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# SURFACE ULTRASTRUCTURE OF HUMAN SKIN

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*Abstract.* The normal human skin surface was studied with a combination of scanning electron microscope, surface replication method, and transmission electron microscope. All specimens were prepared with the critical point drying (CPD) method. Horny cells were found to stack up in columnar fashion with their peripheries overlapping. The surface of a non-nucleated horny cell, therefore, showed a dome-shaped elevation in the center, transmitting the nuclear bulge of the underlying granular cells; parallel double lines at the periphery representing its true cell border and depressed, step-like imprint of the cell border of the cell which once overlapped; and on high magnification the true surface of the separated desmosomes. The surfaces of the horny cells showed many bizarre marks but were relatively smooth. The underside of the surface horny cells, however, bore villi except in areas overlapping the periphery of the underlying cell. Tissue cultured keratinocytes also stacked up when they keratinized and the cell periphery also overlapped. However, the overlapped cell border did not leave the imprint on the underlying cell, probably because of the lack of pressure from above.

*Key words:* Freeze-etching; Desmosome gap junction; Scanning electron microscopy; Replica; Cell membrane; Cell surface

The surface of the normal human epidermis has been studied by many light microscopists (5, 6, 8, 9, 11, 16–20). They used various malleable or hardenable semi-liquid substances to obtain a negative imprint of the skin surface. With the advent of the scanning electron microscope (SEM), a host of publications (1, 5–7, 12–14) appeared in a short time. These works disclosed in three dimensions the details of surface ultrastructure hitherto unobserved. However, the instrumentation, technique and particularly the preparation methods initially available were not adequate for present day standards; most of the published pictures show artefactial shrinkage, contamination of the surface, etc. None of these workers used the critical point drying (CPD) method to prevent tissue damage. The CPD is now routinely used and is mandatory to avoid shrinkage and distortion of the specimen.

Even with CPD and improved instrumentation, the resolution limit of the SEM for biological specimens continues to be a problem because it is still no better than 75–100 Å, with the exception of the field emission SEM. Although the stereoscopic effect (depth of focus) is inferior, it was therefore thought that transmission electron microscopic (TEM) observation of metal replicas should be re-evaluated as an alternative to or as a better method than SEM for high resolution work.

Studies of cell surface ultrastructures including those of the cellular junctions have become increasingly important in many fields of research, such as malignant transformation. Skin has been utilized as a model more and more in recent years because of its easy accessibility and accurate observability of the results. For these reasons, the human skin was processed with a CPD tissue preparation method and examined by a reciprocal utilization of SEM and replica methods. It is hoped that this study will provide a set of basic information on the normal human skin surface for future studies on many abnormal conditions.

## MATERIALS AND METHODS

All materials were excised under 1% procaine anesthesia from the right anterior chest of a 21-year-old white male. The tissue was immediately cut into 1 or 2 $mm^3$ blocks and fixed in 1% glutaraldehyde in 1/10 M cacodylate buffer (pH 7.4) for 2 hours.

### *SEM specimen preparation*

The epidermis and upper dermis were separated from the subcutis with a sharp razor blade and sequentially fixed in 2, 3, 4, and 5% glutaraldehyde with minimum fixation of 1/2 hour in each concentration. Osmic acid fixation was avoided. Specimens thus fixed were rinsed overnight in the

