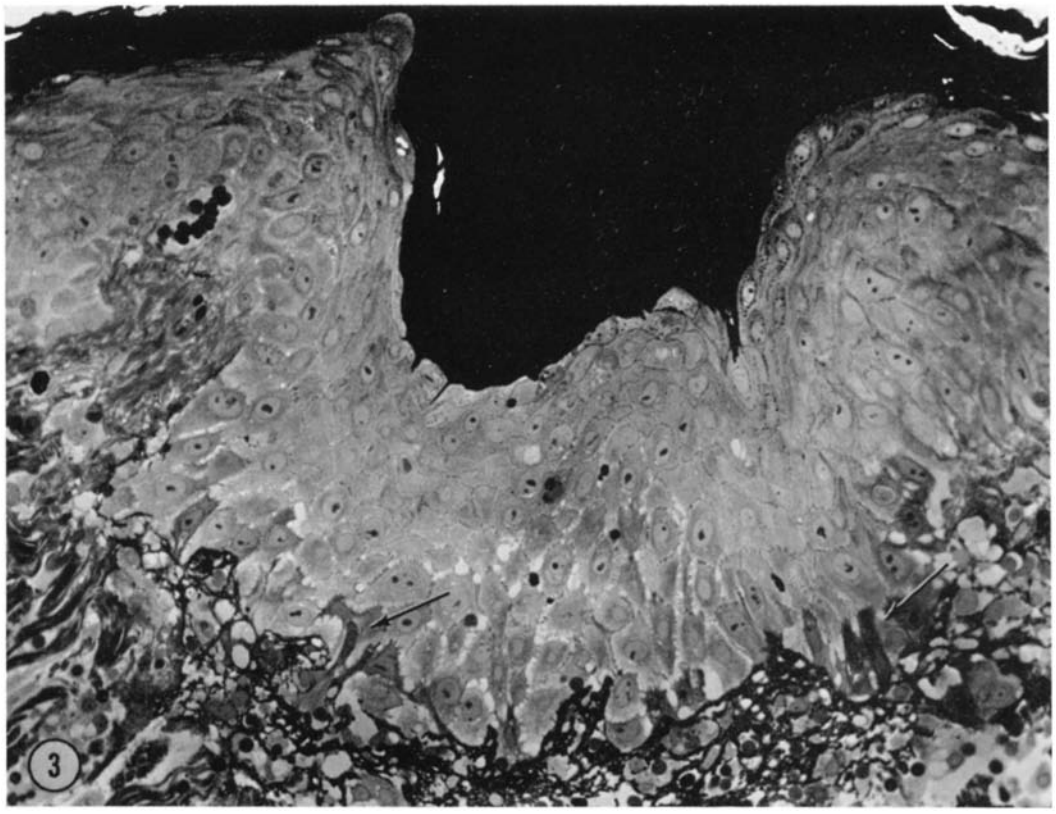

FIGURE 3 Photomicrograph of the hyperplastic epidermis of a newly epithelized 3 day old wound. Keratohyalin granules are seen in the more differentiated upper strata beneath the crust near the wound margin, but not in the center of the wound. Arrows indicate individual degenerated cells in basal and suprabasal regions. Careful examination may permit the viewer to perceive pale surface projections of cells at the basal surface and between the lower strata of epidermal cells. The dark outlines of cells in the upper strata near the center of the wound can be attributed to numerous desmosomes. × 260.

except rosettes of free ribosomes and a scanty dispersion of tonofilaments. As the advancing edge of the epithelial sheet subsequently moves across the wound, these features of the cell cytoplasm persist and the cells frequently have pale cytoplasmic projections at their surfaces (Figs. 1 and 2). Pale projections of the cortical cytoplasm prevail especially at the free surfaces of the epithelial cells (Figs. 4 and 5) but are also present between stratified epidermal cells several cells away from the advancing margin (Figs. 2 and 6). The size of the cellular projections varies from that of ordinary microvilli to that of much larger blunt processes, which appear to pervade the intercellular space (Figs. 2, 5, 6) as well as to protrude into the milieu of the wound exudate.

The early migration of the epithelium takes place in a milieu of homogeneous, dense exudate which is interpreted to be a serous exudate (Fig. 1). In the uppermost portions of the clot this exudate contains cell and tissue debris, whereas beneath the plane of the migrating epithelium the exudate encloses fibrin strands. The fibrin subsequently forms a loose mesh and becomes an increasingly predominant feature of the extracellular matrix as the wound increases in age from 24 to 72 hr (see Figs. 5 and 7). In the early stages of regeneration, the homogeneous serous exudate appears to be the predominant component of the milieu in immediate contact with the advancing epithelium (Figs. 4, 5). This exudate, together with strands of fibrin, is readily observed within the markedly widened intercellular spaces which characterize the advancing epithelial sheet

