ULTRASTRUCTURAL CHANGES IN STRATUM CORNEUM INDUCED BY ULTRAVIOLET LIGHT*

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Scaling of the skin is an almost invariable result of moderate sunburn. The histology of the epidermal reaction to ultraviolet exposure has been thoroughly studied (by light microscopy) by several investigators. (1, 2, 3, 4) Stratum corneum thickening has been observed as one of the features of the epidermal response to ultraviolet exposure. Since this thickening is a reflection of altered keratinization, an investigation of events by electron microscopy was made in an attempt to further the understanding of the keratinization process.

MATERIALS AND METHODS

Four times the minimal erythema dose of medium pressure, mercury vapor irradiation filtered through 2 cm of water was administered to the left lumbar area of a 24 year old healthy white man. Biopsy specimens were excised without anesthesia from the irradiated site and symmetrical control site at intervals of 1, 3, 12, 72 hours, and 1 week. Tissues were fixed immediately in cold, 1% phosphate-buffered (pH 7.3) osmium tetroxide solution, postfixed in neutral, buffered formalin, and dehydrated in graded alcohols. Specimens were embedded in Maraglas epoxy resin (5), followed by curing at 60 degrees C for 48 hours. Thin sections for electron microscopy were cut on a Porter-Blum microtome and placed on copper grids. A Formvar supporting film was used on some grids. After uranyl acetate staining the specimens were studied in an RCA EMU-3F electron microscope at 50 KV.

RESULTS

One, Three, and Twelve Hour Specimens

Mild erythema was present one hour after irradiation. Marked erythema was present at three and twelve hours, and mild edema at twelve hours. Irradiated and control specimens taken at one, three and twelve hours after ultraviolet exposure revealed no changes from normal stratum corneum. Our observations confirmed the existence of three sublayers (basal, intermediate, superficial) in the normal horny layer as recently described by Brody (6). Less dense filaments of approximately 70 A diameter embedded in a highly opaque matrix were observed. In most of the cells in the basal layer of the stratum corneum this pattern was uniform, although in occasional cells it was interrupted by irregular spaces which were electron-optically "empty", giving some cells a "spongy" appearance. The basal layer comprised 2 to 6 cells in the normal stratum corneum. Most of the cells in the intermediate layer of the stratum corneum showed the filament-matrix pattern in areas of various sizes, but differed from basal layer cells in having a predominance of electron empty or less dense areas, giving the cells a spongy appearance. The intermediate layer, which contained 4 to 12 cells, was not found to be more opaque than the basal. Although the spongy appearance was characteristic of cells in the intermediate layer, a few cells showing the same dense packing of keratin fibrils as in the basal layer were randomly distributed in the intermediate layer. The 2 to 4 cells comprising the superficial layer had a more homogeneous internal content and in them the filament-matrix pattern was not as clearly discerned.

Seventy-Two Hour Specimens

The skin showed moderate erythema (less marked than at twelve hours), but no scaling at 72 hours. Changes were first observed in the stratum corneum in the biopsies taken 72 hours post-irradiation. At this time a new layer had developed between what was apparently normal stratum corneum and the stratum granulosum. (Figure 1) The new layer differed sharply from the stratum corneum immediately above it in several respects. It was much

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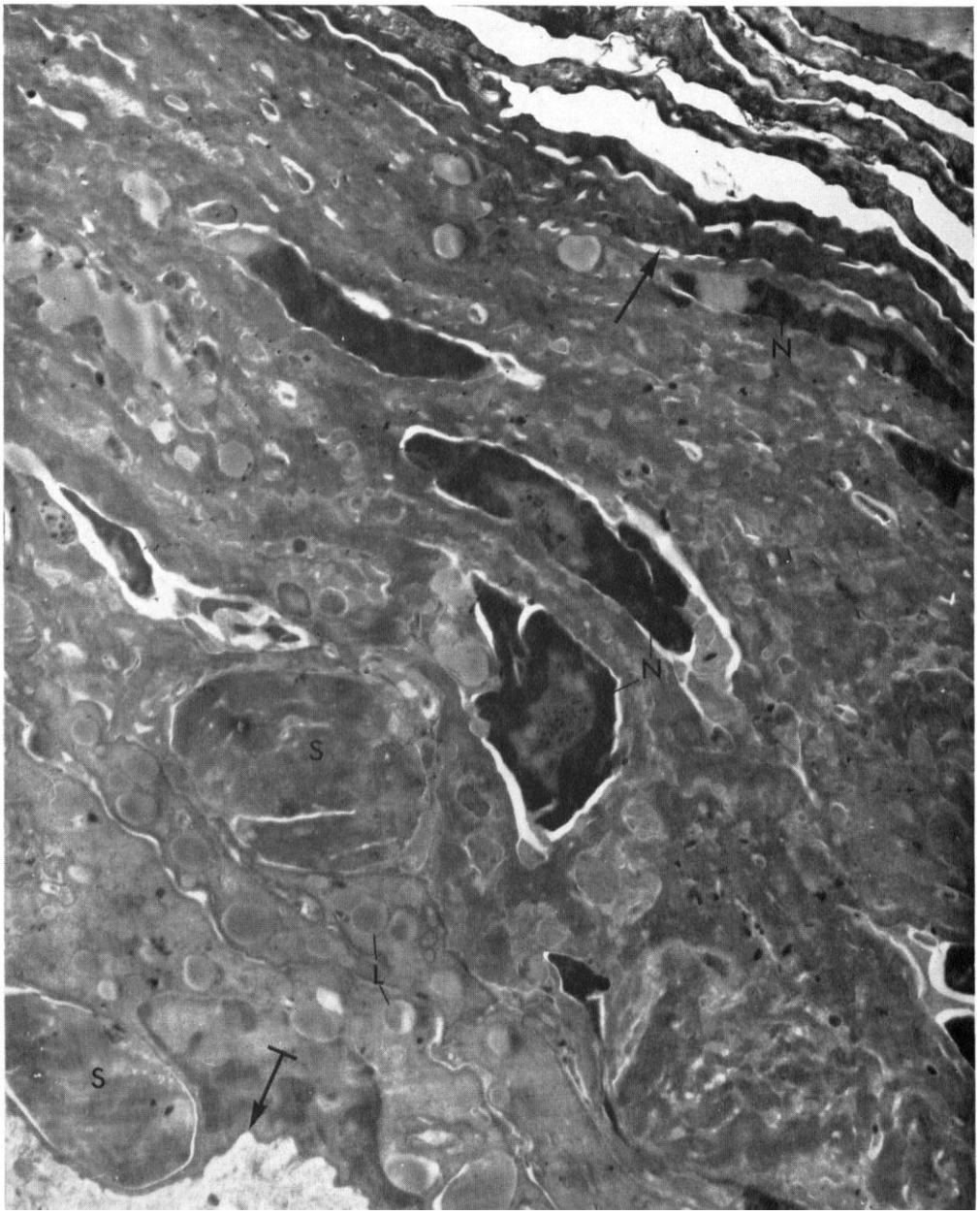

FIG. 1. Entire stratum corneum, 72 hours post-irradiation. Junctions between upper, distal stratum corneum and lower, parakeratotic zones (→) and between parakeratotic layer and stratum granulosum (↦) are indicated. Nuclei (N) tend to be flattened in the upper region of the parakeratotic zone. Spheroidal bodies (S) are present in the lower half of the parakeratotic layer. Numerous lacunae (L) are scattered throughout the parakeratotic zone. × 10,100.

thicker than the superficial layer and had retained many nuclei in its substance. Hence, it has been designated as the "parakeratotic" stratum corneum and the outer, displaced layer has been called "distal stratum corneum," or "distal corneal zone." The parakeratotic zone was much thicker than the distal corneal zone. In most areas measured, the ratio of

parakeratotic zone to distal zone thickness was 2 to 1 or 3 to 1 (average of 12 measurements was 16.7 microns and 6.5 microns, respectively). However, in a few instances the ratio was 4 to 1 (31 microns to 7.5 microns).

