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A Light and Electron Microscopy Study of Normal Human Stratum Corneum with Particular Reference to the Intercellular Space

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ABSTRACT

Intercellular and skin-surface substances, and exfoliating corneocytes, were clearly visualized by both light and electron microscopy. The intercellular space constituted an essential part of the normal human stratum corneum, in the basal and middle zones of which this space was filled with substances producing a compact appearance. The intercellular constituents were a nonhomogeneous substance, intact, single and "compound" lamellar granules and an intensely stained, membrane-like material that in some parts, but not in others, had a lamellar pattern. The artifacts produced by ultrathin sectioning for electron microscopy were too small to provide sufficient explanation for the porous appearance of the superficial zone. More important factors seemed to be enlargement of the intercellular space with decrease in the number of desmosomes and alterations of the intercellular substances, with decrease in the amount of nonhomogeneous substance and transformation of the single and "compound" lamellar granules into single and "compound" vesicular bodies.

The hypothesis is advanced that the single and "compound" vesicular bodies together with the decreased amount of nonhomogeneous substance may contribute to maintain the patency of the intercellular space in the superficial zone (stratum disjunctum), thereby facilitating absorption of surface-applied agents into the stratum corneum by some shunt mechanism, while the content of the intercellular space in the basal and middle zones (stratum compactum) forms the principal barrier to free diffusion.

INTRODUCTION

Electron microscopy of the intercellular space of normal stratum corneum (5) revealed a nonhomogeneous substance, granules of varying size, shape and structure, and also a membrane-like structure often associated with the granules. The nonhomogeneous substance constituted most of the intercellular material. Some of the granules had a lamellar content similar to that found in the lamellar granules of the cells in the stratum intermedium (stratum granulosum). In other electron microscopy studies, intercellular vesicular bodies were discerned in the middle (6,24) and superficial (3,4) zones of normal stratum corneum.

Other authors have reported presence of lamellar substance in the intercellular space of normal stratum corneum (12,17,18,20,23). That substance was assumed to have been discharged from the lamellar granules in the cells of the stratum intermedium by a process of exocytosis.

Discrepancies between results in different investigations of intercellular content led to further studies, and here is presented the light microscopy of the normal human stratum corneum, with a detailed ultrastructural description of its intercellular space.

MATERIALS AND METHODS

With local anesthesia (Carbocaine 10 mg/ml), punch biopsy specimens (3 mm) were taken from the shoulder, sacral and abdominal regions of 19 healthy subjects (14 men, 5 women) aged 22 to 51 years.

The specimens were fixed in a 1 % isotonic solution of osmium tetroxide buffered at pH 7.2-7.4 (9). They were rinsed in Tyrode's solution and dehydrated in graded steps of ethanol and in propylene oxide. The temperature during fixation, rinsing and dehydration to the stage of 95 % ethanol was kept at about 4° C. The specimens were embedded in Epon. They were sectioned transversally to the skin surface. For light microscopy, semithin (1-2 μm) sections were stained with 0.1 % aqueous solution of toluidine blue (7) and examined in a Leitz' Orthoplan-Orthomat photomicroscope. The ultrathin