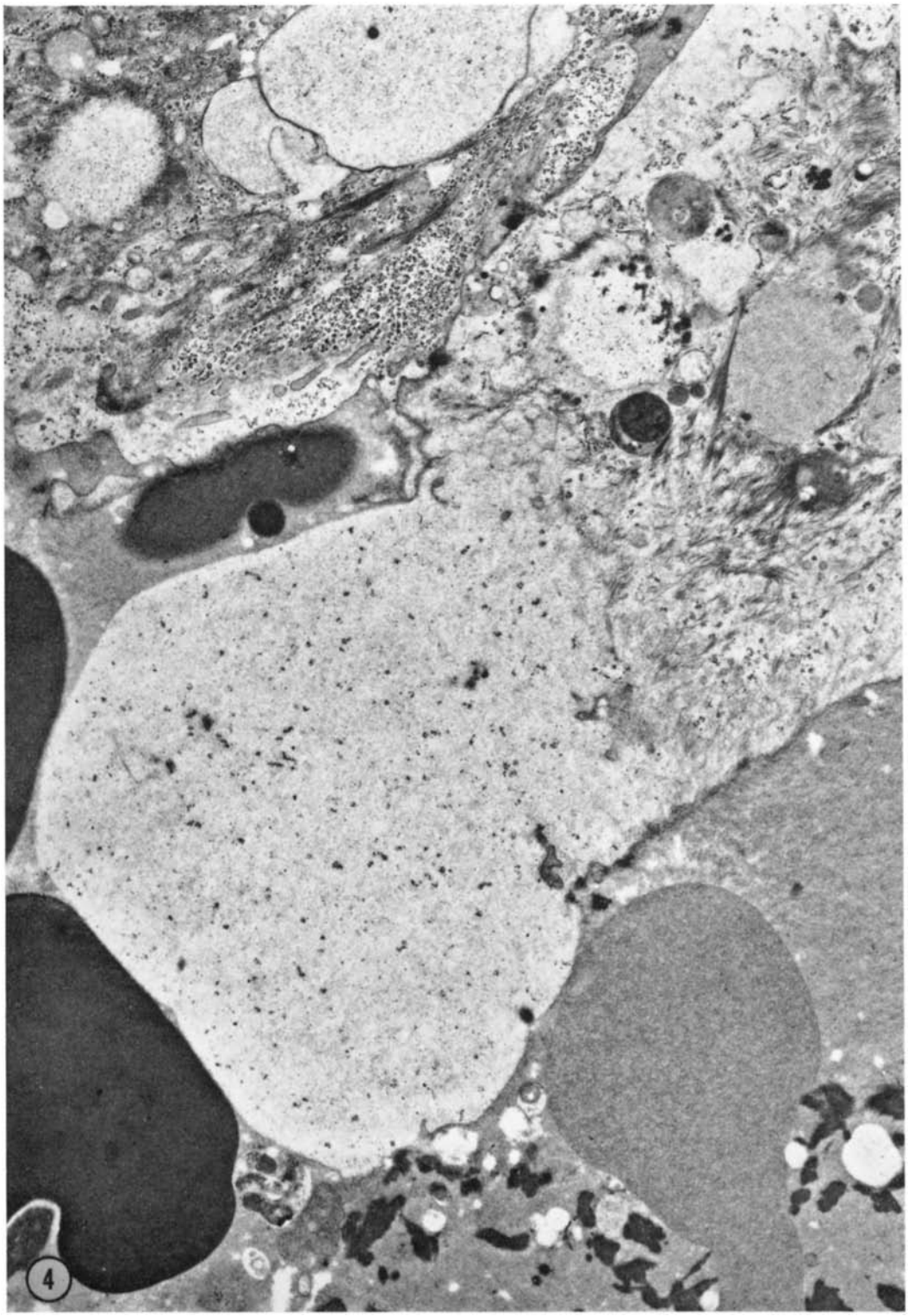

FIGURE 4 Cortical cytoplasmic process at advancing edge of epidermal cell at tip of migrating epidermal sheet in 24 hr wound. Note paucity of filaments and organelles in process. Note that process is in immediate proximity to exudate and erythrocytes in exudate. Bits of fibrin appear at the lower right. × 10,500.

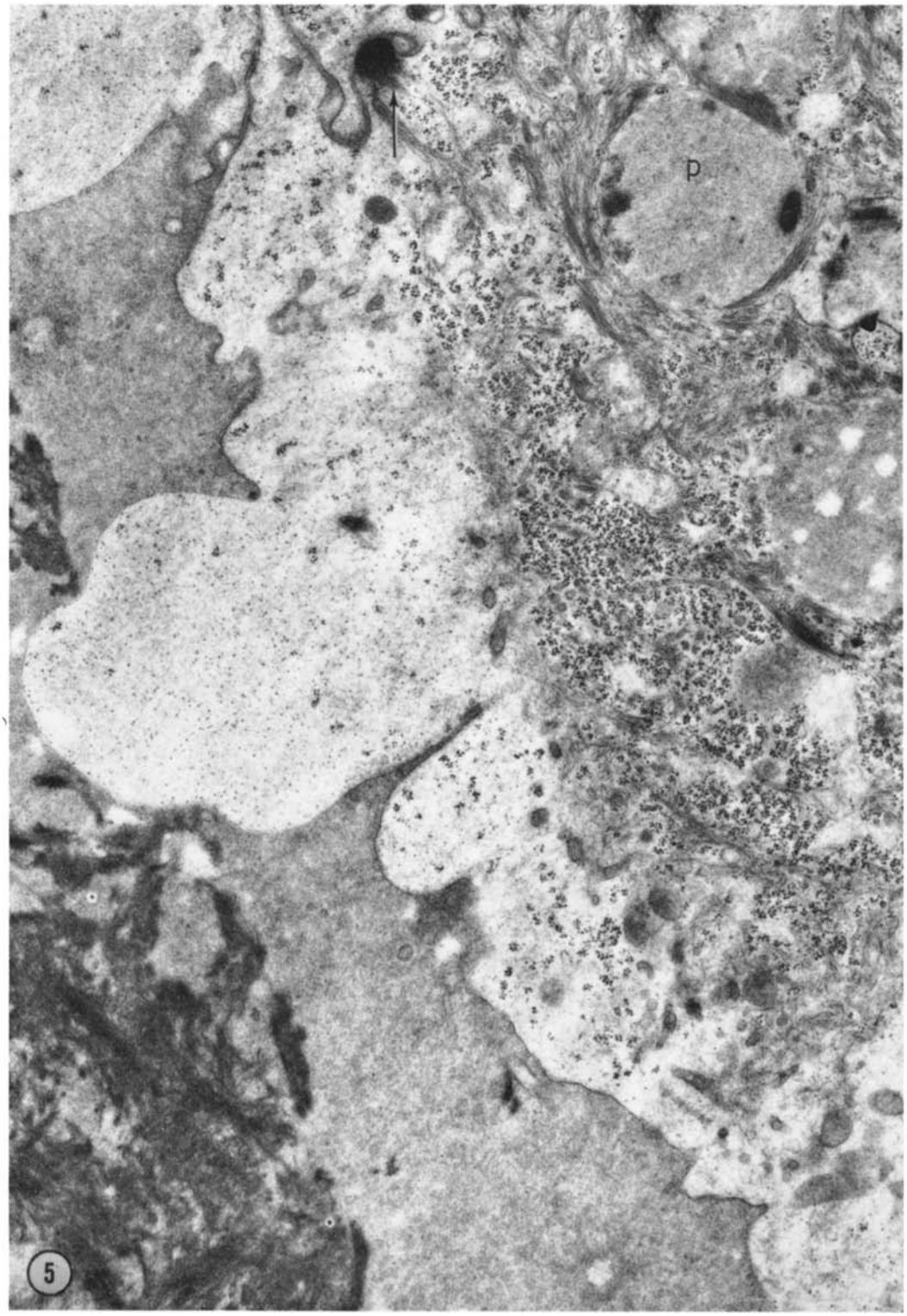

FIGURE 5 24 hr wound. Cortical cytoplasmic projections at basal surface of advancing margin of epidermis. This figure is a higher magnification view of the cell appearing at the extreme lower left corner of Fig. 1. Note paucity of structured elements in cell processes; observe cytoplasmic filaments, ribosome clusters, and phagocytic inclusions (*p*). *Note fibrin strands in exudate* at lower left. Arrow indicates internal face of desmosome and associated filaments. × 17,000.