The structure of the parakeratotic zone differed in many other respects from the apparently normal corneum immediately overlying it. Nuclei were retained throughout this layer. They had either an elliptical or flattened shape in the lower portions; all became flattened as they approached the junction with the distal corneal zone. (Figure 1) Some nuclei were embedded in a less opaque surrounding zone which gave no hint of any resemblance to a cell body. (Figure 2) However many nuclei were situated within spheroidal bodies. (Figure 2) These bodies were sharply defined from neighboring corneum because they differed in opacity, usually being less opaque than the structures enclosing them. Electron-optically empty clefts separated some nuclei from surrounding corneum. (Figures 1, 2) Within some nuclei were clusters of dense particles measuring about 900 A in diameter. (Figure 3) Although resembling melanin granules in density they differed from them in tending to be spherical rather than elliptical, lacking in lamination and in being smaller than melanin granules.

The bulk of the parakeratotic zone was a highly complex system of fibrous bundles with spheroidal bodies. These bodies, some of which contained nuclei, as noted above, were scattered in an apparently random manner in this layer. The familiar, neat, lamellar arrangement of corneous squames was absent except for a few such squames occasionally seen at the lowermost and uppermost edges of the parakeratotic zone. (Figures 1, 2) The spheroidal bodies were sharply demarcated from surrounding corneum, not only by differences in density (Figures 2, 4, 5), as previously noted, but also, in some cases, by a cleft which was less electron dense than either the spheroid or the surrounding corneum. (Figure 2) Clearly defined membranes (measuring 168 A thick) were evident about some of the spheroids in the lower third of the parakeratotic zone (Figure 4) but none were observed about those located in the upper two thirds of this zone. Small, discrete, round areas of lesser opacity were observed within some of the spheroids. (Figure 4) A perinuclear zone of slightly less opacity than other areas within the spheroid has been observed. (Figures 4, 5) Large oval lacunae (long axis 0.48 micron) and groups of dense, laminated melanin granules (long axis 0.21 micron) have been observed in spheroids. (Figure 3) Complex relations of one spheroid to another were commonly observed in which a smaller one was partially enfolded within a larger. (Figure 5) Adjacent spheroids sometimes differed in density. (Figure 5) Complex interdigitating processes separated some spheroids from surrounding corneum. (Figures 2, 4) Similar interlocking processes were present in the zones of corneum surrounding spheroids. (Figures 2, 4, 12) The spaces between interlocking processes were usually narrower than spaces between cells in the normal stratum corneum. Desmosomes were present in small numbers in these spaces. (Figure 6) Plasma membranes were not observed in the parakeratotic zone except about a few squames in the lower margins of the zone. (Figure 5) Membranes of similar thickness surrounded occasional spheroidal bodies in the lower portion of the parakeratotic zone. (Figure 4)

Oval spaces of lesser electron density were scattered throughout the parakeratotic zone but concentrated primarily in the lower portions. (Figures 1, 2, 3, 4, 7) The long axis was about 0.48 micron. We have designated these oval spaces as "lacunae." Many were partially filled with a dense, homogeneous material. In some lacunae this dense material had lost homogeneity and possessed alternating light and dense areas attributable to chatter artifact. (Figure 4) Opaque membranes (140 A thick) surrounded some, but not all, of the lacunae. (Figures 4, 5) Membrane-lined structures of similar size, shape, and eletcron density were prominent in the granular layer. (Figures 2, 7) However, the structures in the granular layer differed in that their boundaries were usually irregular whereas the margins of the lacunae were perfectly smooth.

Groups of dense particles measuring 130 to 280 A were present within the parakeratotic layer. (Figures 2, 3, 7) These were similar to particles observed in large numbers throughout the noncornified portions of the epidermis. These were similar in size and density to particles identified in other tissues as ribosomes.

The typical filament-matrix keratin pattern observed in the normal stratum corneum was not present in the parakeratotic zone. This

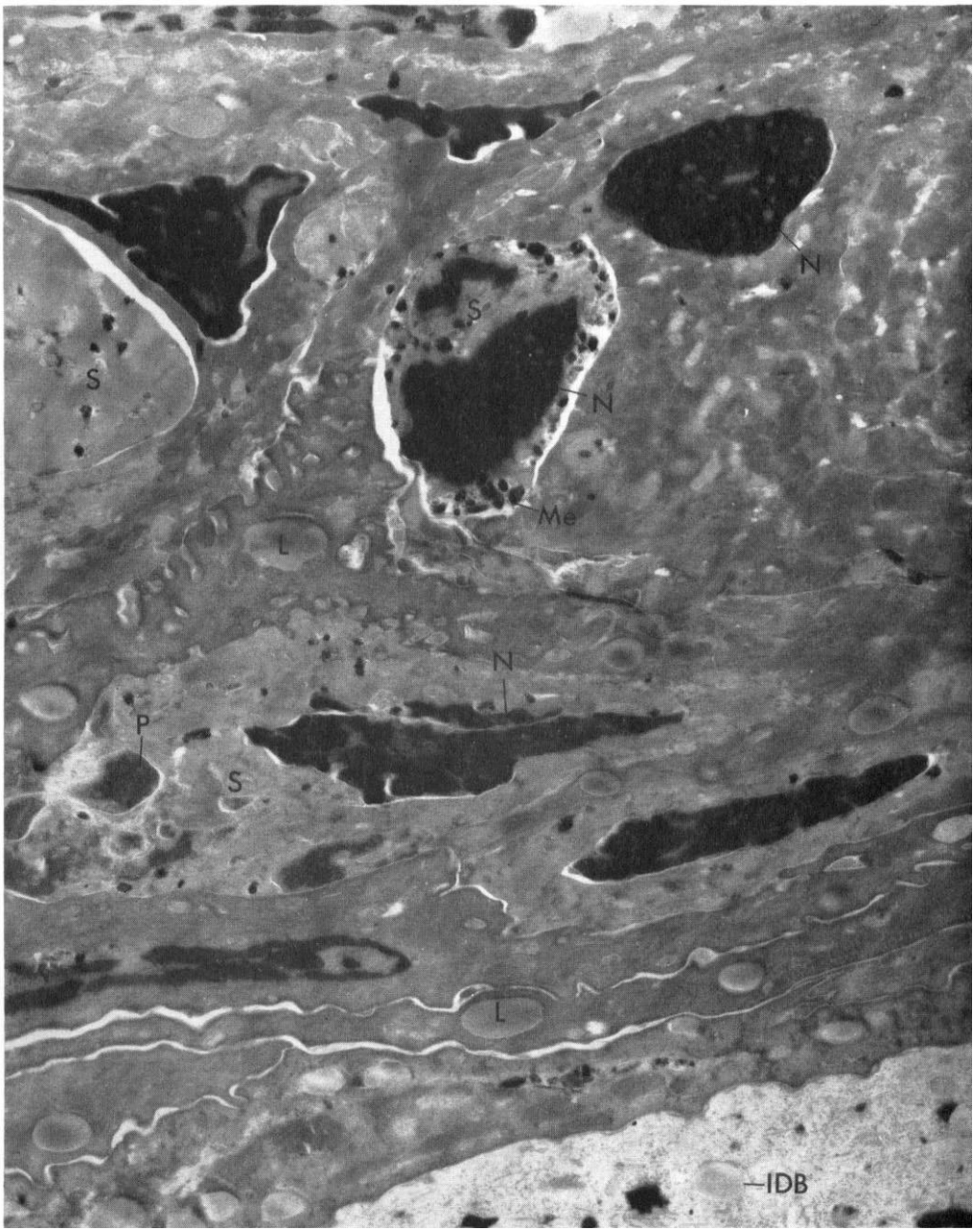

FIG. 2. Parakeratotic zone, 72 hours post-irradiation. A number of spheroid (S) structures contain nuclei (N), melanin granules (Me), and clusters of 130 to 280 A particles (P). Spheroids are less dense than the surrounding corneum, and in some cases are separated from it by a widened "empty" cleft. However, interlocking processes with a narrow intervening space join them in some areas. In right upper portion of figure is a nucleus which is not enclosed within a spheroid. Lacunae (L). Irregular dense body (IDB) in stratum granulosum. × 12,000.

difference in keratin pattern was apparent in micrographs of the junction between parakeratotic and distal corneal zones. (Figure 6) The parakeratotic zone was less dense than the distal corneal. Although the individual keratin filaments were easily distinguished in the basal cells of the distal stratum corneum, they were less readily discerned in the parakeratotic zone.