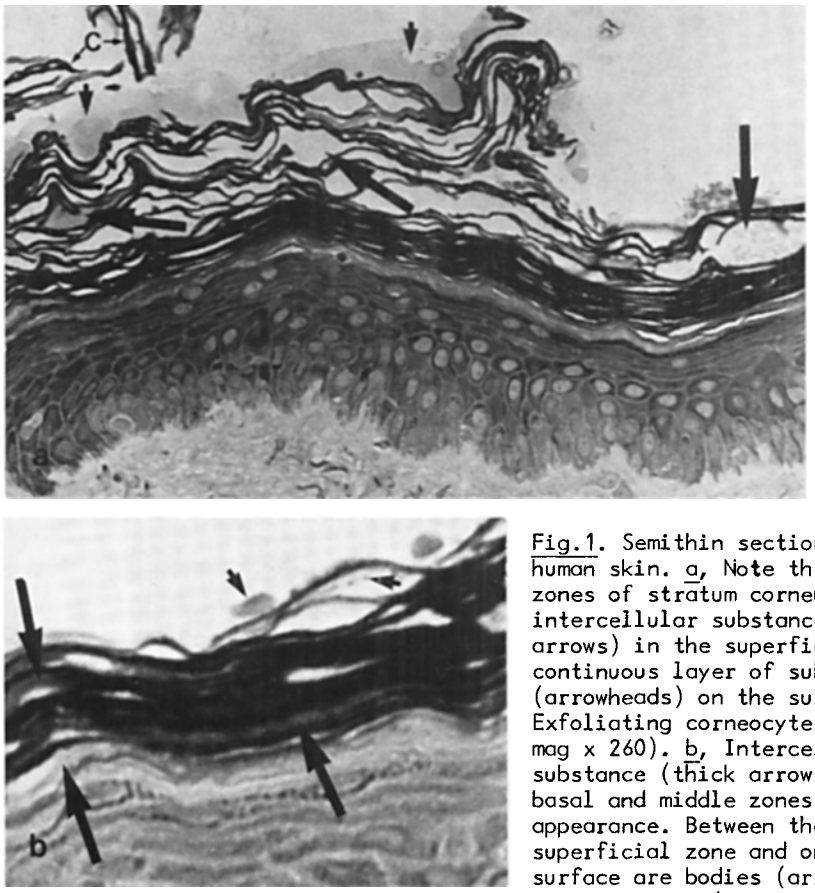

Fig. 1. Semithin section of normal human skin. a, Note three distinct zones of stratum corneum, presence of intercellular substance (thick arrows) in the superficial zone, and continuous layer of substances (arrowheads) on the surface at left. Exfoliating corneocytes (C). (orig mag x 260). b, Intercellular substance (thick arrows) gives the basal and middle zones a compact appearance. Between the cells of the superficial zone and on the zone surface are bodies (arrowheads) of varying size. (orig mag x 760).

sections for electron microscopy were stained with a saturated aqueous solution of uranyl acetate at 70° C for 20 minutes (3) and lead citrate (26).

RESULTS

Light microscopy - The normal human stratum corneum comprised three zones - basal, middle and superficial (Fig. 1a). The thickness of the superficial zone varied from 1-2 to 15 cell layers. Staining was least in the basal zone cells and heaviest in those of the middle zone, while the superficial zone cells were mostly somewhat less stained than the cells in the middle zone.

The basal and middle zones had a mainly compact appearance (Fig. 1a), due to the presence of intercellular substance (Fig. 1b). This substance was still present in areas where the corneocytes were distinctly separated from each others by cutting artifact.

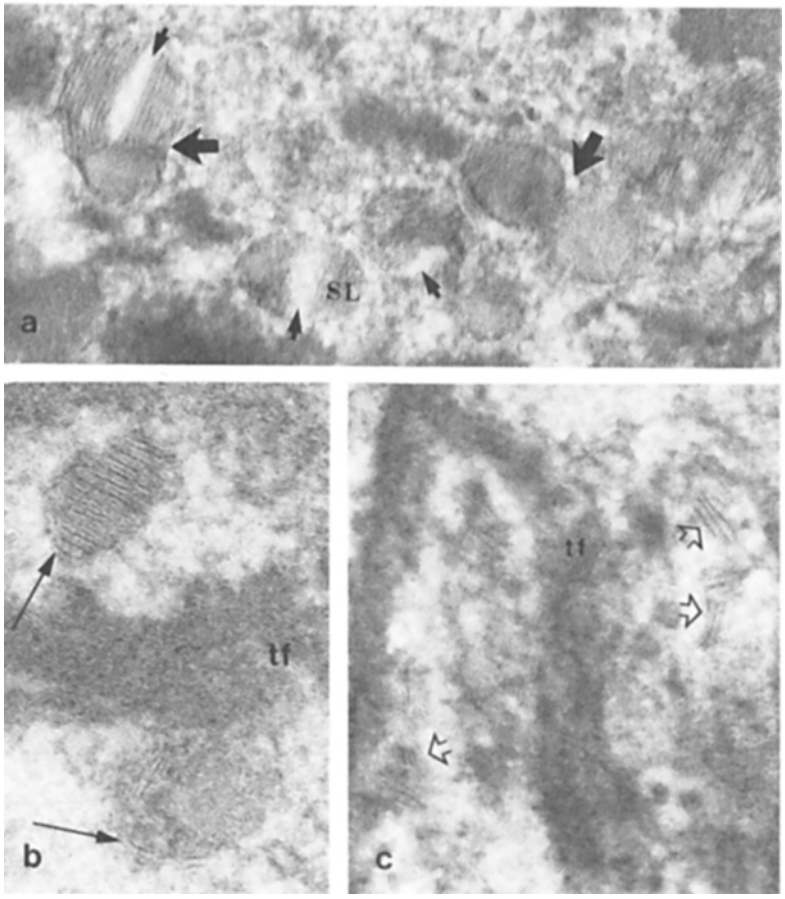

Fig. 2. Electron microscopy of intracellular lamellar granule material in stratum intermedium. a, Single (SL) and "compound" (short, thick arrows) lamellar granules. In some granules, small empty areas (arrowheads) occur. (orig mag x 150 000). b, The lamellar granules are bounded by an outer, trilaminar membrane (long, thin arrows). tf, tonofibril. (orig mag x 234 000). c, Lamellar material (open arrowheads) lying free in the cytoplasm without any encasing membrane. tf, tonofibril. (orig mag x 195 000).