(Fig. 6). Occasionally inflammatory cells also distend the intercellular spaces.

FORMATION OF BASEMENT LAMINA: The only apparent initial support for the advancing margin of the migrating epithelial wedge consists of the desmosomes between adjacent epidermal cells (Figs. 1 and 6). Subsequently, the basal surface of the epidermal sheet establishes contact with strands of fibrin in the clot (Fig. 2). At such points the cytoplasm of the cell appears differentiated and contains small aggregates of filaments in the otherwise watery cortical cytoplasm. In these regions the external milieu is modified by the appearance of the lucent zone of basement lamina structure at the site of hemidesmosomes. The basement lamina and hemidesmosomes are formed in the vicinity of fibrin and bear no observable relationship to either collagen or fibroblasts. In those areas where the fibrin net is discontinuous, the dark component of the basement lamina may be seen. With subsequent replacement of the fibrin by newly formed collagen, the hemidesmosomes and basement lamina resume their normal relationship to the collagenous stroma (see Figs. 7 and 8).

PHAGOCYTIC INCLUSIONS IN REGENERATING EPIDERMAL CELLS: A striking characteristic of the regenerating epithelial cells, which has not previously been recognized in mammalian epidermis, is their capacity to phagocytize particulate debris from the milieu, including elements of the same density as the serous exudate together with bits of fibrin which surround the cells (Figs. 2, 5, 6). The intracytoplasmic inclusions of fibrin and serum protein are commonly bounded by membranes. In addition, large aggregates of melanin particles are enclosed by membranes in the cytoplasm of advancing epidermal cells. A number of cells are seen to contain dense, matted aggregates of tonofilaments without apparent attachment to desmosomes at the cell periphery.

DEGENERATION OF MIGRATING EPIDERMAL CELLS: Some epidermal cells degenerate during the early stage of wound repair. The characteristics of cells interpreted to be degenerating (Fig. 1) are pycnotic nuclei with clumped chromatin, decreased cytoplasmic volume, aggregation of previously differentiated intracellular filaments, large numbers of ribosomes, and structures which resemble either vacuoles or mitochondria without demonstrable cristae. Individual cells within the epidermal sheet may show these changes in the early stages of wound epithelization, whereas most of the adjacent cells possess the cytoplasmic characteristics described for migrating epidermal cells. Degenerating cells appear at the advancing margin of the epithelial tongue (Fig. 1). They commonly appear at the surface of the epidermis in the original wound margin and continue to appear, in 3-day-old wounds, in the basal and suprabasal regions of the hyperplastic epidermal elements which characterize the fully but recently epithelized wound surface (see Figs. 2 and 3).

DIFFERENTIATION: Cytological features interpreted as differentiation of regenerating epidermal cells appear first *during the stage of migration* at a location several cells back from the advancing epithelial edge, with the concomitant appearance of hemidesmosomes and basement lamina overlying the fibrin network. The basal epidermal cells evolve broadly developed cisternae of rough endoplasmic reticulum and numerous clusters and aggregates of ribosomes in spiral array (Fig. 7). The mitochondria tend to have a larger diameter than normally encountered in the epidermis, and many of them are longer than usual. During this period an increasing number of tonofilaments becomes apparent within the cytoplasm of the cells.

In the hyperplastic epidermis of 3–4-day-old wounds which have completely reepithelized, the intercellular space becomes narrowed to normal dimensions (Figs. 3, 9, 10). In such wounds, differentiation in the cells of the spinous layer, prior to their maturation, is marked by the appearance of many aggregates of three to eight ribosomes, most of which lie free within the cytoplasm (Fig. 9). Subsequently the clusters of ribosomes are a less prominent component of the cytoplasm in cells which become increasingly filled instead with cytoplasmic filaments, many of which form large parallel strands (Fig. 10). During the early stage of differentiation, the epidermal cell shows increments of tonofilaments in the cytoplasm, and the perinuclear region comes to be occupied by prominent mitochondria with irregularly disposed cristae (Fig. 7). Significant development of a rough endoplasmic reticulum is rarely encountered in normal epidermis, but it is commonly found in the basal and suprabasal regenerating epidermal cells during differentiation.