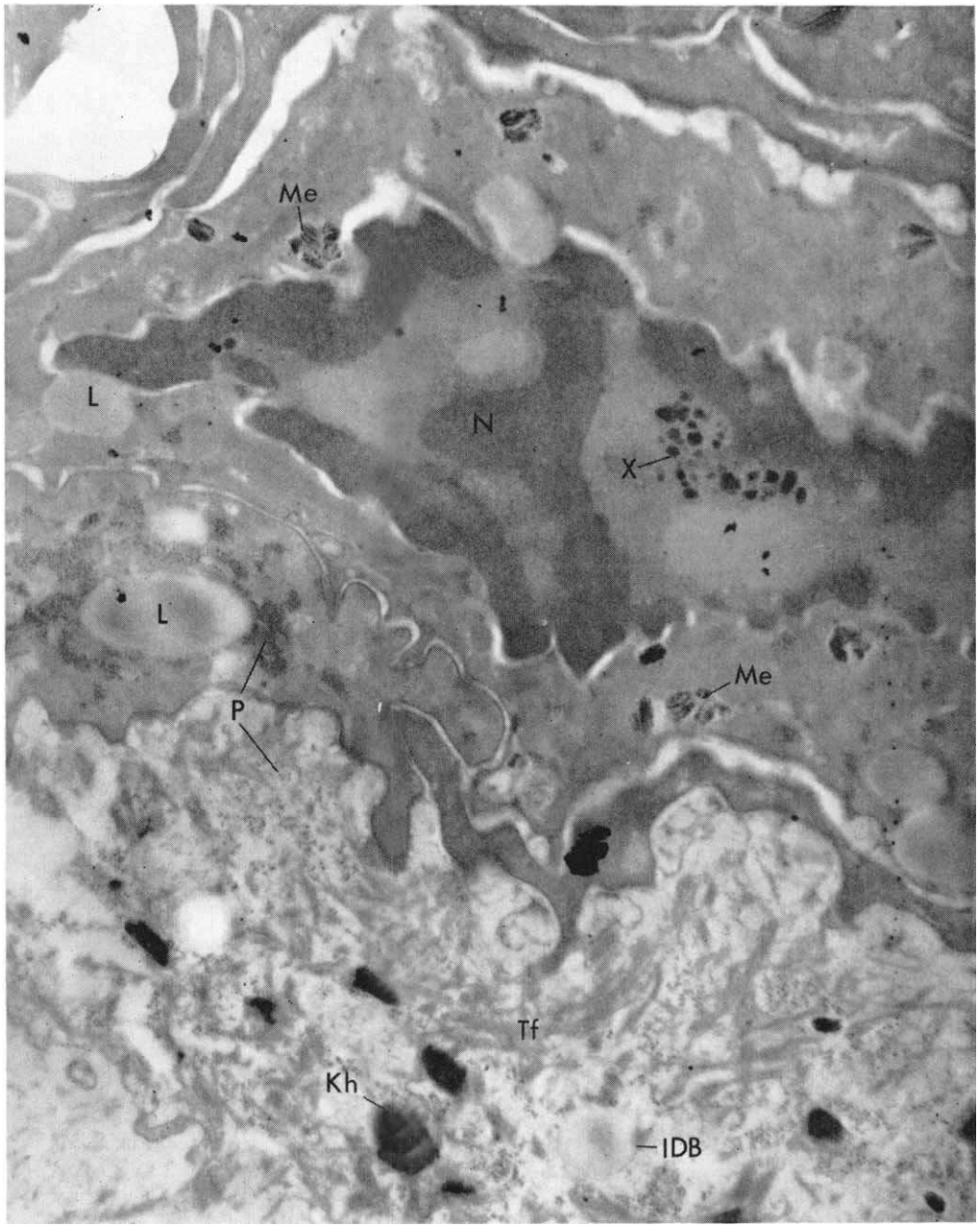

FIG. 3. Junction of parakeratotic zone with stratum granulosum, 72 hours post-irradiation. A nucleus (N) contains a number of dense, 900 A particles (X) which differ from melanin granules in size, shape and lack of lamination. Groups of smaller (130 to 280 A) particles (P) are present within stratum corneum and stratum granulosum. Lacunae (L). Keratohyalin granules (Kh). Tonofibrils (Tf). Irregular dense body (IDB) in stratum granulosum. × 33,000.

This difficulty in discerning filaments in the parakeratotic zone was due to the low density of its interfilamentous matrix.

Measurements were made of filaments in the parakeratotic zone and in the three sublayers of the distal stratum corneum. Measurements indicated that filament diameters in the parakeratotic zone were similar to those in the various sublayers of the distal corneal zone. They were also similar to filament diameters in

FIG. 4. Junction of parakeratotic zone with stratum granulosum, 72 hours post-irradiation. Spheroid (S) possessing nucleus (N), discrete electron-light areas (Z), perinuclear "clear" zone (C), and well-defined membrane (Mb) over part of its surface. Lacuna (L) showing outer membrane. Some lacunae show inhomogeneity of internal content due to chatter artifact. Interlocking processes (IP) in corneum. Keratohyalin granules (Kh). × 20,900.

the stratum corneum of unirradiated control (see Table I).

A cell with a long, dendrite-like process and melanin granules was seen in the lower portion of the parakeratotic zone. (Figure 12) It resembled a melanocyte.

At 72 hours, the stratum corneum from the unirradiated control site had a normal appear-