At a first glance the intercellular space of the superficial zone seemed empty of substance. Closer scrutinity, however, revealed a varying amount of substance, which even completely filled some areas of the intercellular space (Fig. 1 a). Between the uppermost cell layers of the superficial zone, in both scantily and multilayered areas, small bodies were discerned in the intercellular space (Fig. 1b).

On the surface of the stratum corneum, in addition to exfoliating corneocytes, were bodies of varying shape and also a substance (Fig. 1a). In sections with a well developed superficial zone, the bodies and the substance

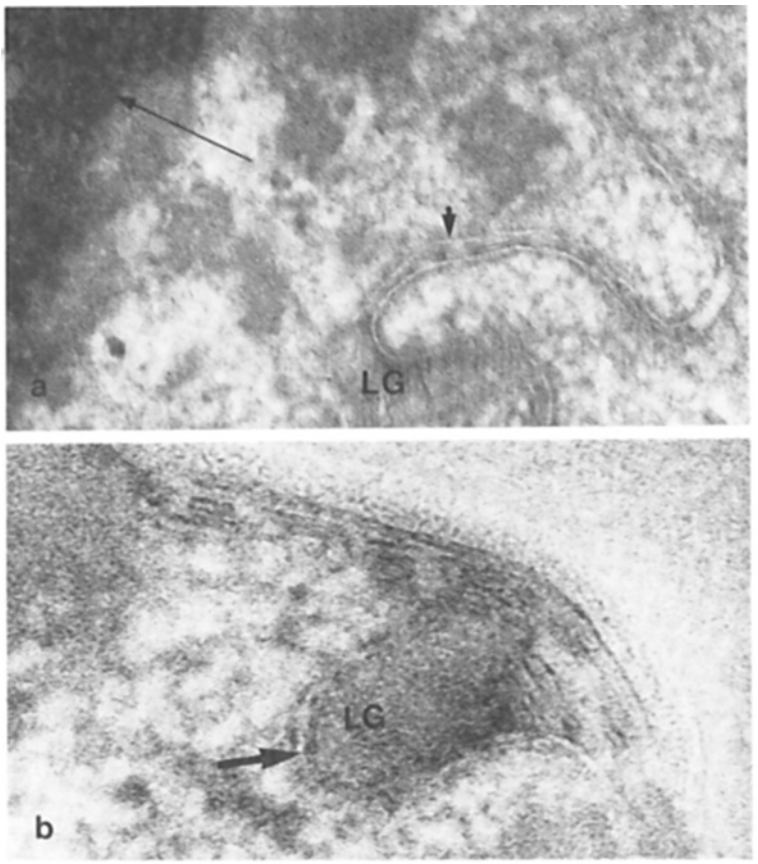

Fig. 3. Electron microscopy of intercellular space in stratum intermedium. a, The narrow intercellular space contains to a large extent a lamellar material. Secretion (?) of a lamellar granule (LG) into the intercellular space. At left keratohyalin with characteristic pattern (long, thin arrow). (orig mag x 195 000). b, An intact lamellar granule (LG), encased by a distinct trilaminar membrane (arrow), is observed in the intercellular space at the interface between the intermediate and corneal strata. (orig mag x 320 000).

formed a continuous layer on the surface of that zone (Fig. 1a).

Electron microscopy - The stratum intermedium cells contained a large number of lamellar granules. The granules occurred singly or they fused to form "compound" lamellar granules of varying size (Fig. 2a). They were bounded by a trilaminar membrane structure (Fig. 2b). Some granules contained empty areas suggesting a disintegration of the granule content (Fig. 2a). Freely lying lamellar material without any encasing trilaminar membrane was also observed in the cytoplasm (Fig. 2c).