Many of the cells which show signs of early

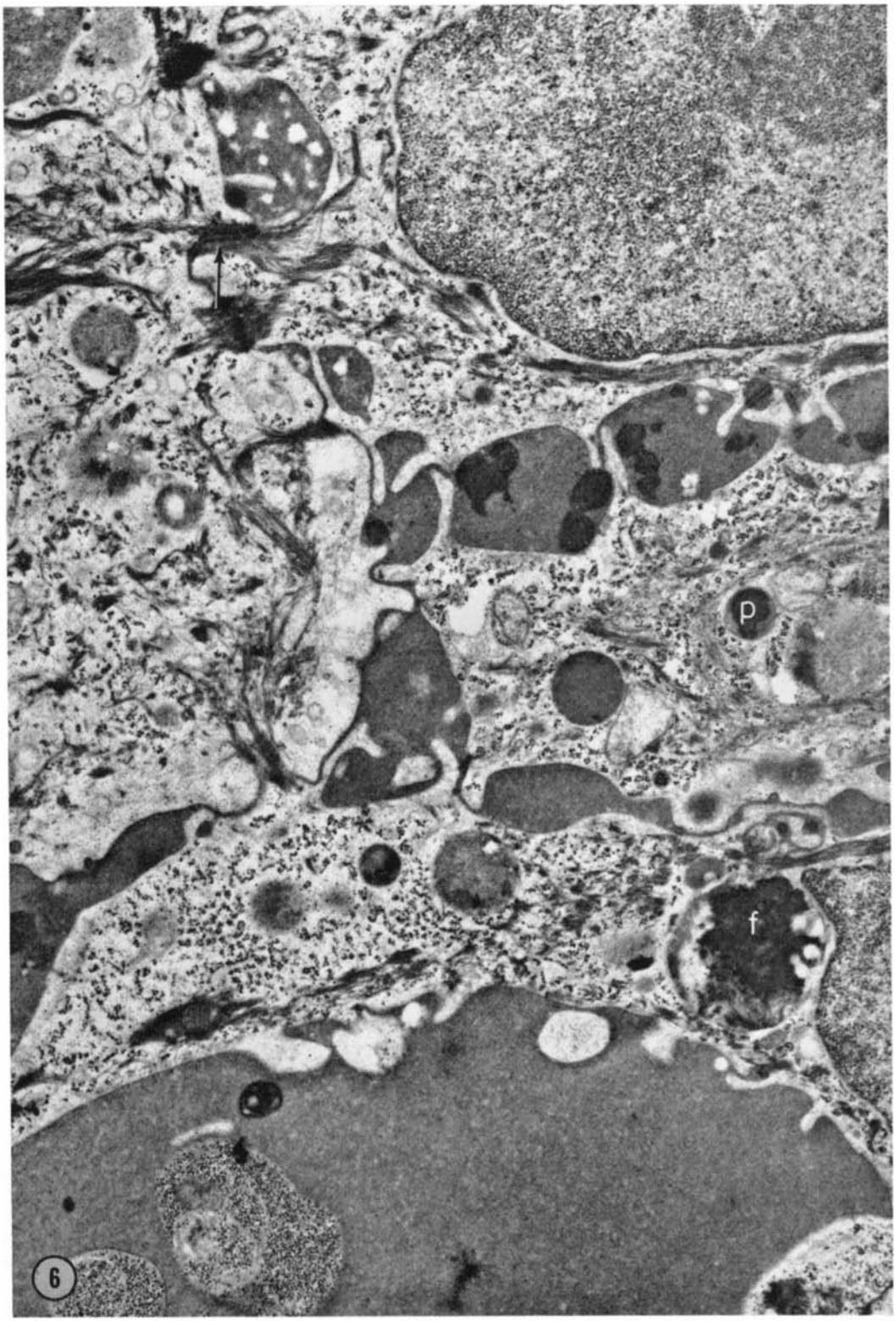

FIGURE 6 24 hr wound. This micrograph reveals particularly the exudate and fibrin strands between migrating epidermal cells, portions of three of which are shown. Phagocytic inclusions (*p*) are aggregates of matted tonofilaments (*f*) and exudate with bits of fibrin. Arrow indicates desmosome. Note also pale cortical cytoplasm of cell in left center. × 15,000.

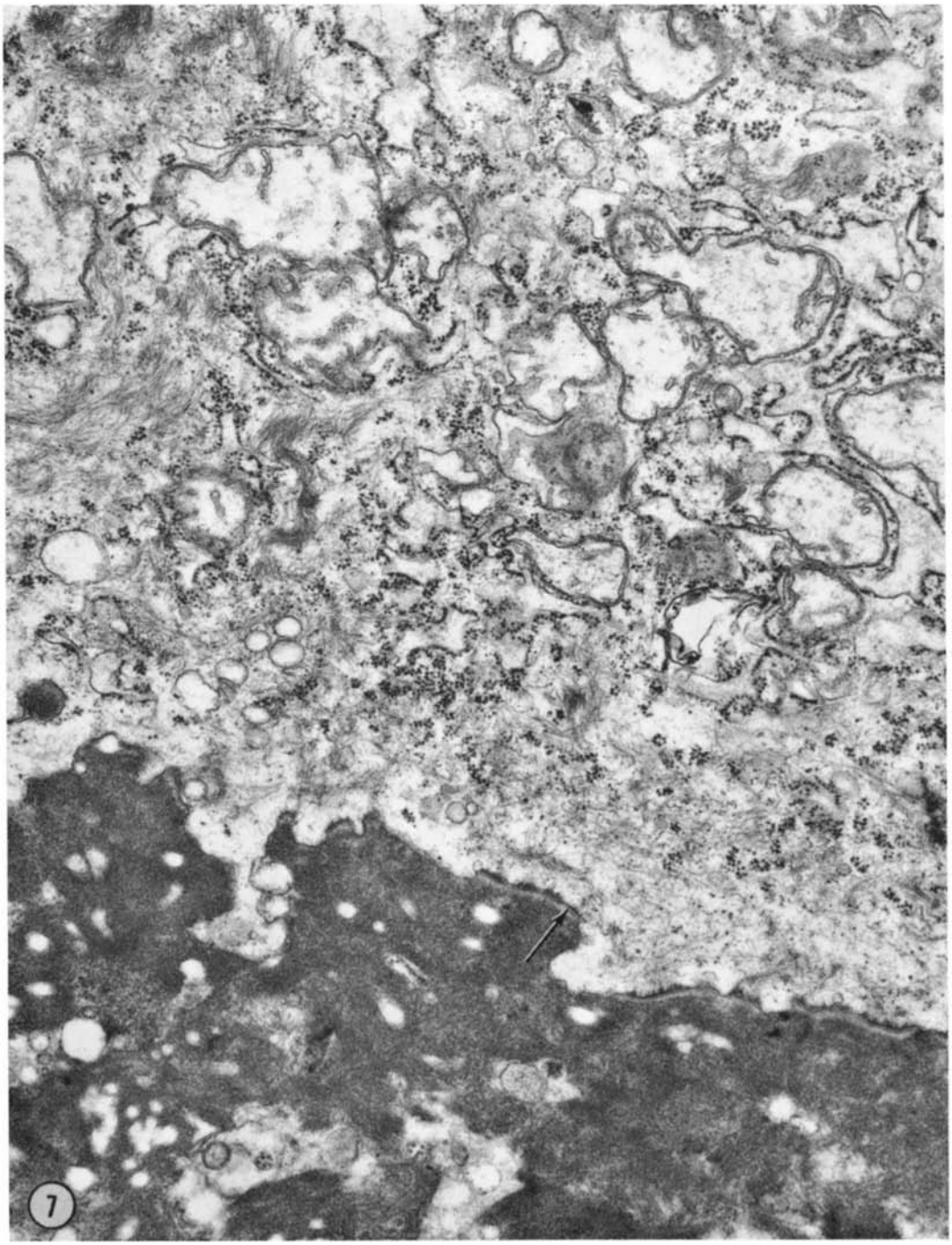

FIGURE 7 This micrograph of the basal surface of epidermis in a 2 day old wound demonstrates the formation of hemidesmosomes and the lucent component of the basement lamina (arrow) in relationship to the fibrin upon which the epithelial sheet migrates. It further reveals features of early differentiation of the cytoplasm as manifested by numerous large mitochondria, rough endoplasmic reticulum, spiral arrays and clusters of ribosomes, and fine cytoplasmic filaments. × 23,500.