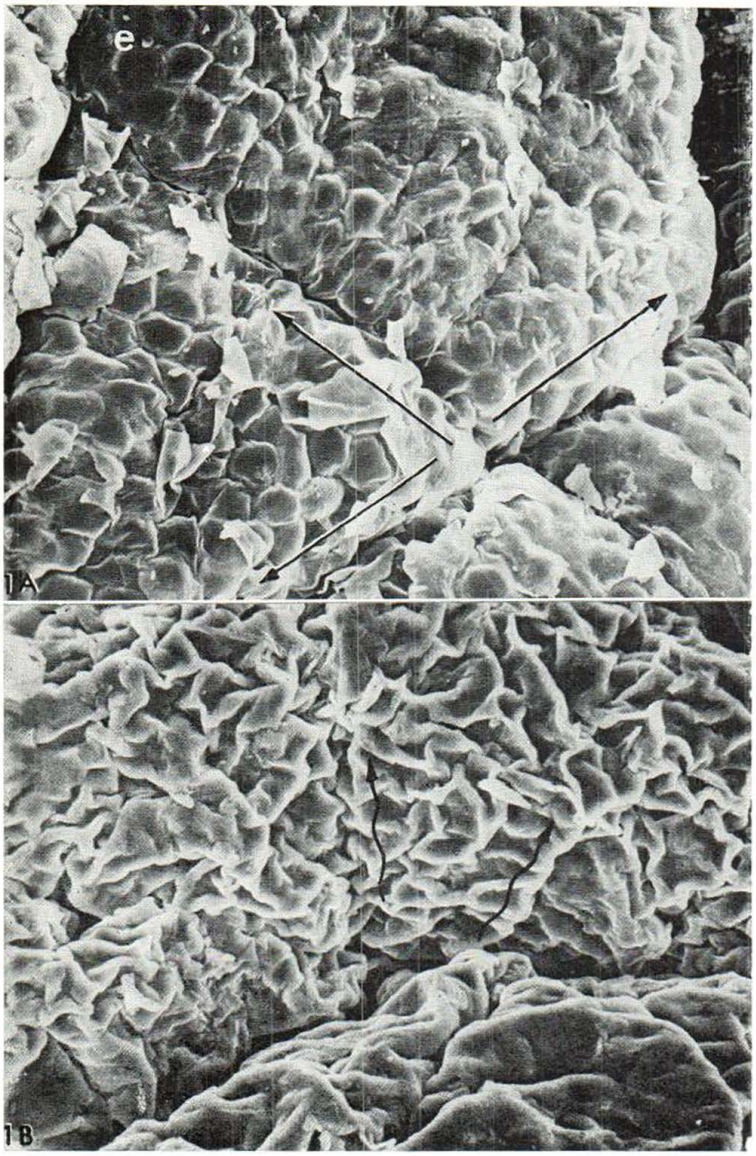



*Fig. 1A.* A low magnification view of CPD-prepared epidermis reveals primary furrows (*arrows*) dividing the surface into triangular, rectangular or polygonal fields. Each skin field consists of several hundred dome-shaped surface horny cells. Some cells are loosely attached and others are flaking off. *e*, erythrocytes. 25 kV, ×240.

*Fig. 1B.* Non-CPD-prepared epidermis at the same magnification as Fig. 1A reveals extensive shrinkage of the surface; primary furrows (*arrows*) are often indistinguishable from coarse folds and wrinkles. Dome-shaped surface elevations of individual cells are absent. Because of the formation of false primary furrows due to surface shrinkage, more than 10 skin fields are formed in the same surface area as that of Fig. 1, in which only 6 fields were counted. 25 kV, ×240.

same buffer and gradually dehydrated over 12 hours through increasing concentrations of acetone (10–100%). The specimen was transferred in absolute acetone into a critical point drying apparatus (Model E3000, Polyscience). Acetone was replaced by liquid carbon dioxide ($CO_2$) in the apparatus. In order to secure the permeation of $CO_2$, the tissue blocks were flushed with $CO_2$ several times. After incubation in $CO_2$ for one hour, liquid $CO_2$ was transformed into gaseous $CO_2$ at the critical point (1100 p.s.i. of pressure at 31.5°C). Specimens were dried simultaneously. The dried specimens were affixed to copper grids with silver conductive paints (ACME Chemicals and Insulation Co., New Haven, Conn.) and gold-coated in a Hitachi HUS 4GB evaporator equipped with a specimen stage in gimbals at $10^{-6}$ torr. Specimens thus prepared were examined in either an Hitachi HSM-2 SEM or an HSE-1 SEM accessory attached to an Hitachi HU-12 high resolution transmission electron microscope (TEM). The accelerating voltage ranged from 5 to 25 kV. The control specimens were prepared in identical manner except that CPD procedures were omitted.

*Replica preparation*

CPD-prepared specimens were placed in a Denton DV-502 vacuum evaporator and cast with platinum–carbon at an angle of 45° and then with carbon alone. To detach the replicas, the tissue was dissolved with Clorox. The detached replicas were cleansed with 50% Liquinox in distilled water overnight, rinsed twice with distilled water containing 1% Kodak Photoflo and placed on 200 mesh copper grids. The replicas were examined in a Hitachi HU-12 TEM.