Except for the invaginations of the cells, the breadth range of the

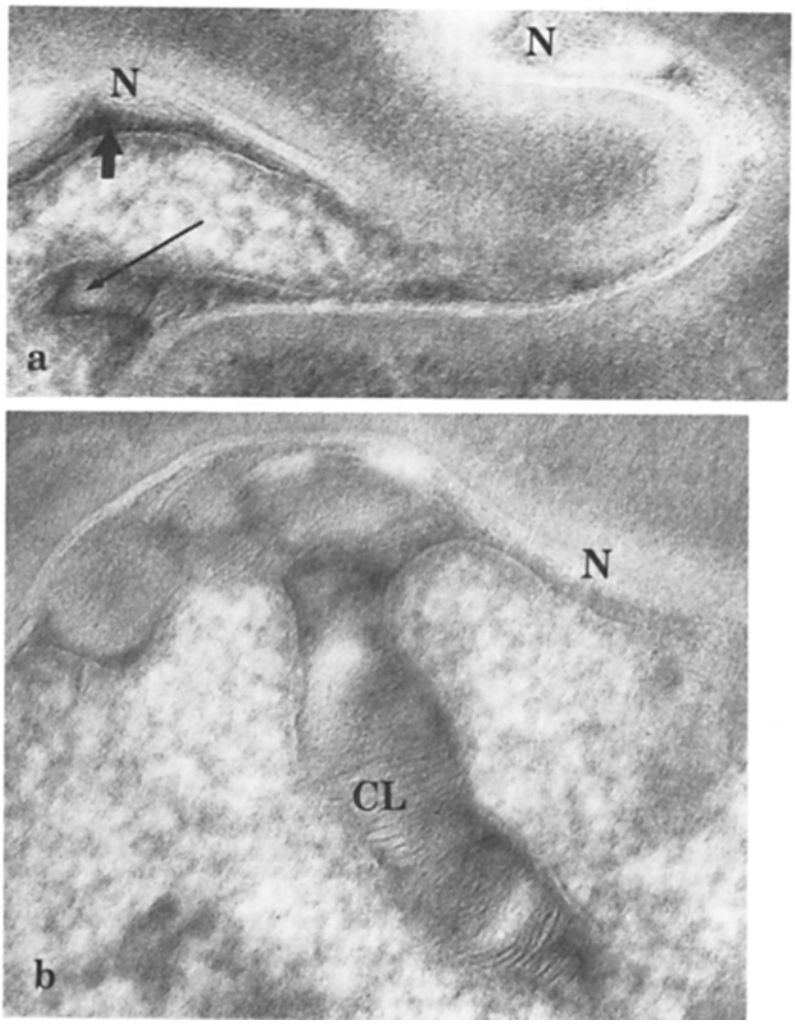

Fig. 4. Electron microscopy of intercellular space at the interface between strata intermedium and corneum. a, The intercellular space contains nonhomogeneous substance (N), a membrane-like structure without lamellar pattern (short, thick arrow) and single, lamellar granule (long, thin arrow). (orig mag x 252 000). b, The intercellular space contains nonhomogeneous substance (N) and an intact, "compound" lamellar granule (CL) showing areas with a less distinct lamellar pattern. (orig mag x 256 000).

intercellular space in the stratum intermedium was only 30-50 nm, and the intercellular space was mostly filled with substance (Fig. 3a). The breadth range of those parts of the intercellular space which were completely or almost completely filled with substance in the basal zone, in the middle zone and in the superficial zone was 30-1000, 30-2000 and 30-3000 nm, respectively. With increasing breadth of the intercellular space towards the skin surface,