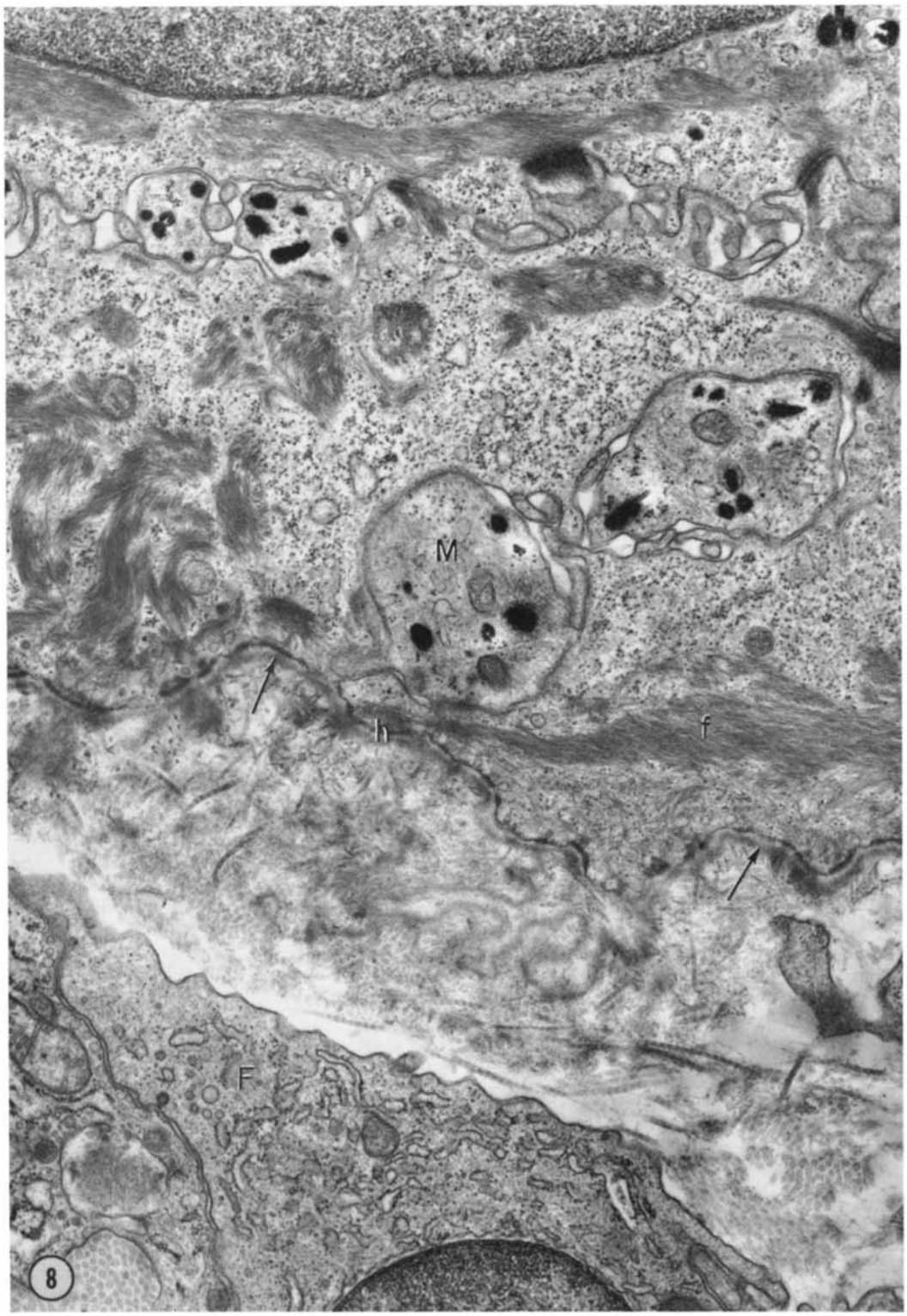

FIGURE 8 This micrograph of a 21 day old wound shows cytoplasm of mature basal epidermal cells and also hemidesmosomes (*h*) and basement lamina (arrows) in the presence of regenerated collagen and a fibroblast (*F*). Bundles of tonofilaments (*f*) and free ribosomes occupy the cytoplasm of the epidermal cells. Processes of melanocytes (*M*) containing melanin particles pervade the intercellular spaces. Compare with Figs. 3 and 7. × 18,500.

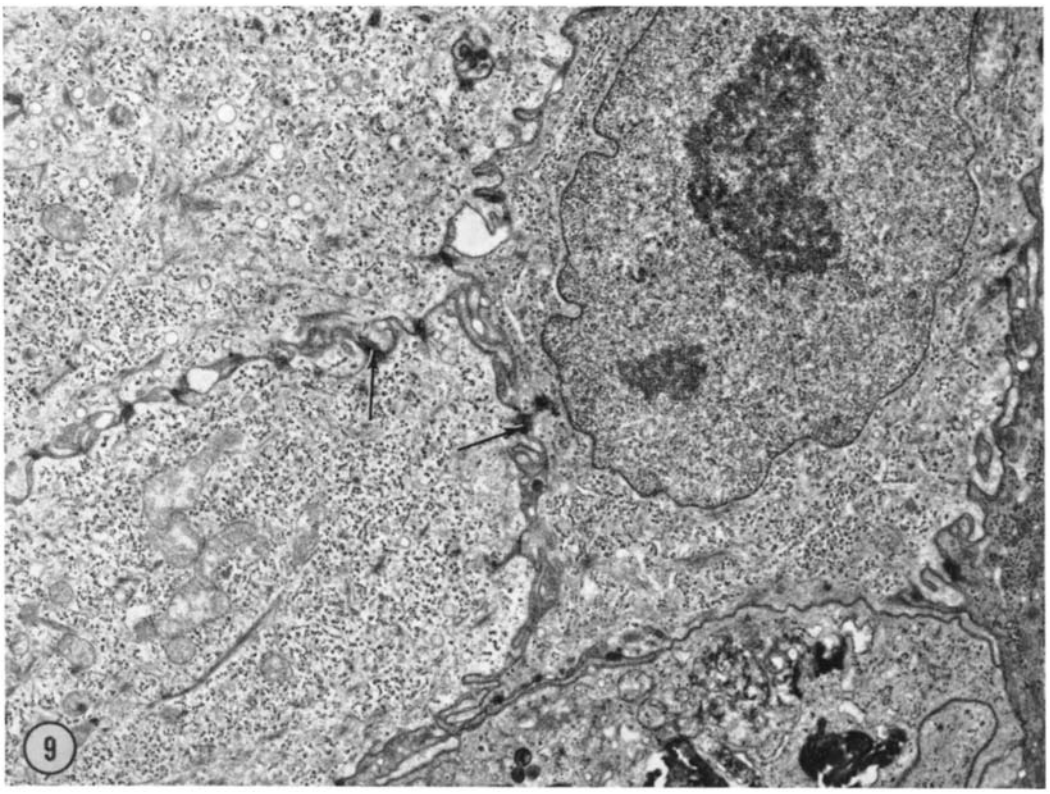

FIGURE 9 Early differentiation in lower strata of epidermal cells near the original margin of a 2 day old wound. This figure depicts changes characteristic of early differentiation in the basal and suprabasal cell layers. Filaments appear diffusely scattered throughout the cytoplasm amidst mitochondria, clusters of ribosomes, and some rough endoplasmic reticulum. Note convoluted cell membranes and paucity of desmosomes (arrows). A small part of the dense cytoplasm of a degenerated cell appears along the right margin. × 8,600.