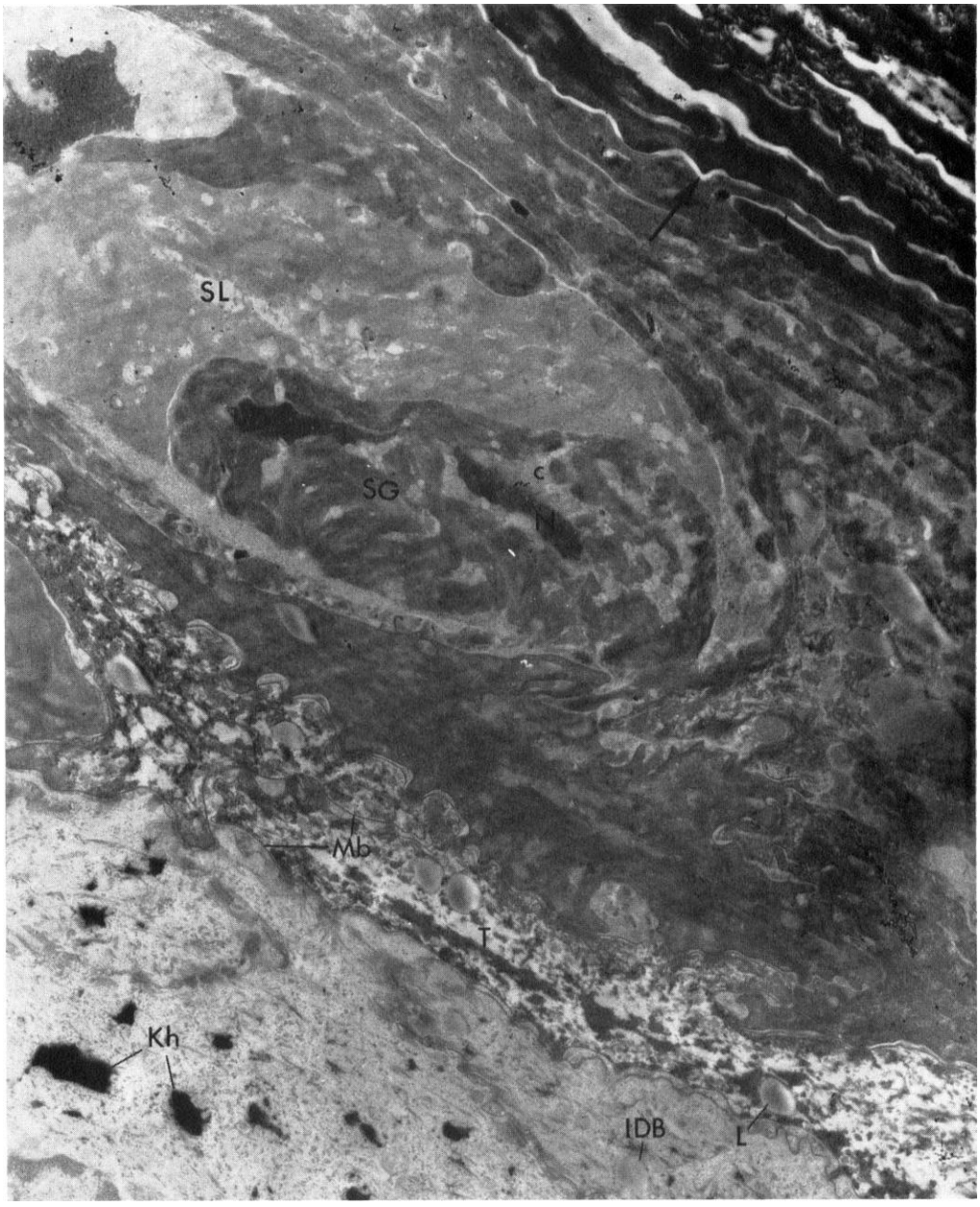

FIG. 5. Junction of parakeratotic zone with stratum granulosum, 72 hours post-irradiation. Spheroid of lesser density (SL) enfolding one of greater (SG). Perinuclear clear area (c) is evident in SG. Junction between parakeratotic and distal corneal zones (→) is marked by contrast between *less dense* parakeratotic and *denser* distal corneal substance. Membranes (Mb) of transition cells (T) are evident. Lacunae (L). Keratohyalin granules (Kh). Irregular dense body (IDB) in stratum granulosum. × 15,900.

ance. It showed no signs of development of a parakeratotic layer or other changes.

One Week Specimens

At one week erythema had been replaced by brown pigmentation and slight scaling was evident. Epidermis examined one week after irradiation exhibited a new zone immediately *beneath* the parakeratotic zone. (Figure 8) We designated this as the "proximal stratum corneum" or "proximal corneal zone." At this time the distal corneal and parakeratotic zones were

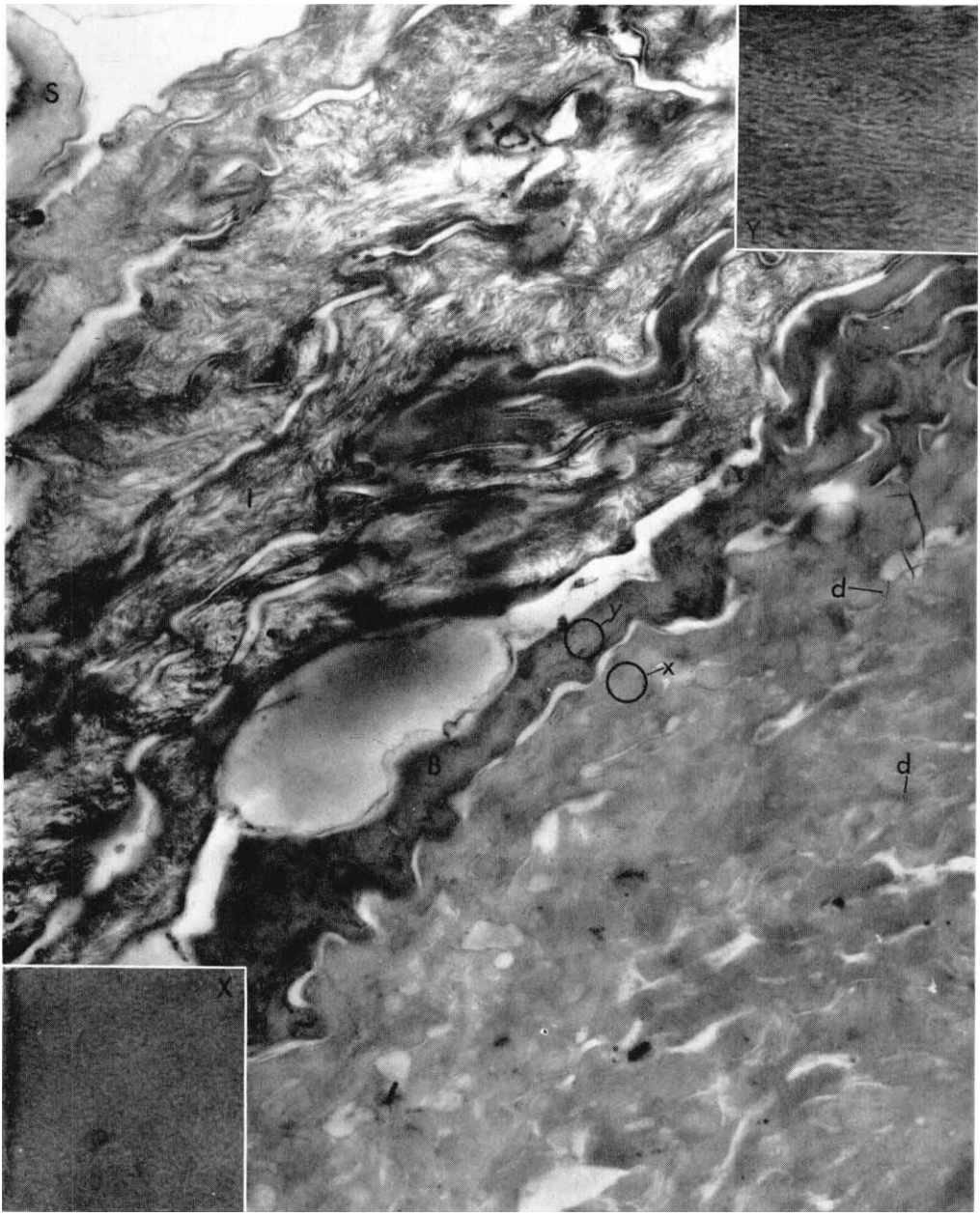

FIG. 6. Junction of parakeratotic zone with distal corneal zone (→), 72 hours post-irradiation. Three sublayers of distal corneal zone are evident: basal (B), intermediate (I), and superficial (S). Striking contrast is present between the less dense parakeratotic zone and the denser distal corneal zone. Inset Y (× 165,000) is from basal squame of distal corneal zone and shows the interfilamentous matrix to be denser than that present in parakeratotic zone picture in inset X (× 165,000). Desmosomes (d) are sparse in parakeratotic zone. × 22,800.

still present. Thus, the stratum corneum was divided into three broad zones.