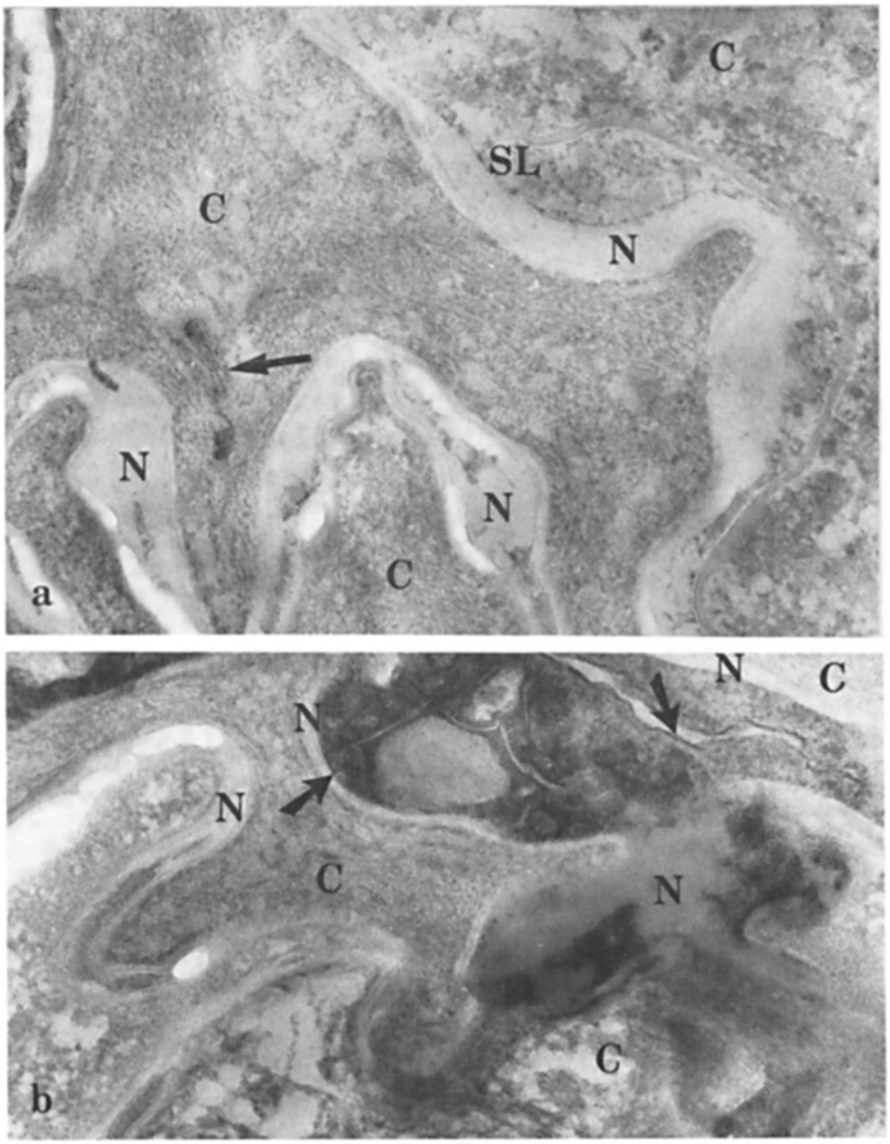

Fig. 5. Electron microscopy of the intercellular space in the basal (a) and middle (b) zones of the stratum corneum. a, The main part of the intercellular space is filled with nonhomogeneous substance (N). SL, intact, single lamellar granule. The cytoplasm of the cells (C) shows areas with a keratin pattern and areas with nonkeratinous substance. An intracellular, lamellar granule (thick arrow) is seen. The cells are bounded by a plasma membrane proper and cornified envelope. (orig mag x 80 000). b, The intercellular space shows an intact, "compound" lamellar granule, encased in a trilaminar membrane (arrows), and nonhomogeneous substance (N). C, cell. (orig mag x 100 000).

the desmosomes decreased in numbers (Brody, I. In preparation).

The invaginations of the stratum intermedium cells, the intercellular space at the interface between the intermediate and corneal strata (Figs. 3b,4), and