differentiation also possess a cortical zone containing a few filaments, a few ribosomes, and surface projections suggestive of cortical cytoplasmic changes described for the migrating epidermal cells (Figs. 3, 7, 9). These features are commonly seen in the basal and suprabasal region of the hyperplastic epidermis. A prominent feature of the newly differentiating epidermal cells is the increased number of desmosomes at their profiles, particularly in the upper spinous layers (Figs. 3 and 11). No attempt has been made to quantitate these findings.

In the upper layers of newly differentiating epidermis, there is no stratum granulosum. Neither keratohyalin granules nor membrane-coating granules are encountered (Fig. 11). The surface cells retain nuclear remnants as they become flattened beneath the wound crust (Fig. 11). Residua of phagocytic inclusions and aggregates of ribosomes (and/or glycogen) are retained within these "parakeratotic" elements. Clumps of densely stained tonofilaments are also present.

Subsequent differentiation is manifested by *keratinization*. It is heralded by the appearance of membrane-coating granules in cells of the upper spinous layers (Fig. 12). Keratohyalin granules evolve in the filament-filled epidermal cells. They appear to be smaller and more numerous in each cell than in the more normal epidermal cells located distant from the area of regeneration (Fig. 3). Overlying these spinous cells are anucleate cells of the stratum corneum. These cells are characterized by the normal cytoplasmic keratin pattern and by the thick plasma membranes which mark the morphologic end point of epidermal cell differentiation (Fig. 12). In the

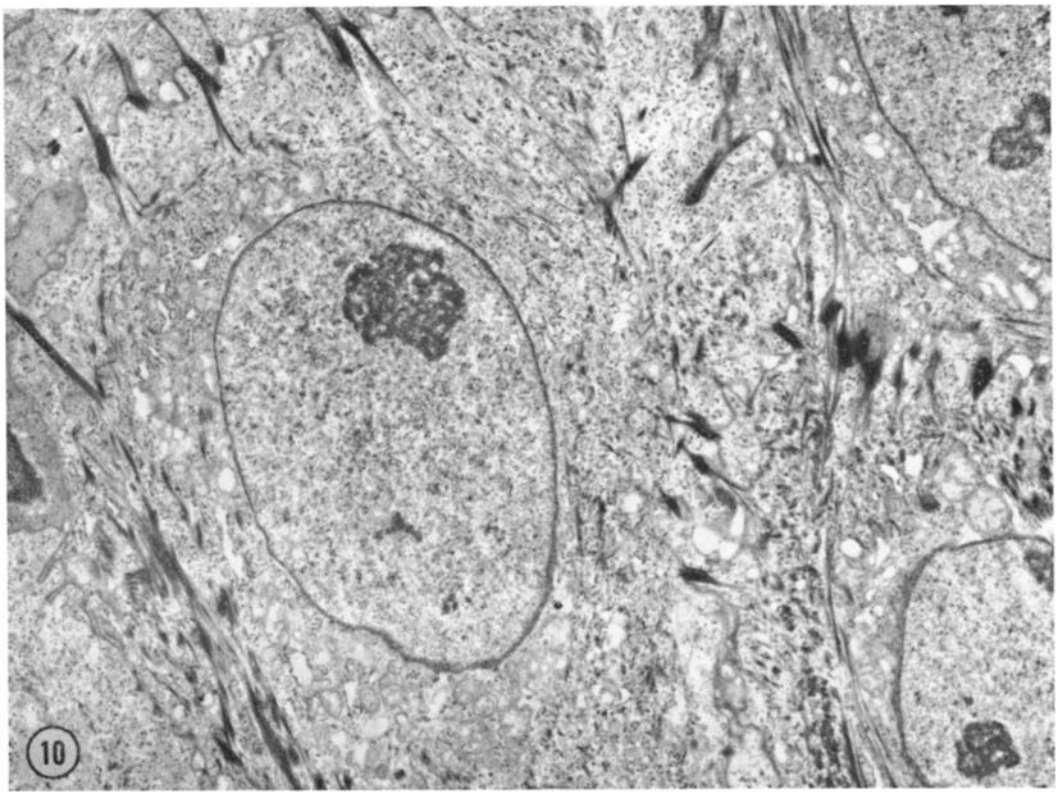

FIGURE 10 Portions of several maturing epidermal cells from the upper spinous layers of hyperplastic epidermis of 3 day old wound. Filaments tend to be aggregated in parallel strands and spare the perinuclear region which is occupied by mitochondria and endoplasmic reticulum. Mitochondria are larger and more numerous than in mature cells. Note increased numbers of desmosomes at the cell profile compared with those in Fig. 9. × 6,200.

present series, the 7-day-old wounds show regeneration and differentiation to be complete and the newly formed epidermis to be indistinguishable from normal epidermis, except for vestiges of incompletely keratinized cells among the superficial layers of the stratum corneum.

DISCUSSION

In their recent histologic analysis of epithelization of small wounds in rabbit skin, Viziam et al. reviewed and clarified the sequence of epidermal responses to superficial wounds (4). They pointed out that during the first 18 hr after wounding no microscopically visible changes are present in the epidermis around the wound. However, by 21 hr after wounding there is an increase in both the size of the cells of the Malpighian layers as well as in the number of cell layers. In their material, epidermal cells commenced migrating across the wound surface after 24 hr, and wound epithelization was completed in 72 hr, at which time the newly regenerated epidermis covering the wound was thickened and hyperplastic. They studied mitosis by the techniques of colchicine arrest and radioautographic marking with tritiated thymidine, and they concluded that epidermal cell migration and mitosis accounted for the cellular closure of the wound and that continued mitotic activity after cessation of migration accounted for the thickened epidermis characterizing the newly epithelized wound.