The proximal corneal zone was composed of flattened cells, most of which resembled those of normal stratum corneum. The proximal stratum corneum also had three sublayers. (Figure 9) It was comprised of from 10 to 14 cells. In the proximal corneal zone the basal

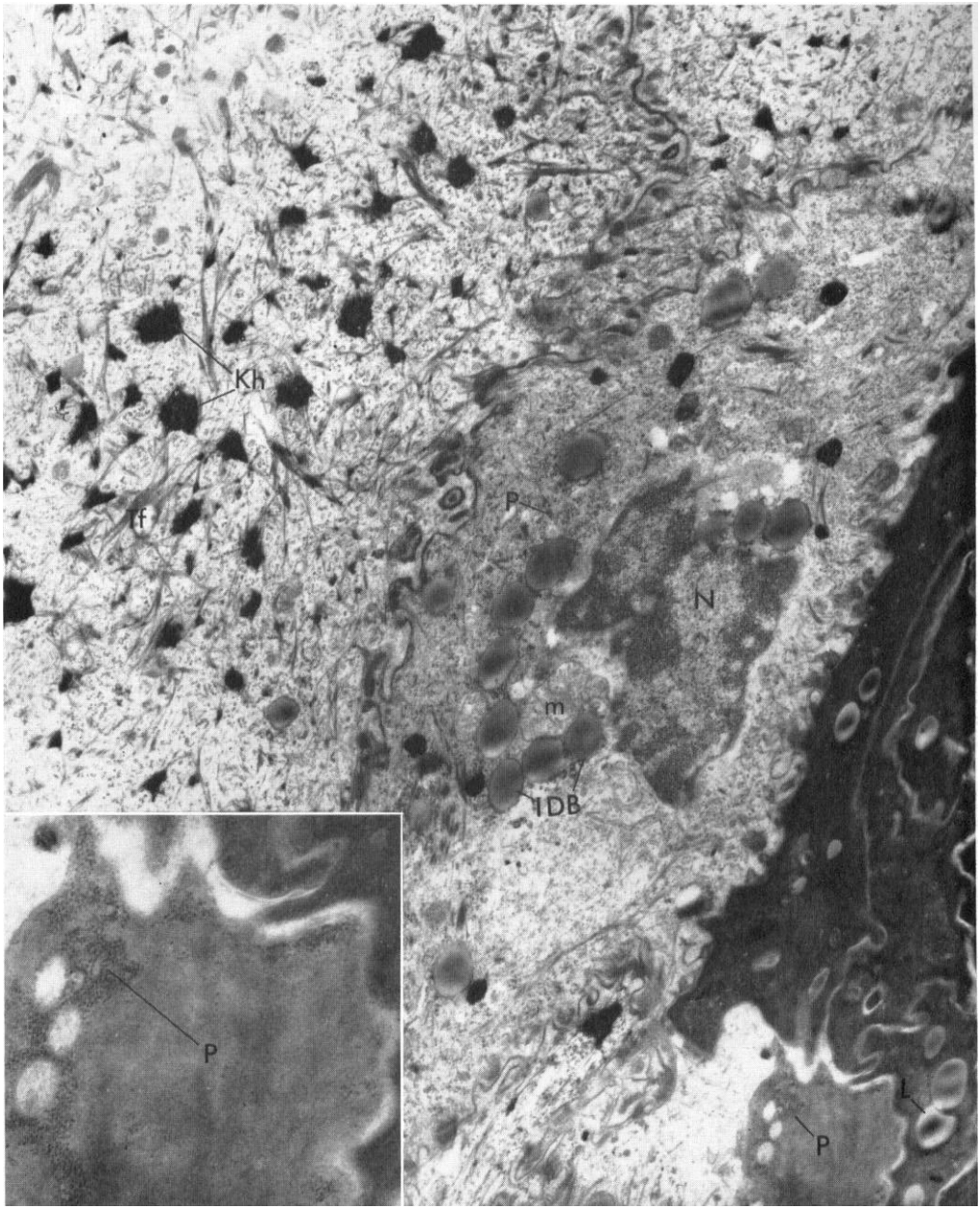

FIG. 7. Junction of parakeratotic zone with stratum granulosum, 72 hours post-irradiation. Lacunae (L) in lower portion of parakeratotic zone resemble irregular dense bodies (IDB) in stratum granulosum in size, shape, and presence of chatter artifact. They differ in that the granular layer bodies usually have slightly irregular outlines while the lacunae are smooth. Clusters of dense particles (P) measuring 130 to 280 A are present in lower portion of parakeratotic layer and in large numbers in the granular layer. Nucleus (N). Mitochondrion (m). Keratohyalin granules (Kh). Tonofibrils (Tf). Inset (× 40,600) shows 130 to 280 A particles in parakeratotic layer. × 15,900.

layer had from 2 to 5 cells, the intermediate 4 to 10, and the superficial 1 to 3. Its thickness varied from 7.8 to 14.1 microns but averaged 9 microns.

In similarity to cells in the basal layer of the normal stratum corneum, some cells in the proximal corneal, basal layer had alternating areas of electron-dense and electron-light sub-

TABLE I

Keratin filament diameters

Measurements of filaments in 72 hour specimens from (a) parakeratotic zone, (b) three sublayers of distal corneal zone ("irradiated basal," etc.), and (c) three sublayers of unirradiated control corneum ("unirradiated basal, etc.").

SPECIMEN	N	MEAN	STD. ERROR
Parakeratotic (Irrad. only)	156	67.3	14.68
Irradiated Basal	100	70.2	11.96
Unirradiated Basal	100	67.1	11.35
Irradiated Intermed.	42	63.6	8.72
Unirradiated Intermed.	49	61.5	12.14
Irradiated Superfic.	50	70.2	14.64
Unirradiated Superfic.	9	58.0	10.27

stance. (Figures 9, 10) However, the cytoplasm of these proximal corneal, "spongy" basal cells had a greater proportion of electron-optically "empty" spaces than the cytoplasm of normal basal layer corneum cells. Spongy basal cells were also more *numerous* in the proximal corneal zone than in normal stratum corneum basal layer. Individual filaments were present within the dense areas of most of these spongy cells in the proximal stratum corneum. (Figure 10)

The typical filament-matrix keratin pattern was present in those basal cells which were of uniform density and in the denser areas of spongy basal cells. (Figure 10) In some areas *no* spongy basal cells were observed and the basal layer was composed only of cells having a uniform cytoplasm of filaments and matrix without "clear" areas. (Figure 11)

Many of the spongy basal cells had an area of distinctly increased density, marginated at the upper plasma membrane. Thus there was a stratification of density within a single cell. (Figure 9)

Desmosomes appeared normal in the proximal corneal zone.

In the lower portion of the parakeratotic zone, at its junction with the proximal corneal zone, numerous lacunae were observed. However, they differed from lacunae observed in the parakeratotic zone in the 72 hour specimens in that their electron density was greatly increased. (Figure 11)

At one week the stratum corneum from the unirradiated control site had a normal appearance.

DISCUSSION

Thickening of the stratum corneum is an essential part of the epidermal reaction to ultraviolet erythema. Miescher (1) found the degree of horny layer thickening to be proportional to the intensity of the reaction. Ultraviolet light administered to skin in daily increments for 14 days in a man treated with psoralens, resulted in hyperkeratosis in an experiment of Becker, Jr. (3) A stratum lucidum was formed but no parakeratosis was evident. Zimmerman (4) performed experiments which demonstrated similar changes in stratum corneum, with the additional feature of parakeratosis one week after the start of daily irradiation with 2800 to 3200 A wavelengths. The light micrographs published by Rost and Keller (7) show the stratum corneum divided into three zones at one week after a mildly blistering dose of ultraviolet light. The middle zone is broad and seems to contain nuclear remnants.