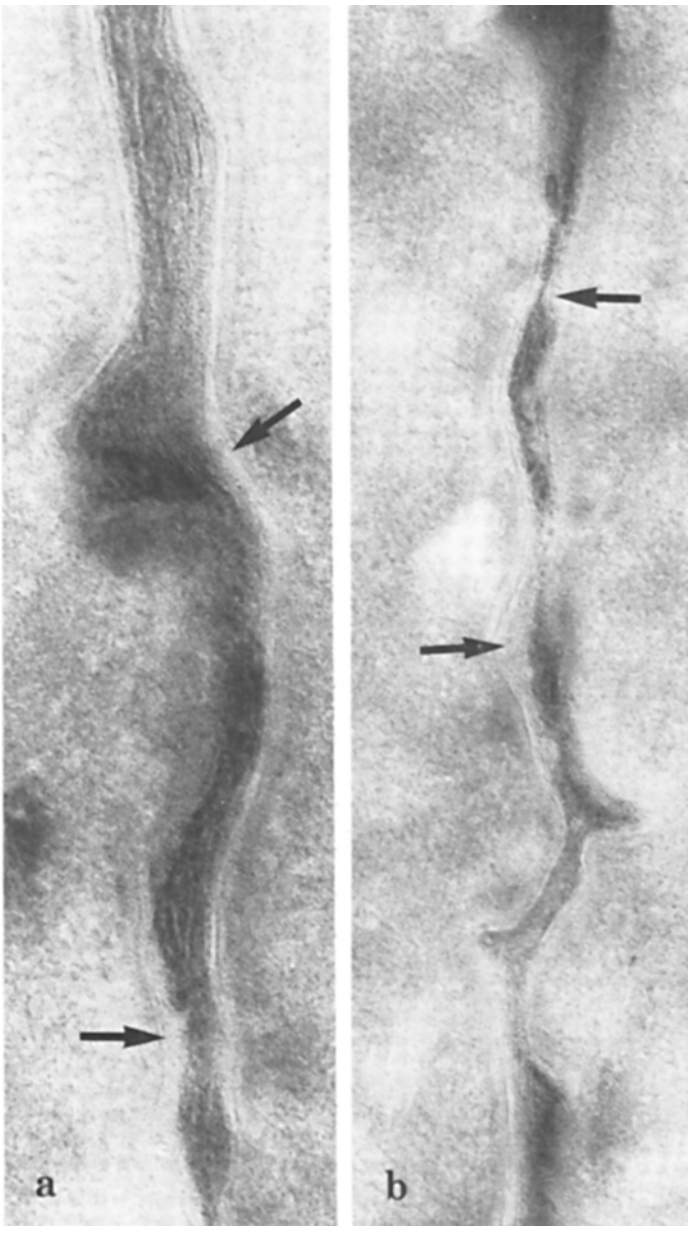

Fig. 6. Electron microscopy of intercellular, membrane-like structures in the basal zone of the stratum corneum. a, A distinct lamellar pattern is seen. Nonhomogeneous substance (arrows). (orig mag x 240 000). b, No lamellar pattern is distinguished. Nonhomogeneous substance (arrows). (orig mag x 175 000).

the intercellular space of the basal and middle zones of the stratum corneum (Fig. 5) contained a nonhomogeneous substance, single and "compound" lamellar granules and a membrane-like structure. The nonhomogeneous substance was the major intercellular constituent in the basal and middle zones. The single and "compound" lamellar granules were encased in a trilaminar membrane structure (Fig. 5). In the "compound" lamellar granules the intervening membranes of the

adjacent granules were intact or were partly or completely dissolved. In some parts, "compound" lamellar granules occupied large segments of the intercellular space (Fig. 5b) or the entire space between the desmosomes. But even in those parts of the space largely filled with single or "compound" lamellar granules or a membrane-like structure, a varying amount of nonhomogeneous substance separated these components from the plasma membranes of the corneocytes (Fig. 5,6). The membrane-like structures had in some parts (Fig. 6a), but not in others (Figs. 4a,6b), a lamellar pattern.

In the superficial zone, the varying amount of nonhomogeneous substance appeared mostly as fields and bars criss-crossing the intercellular space and leaving variably sized, empty loculi (Fig. 7a), or else the space appeared wholly or almost wholly void (Fig. 7b). But, similar to observations at light microscopy (Fig. 1a), some areas of the intercellular space were completely or almost completely substance-filled (Figs. 7b,8). Most of the lamellar granules in the superficial zone were transformed into single and "compound" vesicular bodies (Figs. 7b,c,8). Such bodies were always seen to be separated from the plasma membranes by a varying amount of nonhomogeneous substance (Figs. 7b,c,8). Extending completely or partly across the internal, empty space of the vesicular bodies and subdividing it into smaller, empty compartments (Fig. 7b,c) was intensely stained material - remnants of the encasing membranes in the "compound" lamellar granules and of intervening nonhomogeneous substance.

DISCUSSION

A similar division of the normal human stratum corneum into basal, middle and superficial zones, based on stainability of the corneocytes at different levels, as shown here, was previously reported from electron (4) and light (8) microscopy studies. The content and the breadth of the intercellular space in the basal, middle and superficial zones, as described here, and also changes in cell height and shape towards the skin surface (Brody, I. In preparation), suggest that the intercellular space earlier reported (5) comprised only a stratum corneum with a superficial zone of a few cell-layers' thickness.