The observations afforded by the present electron microscopic analysis do not permit conclusions regarding the role of mitosis. However, it is clear that in human wounds the major events of migration of epidermis and the thickening of

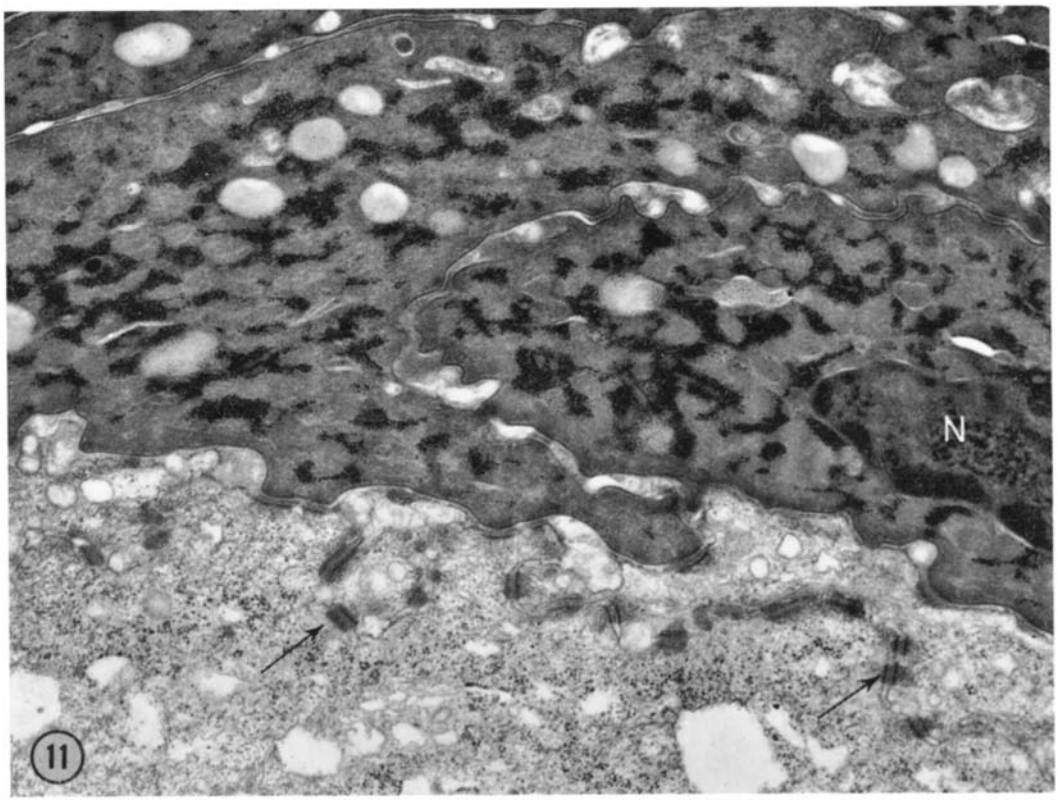

FIGURE 11 Upper strata of incompletely differentiated epidermis near center of a 3 day old newly epithelized wound. The region depicted is comparable to that shown in the center of Fig. 6 just below the wound crust. Note absence of keratohyalin granules and membrane-coating granules in the cytoplasm of the cell shown in the lower one-third of the micrograph. Observe numerous desmosomes (arrows). The cells in the upper two-thirds of the micrograph are nucleated (*N*), "parakeratotic," incompletely keratinized squamae with dense cytoplasmic filaments, aggregates of ribosomes and/or glycogen, and thick, prominent cell membranes. × 20,500.

epidermis after wound closure correspond chronologically to the events described histologically in rabbit skin wounds by Viziam et al. (4).

MIGRATION: This study has revealed that sometime between 12 and 24 hr after wounding, the epidermis commences migration from the margin toward the center of the wound. The movement of the epithelial sheet is associated with distinctive changes occurring about the surface of cells of the migrating epidermis. The surface phenomena are characterized by evolution of cytoplasmic processes at the cell cortex, which are pale in contrast to the normal filament-filled cytoplasm of epidermal cells.

Comparable changes were observed at the basal surface of regenerating epidermal cells in amphibian wounds by Singer and Salpeter (5). The findings in the present study of human epidermis show that these surface changes occur on all surfaces of regenerating epidermal cells, and that they are most prominent at those surfaces exposed to the wound exudate during epidermal cell migration. Our analysis did not include controls to eliminate the possibility that the variation in the size of the cortical projections is not a fixation artifact, but Singer and Salpeter noted comparable changes in amphibian epidermis fixed by different fixatives (5). The surface changes are interpreted to be the "ruffling" undulations of cell membranes observed by cytologists in a wide variety of epithelia and connective tissue cells in culture. They are generally associated with cell movement (6 and 7).