FIG. 8. Stratum corneum, entire, one week post-irradiation. Junction between distal corneal and parakeratotic zones is marked (→). Outermost squame of distal corneal zone is barely visible at right upper corner of figure. The large cleft separates parakeratotic from proximal corneal zone. Junction of proximal corneal zone with stratum granulosum is indicated (↦). Three sublayers are discernible in the proximal stratum corneum: basal (B), intermediate (I), and superficial (S). Spheroid (Sp) in parakeratotic zone. × 10,000.

It has been shown in this study that the newly formed parakeratotic zone present in epidermis 72 hours after irradiation differs markedly in structure from normal stratum corneum. The usual lamellar pattern of stratum corneum is replaced by structure in which there is a conglomerate of nuclei, spheroidal bodies and a surrounding substance in which interdigitating

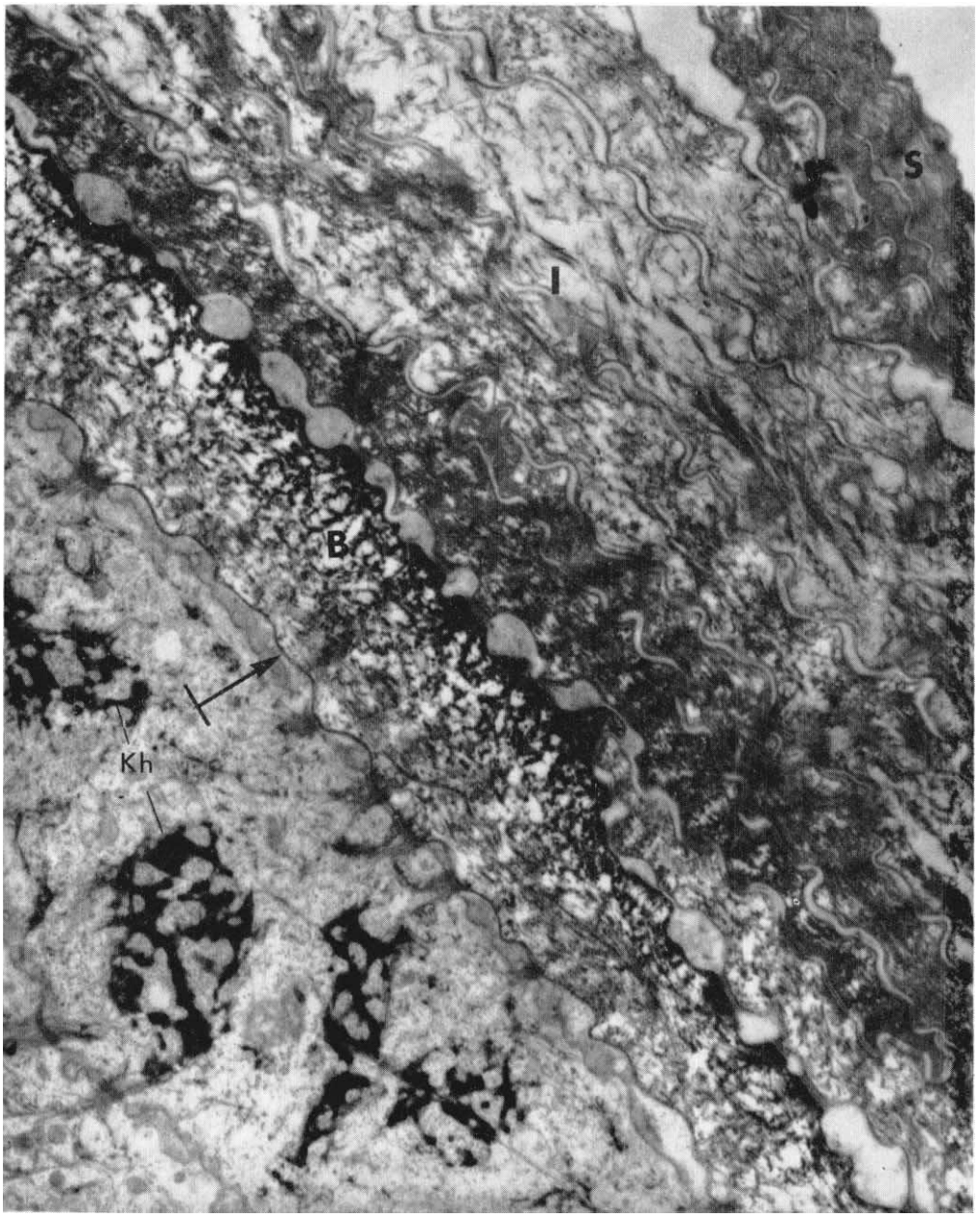

FIG. 9. Junction of proximal corneal zone with stratum granulosum (↦), one week post-irradiation. Stratification of dense matrix material present along upper margin of lowermost cell in basal sublayer (B) of proximal corneal zone. Intermediate (I) and superficial (S) sublayers of proximal corneal zone are present. Keratohyalin granules (Kh). × 21,000.

boundaries have no preferred orientation in relation to the surface of the epidermis. Desmosomes are sparse in the parakeratotic zone but are normal in appearance. The space between interlocking boundaries in the parakeratotic zone is usually very narrow, and contains a minimal amount of homogeneous filler substance. However, occasional, wide clefts, which appear electron-optically empty, separate spheroidal bodies from surrounding structure. The