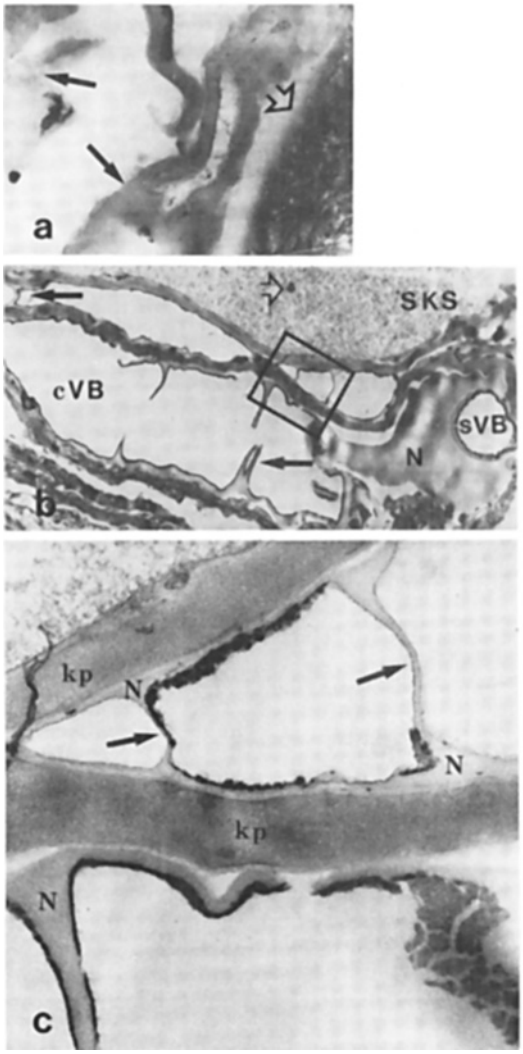

Fig. 7. Electron microscopy of the intercellular space in the superficial zone. a, Nonhomogeneous substance (arrows) appears as strands and fields criss-crossing the intercellular space. Corneocyte (open arrowhead). (orig mag x 48 000). b, At right almost the whole of the intercellular space is filled with nonhomogeneous substance (N) and a single vesicular body (sVB). Otherwise the intercellular space contains "compound" vesicular bodies (cVB) divided by complete or incomplete processes (arrows) into smaller, empty compartments, with a narrow zone of nonhomogeneous substance separating the vesicular bodies from the cell surfaces, or it is more or less empty. The skin surface (SKS) is covered by a loose substance, containing a small body (open arrowhead). (orig mag x 4200). c , Higher magnification of the area within the box in b, showing the organization of a "compound" vesicular body. Processes (arrows) divide the body into smaller, empty compartments. N, nonhomogeneous substance. A distinct keratin pattern (kp) is observed in the outermost corneocytes of the superficial zone. (orig mag x 180 000).

Also agreeing with earlier results (5,6) was the observation of discharged lamellar granules. The present finding that a distinct trilaminar membrane frequently encased the single and "compound" lamellar granules in the intercellular space of the stratum intermedium and of the basal and middle zones in the stratum corneum indicates that the single and "compound" lamellar granules were secreted from the stratum intermedium cells as intact organelles by the mechanism of diacytosis (10,11,27). The present investigation permits no conclusion as to whether the presence of lamellar material in the intercellular space, described in this and earlier (5) reports as a membrane--like structure, resulted from exocytosis of the lamellar granule content of

the cells in the stratum intermedium (12,17,18,20,23), from dissolution of encasing membranes after discharge of intact granules into the intercellular space, or from both mechanisms.

On the basis of histochemically demonstrated acid phosphatase, the lamellar granules have been characterized as epidermal lysosomes (33). Recent studies of lamellar granule-enriched fractions from neonatal mouse epidermis (14) and from fetal rat epidermis (13) revealed presence of acid hydrolases as well as lipids in the lamellar granules. The role of the acid hydrolases in these granules is still unknown (13,14). Further studies are necessary to elucidate whether they are involved in the disintegration of lamellar granules in the stratum intermedium cells,with appearance of lamellar material lying free in the cytoplasm, in the transformation of lamellar granules into vesicular bodies and in the falling amount of nonhomogeneous substance in the horny layer´s intercellular space towards the skin surface.

In percutaneous absorption studies (1,22), based on sequential stripping of the stratum corneum with adhesive tape, it was suggested that the innermost two-thirds of the stratum corneum formed the principal barrier to free diffusion. This hypothesis was in agreement with the histologic concept of a stratum corneum consisting of a proximal, compact stratum compactum and a distal, porous stratum disjunctum (19,25,28,31). In later studies, Blank (2) questioned the histologic view of large "channels" in the stratum disjunctum. He proposed, in agreement with Kligman (16), that the entire stratum corneum forms a barrier and that it is composed of a compact, multilayered tissue. The same opinion was held by Scheuplein & Bronough (29), who also meant that the tightness of the intercellular space in the stratum corneum was of vital significance to permeation. According to Kligman (16), the porous structure of the stratum disjunctum was artifactitious and produced by the histologic custom of sectioning the skin transversally to its surface.

Contradictory to the view of Kligman (16), Blank (2) and Scheuplein & Bronough (29), the present and previous (5) studies suggest that the intercellular space represents an important element in the entire stratum