The epithelial sheet moves into an inflamma-

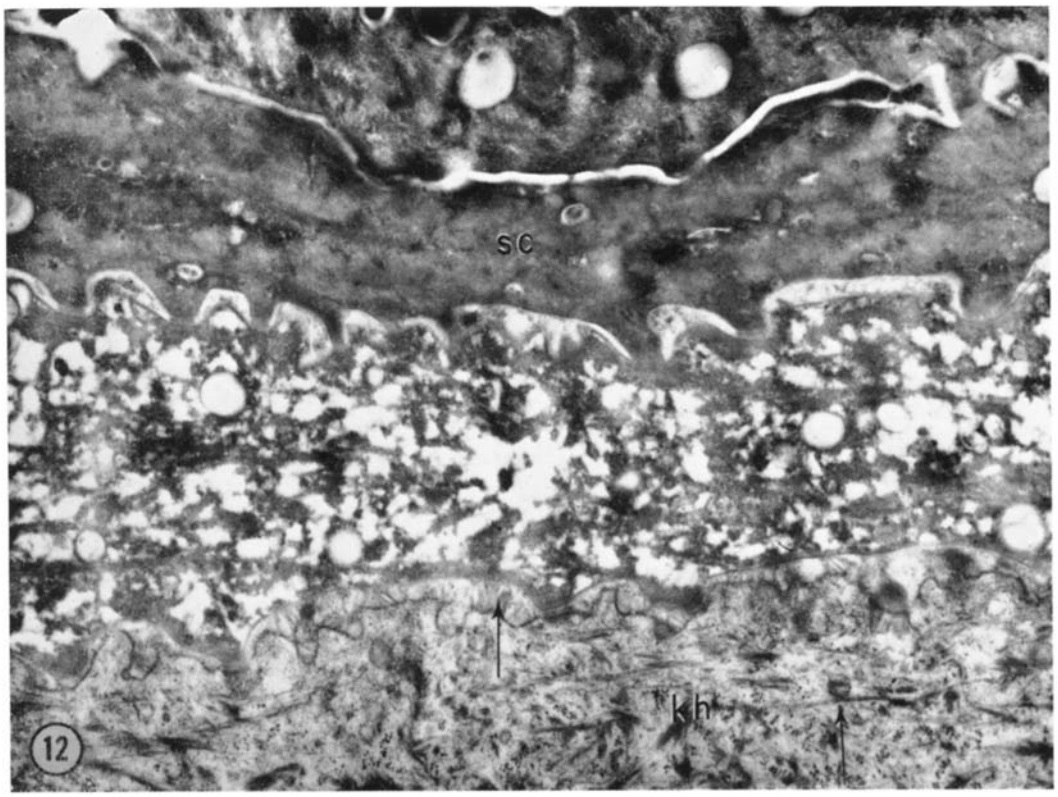

FIGURE 12 Keratinizing elements of the upper strata of more completely differentiated epidermis near the original wound margin of a 3 day old wound. Note keratohyalin granules (*kh*) and filaments and relative paucity of ribosomes in cell at lower margin of micrograph. Note membrane-coating granules in cytoplasm and in intercellular space (arrows). Normally keratinized anucleate cells of stratum corneum (*sc*) have evolved above the newly formed granular cell. × 18,000.

tory milieu, and our studies have shown that the advancing cells appear to be supported only by desmosome contact between adjacent epithelial cells. From histologic analyses various suggestions have been offered regarding the ultimate plane for epidermal regeneration: (*a*) that it is defined by a band of polymorphonuclear leukocytes in the lower portion of the wound crust (4), (*b*) that it passes through dermal fibrous tissue below the original wound surface (8), (*c*) that it is developed by epidermis which enzymatically dissolves its way through the wound clot (9), or (*d*) that it moves through a layer of fresh exudate under the scab (10). Electron microscopic analysis of superficial wounds reveals that the advancing epidermis moves initially through a serous exudate containing fibrin and some red blood cells. Inflammatory cells do not regularly lie in proximity to the advancing elements of the epidermis. The viscosity of the exudate could not be determined, but the cells appeared to be deformed only by structured elements of the exudate.

Wessels has pointed out that basal epidermal cells require attachment to some form of physical substrate in order to undergo normal mitosis (11). It seems clear from the present morphologic analysis that the migrating epidermal cells promptly establish a physical attachment to the fibrin substrate of the wound.

FORMATION OF BASEMENT LAMINA: Subsequently, within this milieu, the formation of a fibrin net provides the first structural element of the temporary wound architecture that appears to be utilized by epidermal cells before they engage in the establishment of hemidesmosomes and a basement lamina. This demonstration of contact of the epidermal sheet with the fibrin net tends to strengthen morphologically the observa-

tions of Weiss and his colleagues (12) who proposed the concept of "contact guidance," implying that early orientation of epithelial elements results from the linear orientation of their substrate. Hay and Revel (13) used electron microscope radioautographic methods to demonstrate apparent synthesis of basement lamella proteins by regenerating salamander epidermis. Their findings may be relevant to the synthesis of basement lamina protein by epidermal cells. The broader concept that epithelial cells synthesize basement lamina is reviewed and reinforced in the work of Pierce and colleagues who utilized immunofluorescent techniques (14). Hence, it is of current interest to note in the present study that regenerating human epidermal cells appear to produce component structures of basement lamina in the absence of fibroblasts and that they do so at a time when the earliest signs of differentiation appear in their cytoplasm; these signs are the organelles generally associated with synthesis and secretion of extracellular proteins.

PHAGOCYTOSIS BY EPIDERMAL CELLS: A phagocytic capacity of regenerating epidermis was observed by Taban (15), who noticed liquefaction of a blood clot by the wound epithelium in a salamander and who also observed debris of various types within the epidermal cells. Weber (16) noted epidermis of regenerating amphibian skin to be phagocytic in the earliest days after wounding and immediately after wound closure. Our findings confirm and refine these observations and extend them to human epidermis.

DIFFERENTIATION OF EPIDERMIS: In the present study the cells a short distance back from the advancing epidermal edge show characteristics interpreted as early differentiation, even while some of the epidermal cells are migrating and, according to other investigators, dividing. The early differentiation is accompanied by synthesis of basement lamina and by formation of new hemidesmosomes and of characteristic intracellular filaments. The markers of differentiation, appearance of a rough endoplasmic reticulum and numerous free ribosome clusters, were observed formerly in regenerating amphibian epidermis by Singer and Salpeter (5).

The thickening of epithelium newly covering a wound surface appears characteristic of 3-and 5-day-old wounds in the material presented herein. The cellular kinetics in a similar epithelial thickening in rabbit wounds (4) suggests that mitosis continues at a high rate after wound closure, with a resultant heaping up of cells still attuned to the proliferative demands of wound closure. The persistence of pale projections of the cortical cytoplasm of cells in the lower layers of epidermis at this stage also may be interpreted as response to proliferative demand. The subsequent appearance of increased numbers of desmosomes in the upper layers may reflect a residual cell surface activity, an interpretation which is consonant with the conclusions of Vaughn and Trinkaus (17) that the ruffled membranes of epithelial cells are particularly adhesive parts of the cell, and that stable adhesions tended to be formed rapidly between contacting cells of epithelial cell masses. Those authors noted that the formation of these adhesions was associated with a cessation of membrane activity.

Subsequent features of differentiation in the newly regenerated hyperplastic epidermis relate to stratification of cells and evolution of increasing numbers of filaments within cells in the upper strata of the epidermis. Those cells which flatten or become squamous appear to do so without the interposition of keratohyalin granules. The resultant squamous cell contains incompletely consolidated intracellular filaments in the presence of vestiges of cell organelles and condensed nuclear material.

KERATINIZATION: Ultimate differentiation of the regenerating epidermal cells proceeds near the wound margins before epithelization is completed. This event, keratinization, is heralded in the upper strata of the regenerating epidermal cells by the appearance of submicroscopic lamellar granules within the cytoplasm (see membrane-coating granules; reference 18). Concomitantly, small keratohyalin granules appear in the cytoplasm of the nucleated cells of the uppermost layers, and the characteristic structural image of fully keratinized stratum corneum is evolved. The observations made after experimental disruption of the chronology of epidermal cell differentiation by the technique of wounding permit the conclusion to be drawn that the appearance of membrane-coating granules coincides with the evolution of keratohyalin granules and, consequently, of normal keratinization.

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