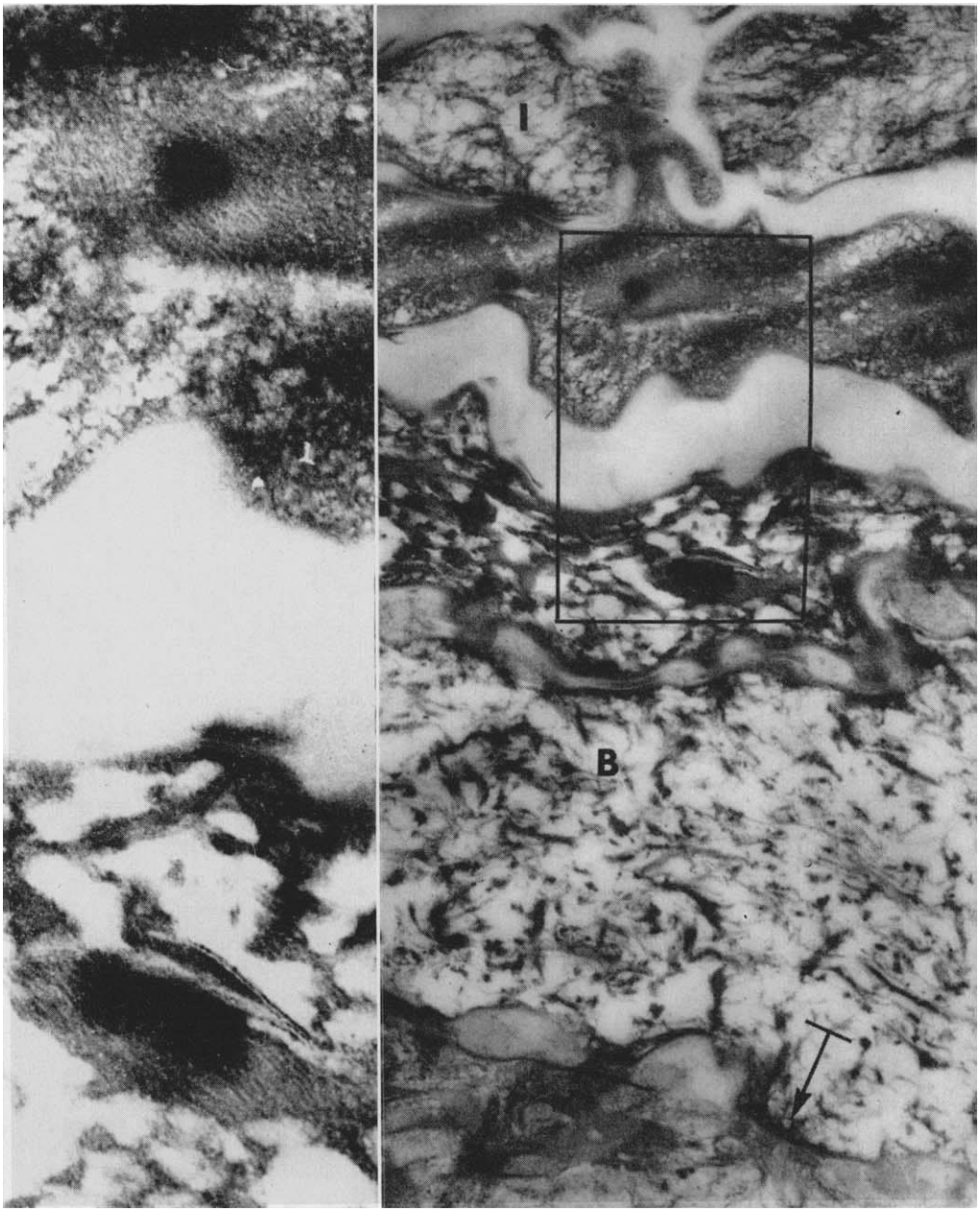

FIG. 10. Junction of proximal corneal zone with stratum granulosum (↦), one week post-irradiation. Large volume of electron-light material in basal spongy cell (B). Inset (× 117,800) illustrates filament-matrix keratin pattern in basal cells. Cell (I) in intermediate sublayer of proximal corneal zone. × 39,900.

spheroidal bodies, which are present in the lower half of the parakeratotic zone, possess features suggesting that they are modified cells. Not only have they often been observed to contain nuclei, but the presence in some of an overall dense content, contrasting with a perinuclear less-dense zone, is similar to the pattern of tonofibrils and perinuclear fibril-free zone in stratum spinosum cells. That some of the spheroids have a surrounding membrane resembling in density and thickness (170 to 190 A), the plasma membranes of cells in the transi-

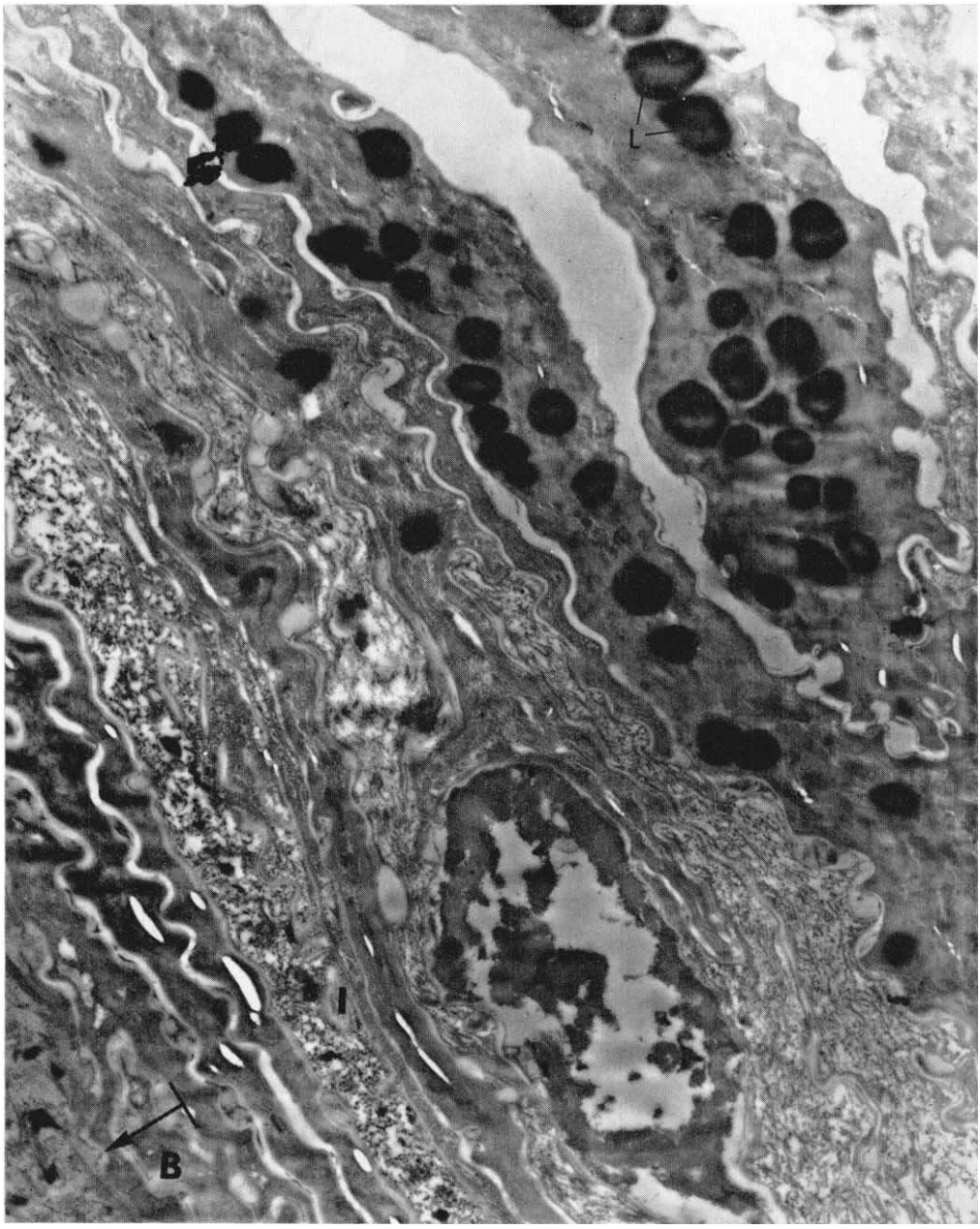

FIG. 11. Parakeratotic, proximal corneal zones and stratum granulosum, one week post-irradiation. Junction with stratum granulosum (↦). Basal (B) and intermediate (I) sublayers, proximal corneal zone, are present but superficial layer is not identified in this micrograph. Lacunae (L) have become extremely electron-dense at this time. × 20,900.

tion zone between stratum granulosum and stratum corneum is further evidence that the spheroidal structures are modified cells. In the upper portions of the parakeratotic zone, the spheroidal bodies are no longer evident, but there are flattened nuclei embedded in broad zones having poorly defined boundaries.

Melanin granules with well-preserved lamellar structure are present within spheroids and in non-spheroidal corneum in the parakeratotic