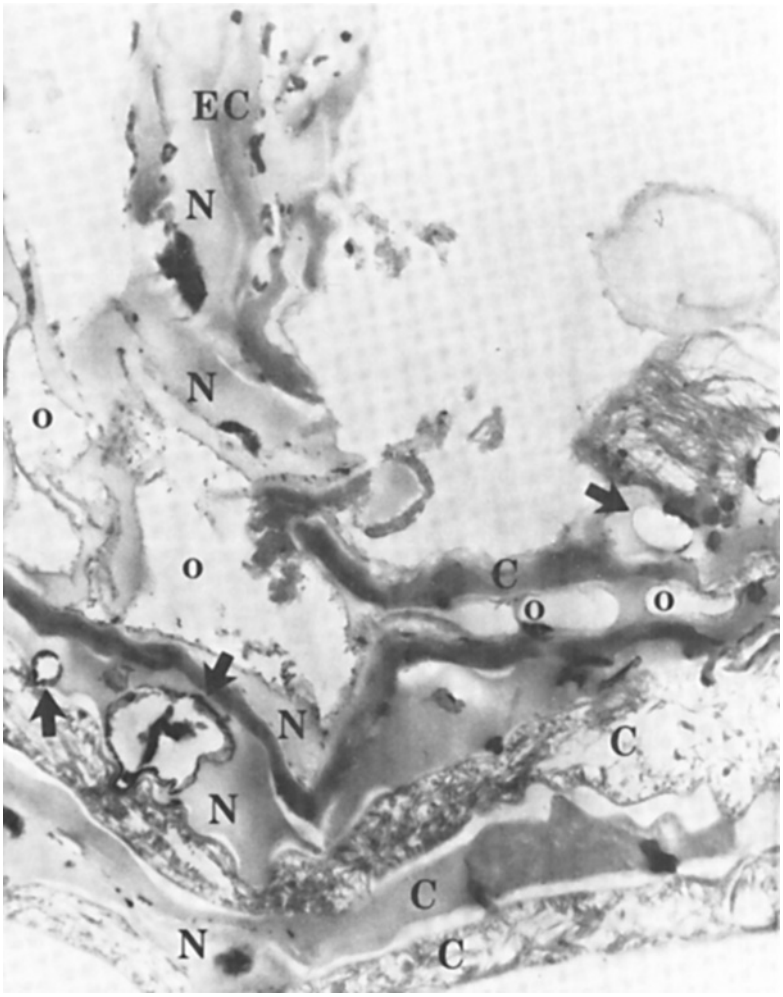

Fig. 8. Electron microscopy of outer part of a multilayered superficial zone, with a system of extracellular, single (short, thick arrows) and "compound" (o) vesicular bodies embedded in nonhomogeneous substance (N). C, corneocyte. EC, exfoliating corneocyte. (orig mag x 17 000).

corneum. The basal and middle zones seem to correspond to what histologists (19,25,28,31) have termed stratum compactum, which according to Blank's (1,22) original hypothesis represented the main barrier to free diffusion. As shown here, the intercellular content of nonhomogeneous substance, lamellar granules and a membrane-like structure, with predominance of the nonhomogeneous substance, was responsible for the compact appearance of the basal and middle zones. In this study large cutting artifacts occurred in the semithin sections for light microscopy, but cutting artifacts in the ultrathin sections for

electron microscopy were too small to explain the porous appearance of the superficial zone (stratum disjunctum). More important factors seem to be the enlargement of the intercellular space, due to numerical decrease in the desmosomes (Brody, I. In preparation), with concomitant reduction in the amount of nonhomogeneous substance, and the transformation of single and "compound" lamellar granules into single and "compound" vesicular bodies, as demonstrated here. Lately, Hirose & Kligman (15), in contradiction to Kligman's (16) original view, recognized the occurrence of a stratum disjunctum in an electron microscopy study of normal human skin and low--humidity dry skin of the leg.

It is here of interest to mention Scheuplein's and Bronough's (29) remark that if, contrary to their own opinion, the stratum corneum membrane is porous, "then shunt diffusion can contribute to the rate of permeation. Because shunt diffusion in effect bypasses the tissue, it is not detected in sorption studies". The function of the vesicular bodies in the superficial zone (stratum disjunctum) is unknown. A possibility which merits exploration is that they together with remaining nonhomogeneous substance may contribute to maintain the patency of the intercellular space, thereby facilitating absorption of substances applied to the skin surface by some shunt mechanism. It may also be of interest to study whether the vesicular bodies may be involved in the reservoir function (32) of the stratum corneum.

The composition of normal human stratum corneum has been stated as 50 % fibrous protein, 38 % and 23 % water-soluble substances in the proximal and distal zones, respectively, 2 % lipids in the proximal layers, 20 % lipids on the skin surface, and 7-10 % water (30). The lipid content of the stratum corneum has been located to the extruded lamellar substance in the intercellular space and to the plasma membranes (12). If there are no major species-related differences regarding lipid content of the lamellar granules between normal human epidermis and epidermis from neonatal mouse (14) or fetal rat (13), it seems reasonably to assume that the intact, single and "compound" lamellar granules in the intercellular space may also contribute to the lipid

content. As shown here, and in a previous study (5), the single and "compound" lamellar granules, the membrane-like structure and the single and "compound" vesicular bodies were always found in close association with the nonhomogeneous substance. The decrease in amount of water-soluble substances towards the skin surface (30) and the close association of lipids and water--soluble substances observed in studies on the mechanism of water binding in the stratum corneum (21) may suggest that the nonhomogeneous substance represents water-soluble substances.

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