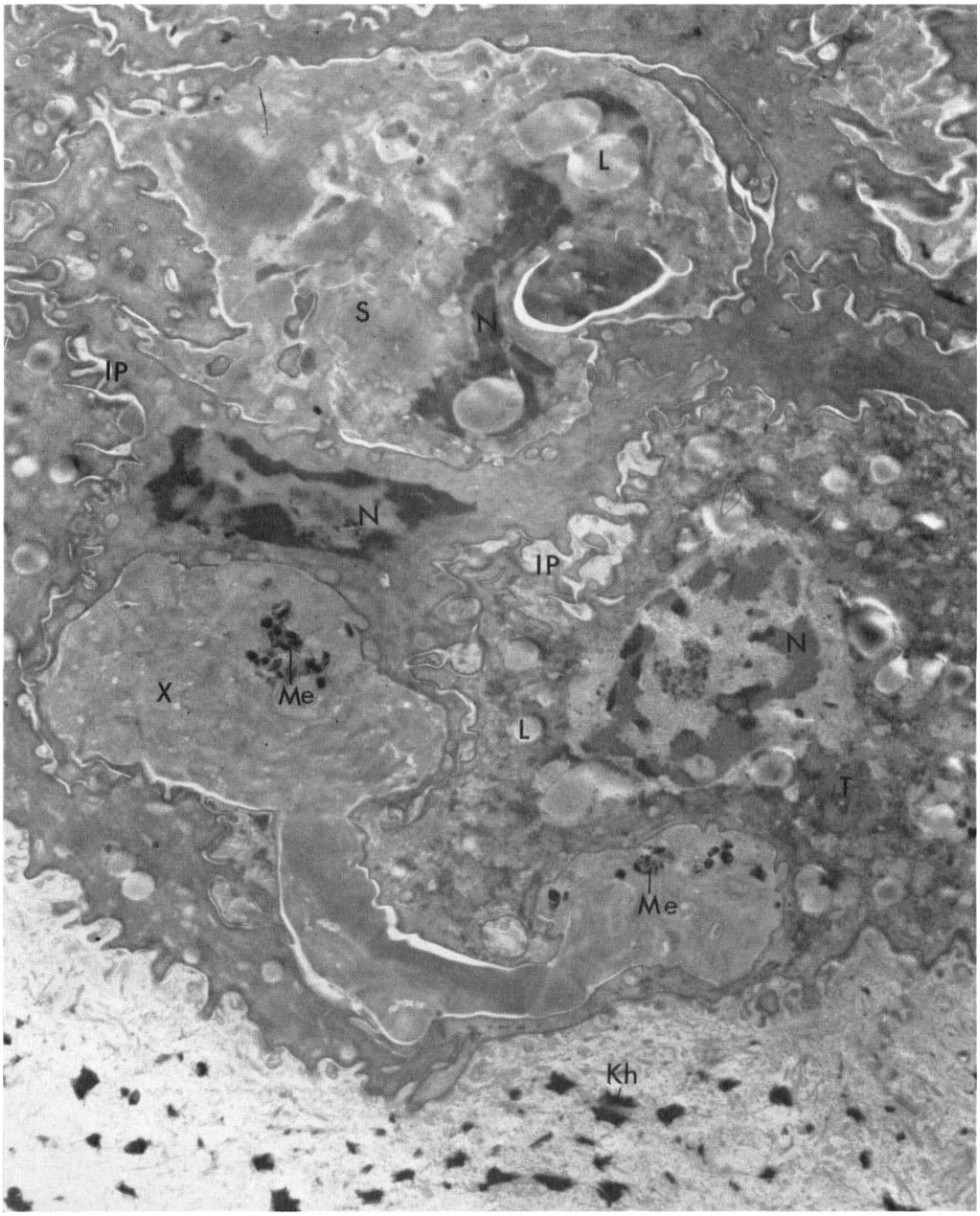

FIG. 12. Junction of parakeratotic zone with stratum granulosum, 72 hours post-irradiation. Large cell (X) with dendrite-like process containing melanin granules (Me) is present in lower parakeratotic corneum. Transition cell (T). Spheroid (S). Nuclei (N). Lacunae (L). Interlocking processes (IP). Keratohyalin granules (Kh). (× 9400).

zone. A large melanin-containing cell with a long, dendrite-like process has been observed in the parakeratotic zone and may be a melanocyte corpse.

Peculiar, oval spaces, less electron-dense than surrounding corneum have been observed and are similar to the structures in the stratum corneum of parakeratotic psoriasis without keratohyalin and in hyperkeratotic psoriasis described by Brody. (8, 9) These lacunae are

similar in size and density to irregular bodies occurring in large numbers in the stratum granulosum in epidermis 72 hours after ultraviolet irradiation. They differ from the irregularly shaped structures in the stratum granulosum by having a perfectly smooth outline. In the lowest portions of the parakeratotic zone in the one week post-irradiation specimens, the lacunae have undergone an extreme increase in density so that they have become almost totally electron-opaque.

Groups of dense particles measuring 130 A to 280 A are present in the parakeratotic zone. These are similar in size and density to particles in the cytoplasm of non-cornified epidermal cells. These are thought to be ribosomes.

The formation of a third or proximal corneal zone, located beneath the parakeratotic zone is evident in the one week postirradiation specimens. The proximal corneal zone is similar to the presumably normal horny layer distal to the parakeratotic zone in thickness, number of cells and in the presence of three sublayers (basal, intermediate, and superficial). It differs from normal or distal stratum corneum in having a greater proportion of electron-light cytoplasm than is ever observed in the basal cells in normal corneum. A peculiar stratification of dense cytoplasm along the upper plasma membrane in some of the proximal corneal, basal cells is another feature which is not observed in basal cells of normal corneum. The rapid formation of the proximal stratum corneum, similar in many respects to unirradiated corneum, implies an accelerated epidermal renewal time. (10)

Perhaps one of the most significant aspects of altered structure in the parakeratotic zone is the filament-matrix pattern of the keratin. It differs from the normal keratin pattern. The principal difference is the diminished contrast between the filaments and the interfilamentous matrix. In normal unirradiated, in distal corneum, and in proximal corneum, the filaments measure about 70 A. They are electron-light and they contrast sharply with the surrounding electron-dense matrix. However, in the parakeratotic zone the matrix seems less dense than in these other zones. Therefore the interfilamentous matrix contrasts less well with the electron-light filaments in the parakeratotic zone. The filaments in the parakeratotic zone measure about 70 A, and thus have a diameter comparable to those in pre- and postparakeratotic zones. Filaments are present both in spheroids and in the surrounding non-spheroidal part of the parakeratotic zone.

The only similarities between the parakeratotic zone of irradiated stratum corneum and the stratum corneum of parakeratotic psoriasis without keratohyalin, described by Brody, (8) are the nuclei, lacunae, and groups of 130 A to 280 A dense particles found in both. Parakeratotic, postirradiated corneum differs significantly from parakeratotic, psoriatic corneum in the filament-matrix keratin pattern. In the psoriatic corneum the individual filaments within fibrils are electron dense, measure about 50 A in diameter, and resemble tonofilaments of the stratum basale of the epidermis. Between filaments in psoriatic corneum, a less-dense material is present. Therefore, the typical keratin pattern of less-dense filaments embedded within a denser matrix is absent in psoriatic corneum.

The existence of less-dense filaments measuring 70 A in diameter in the post-irradiated parakeratotic corneum indicates that the mechanism for keratin filament synthesis is intact. The lower-than-normal density of the interfilamentous matrix is the distinctive change in in keratinization induced by ultraviolet irradiation. At present the molecular structures of both the keratin filament and the interfilamentous matrix are poorly understood. Several recent reviews are available (11–16) which detail the various theories of alpha-keratin molecular structure.

SUMMARY

The ultrastructure of stratum corneum has been studied at intervals following a single ultraviolet exposure (4 times minimal erythema dose). A thick parakeratotic zone formed beneath the normal stratum corneum (distal corneal zone) at 72 hours post-irradiation. At one week after irradiation a third layer (proximal corneal zone) formed beneath the parakeratotic layer and is comparable in thickness to the distal corneal zone, differing from it in only minor structural details. The rapid formation of this proximal corneal zone implies an accelerated epidermal renewal time.

The parakeratotic zone possesses several distinct differences from normal stratum corneum. Not only are nuclei retained, but the normal

lamellar organization of horny layer is replaced by large spheroidal and less well-defined cells which are believed to be incompletely keratinized. The keratin pattern of the parakeratotic zone is different from normal, unirradiated stratum corneum, and from distal and proximal corneal zones. Although keratin filament diameter appears normal (70 A) in the parakeratotic zone, the interfilamentous matrix is less dense than normal, implying that some alteration in synthesis of this matrix has been induced by ultraviolet light.

Other alterations in parakeratotic postirradiated stratum corneum include the presence of peculiar oval lacunae (long axis 0.48 microns), well-preserved melanin granules, and groups of dense particles (130 A to 280 A diameter) thought to be ribosomes. A cell possessing a long dendrite-like process and melanin granules has been observed in parakeratotic stratum corneum and may be a melanocyte corpse.

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