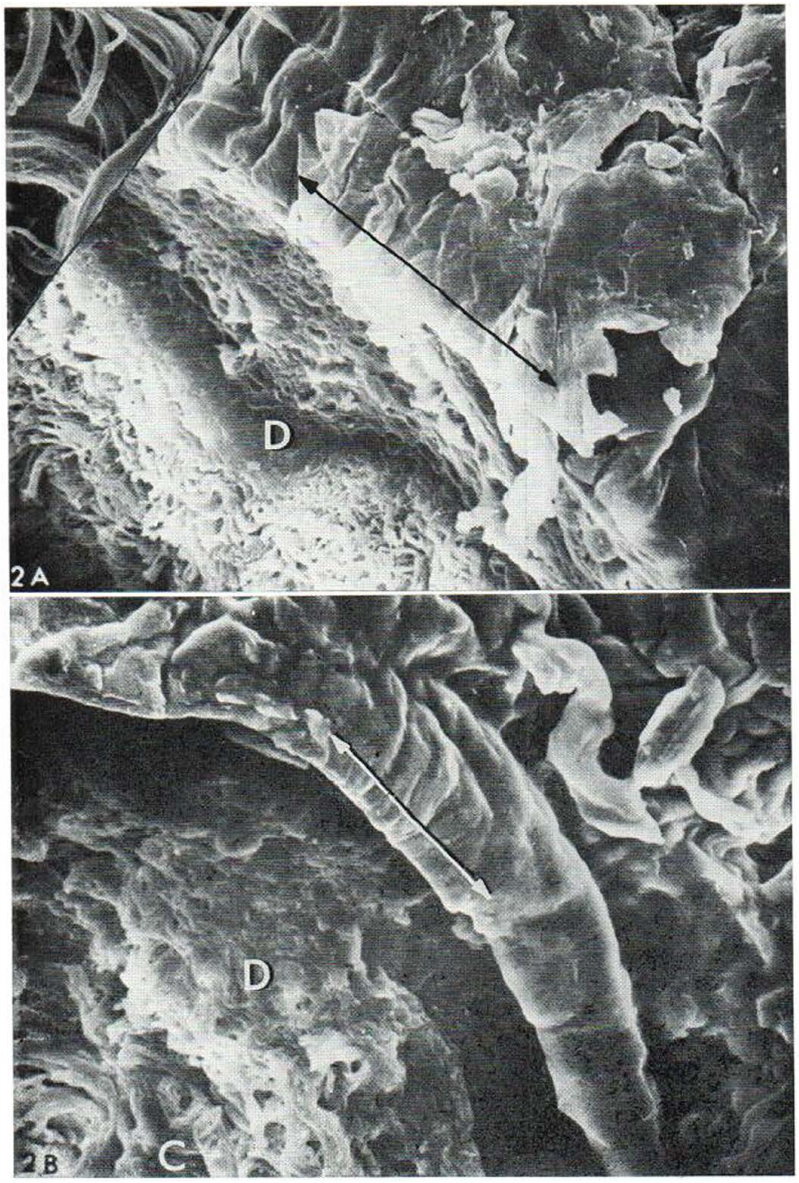



*Fig. 2A.* A CPD-prepared specimen shows a sharp edge of the section (*arrows*) and well-demarcated, dome-shaped surface horny cells. Individual fibers of the dermis (*D*) are visible and even fine pericollagenous filaments are clearly visible (inset, upper left). ×1700, 25 kV.

*Fig. 2B.* A non-CPD-prepared specimen, in contrast, shows a rolled-in epidermal edge (*arrows*) and shrunken dermis (*D*) underneath. Contour of surface horny cells is not sharp and dermal collagen (*C*) appears coagulated. ×8 000, 25 kV.

*Freeze-fracture method*

Glutaraldehyde-fixed tissue was placed in a Denton DFE-2 freeze-etch apparatus attached to a DV-502 evaporator. Specimens were fractured at −170°C and cast as in replica preparation at −120°C. The procedure of recovery of the replicas was the same as above.

*TEM specimen preparation*

Glutaraldehyde-fixed and buffer-rinsed specimens were post-fixed with 1% osmic acid in the same buffer and embedded in Araldite. Thin sections were cut in a Porter–Blum MT-2 ultramicrotome at 600 Å and stained with 1% uranyl acetate in 13% methanol and then stained with lead citrate (15).

*Tissue culture studies*

Normal epidermal keratinocytes from several healthy individuals were cultured according to our previous method (10). Keratinized specimens were prepared for TEM examination as well as for replica production by the same methods as above and as described previously (10), except that fixation in 1% as well as 5% glutaraldehyde was used.

## RESULTS

### I. *SEM observations*

At a very low magnification, the specimen prepared with the CPD method revealed a non-shriveled sur-

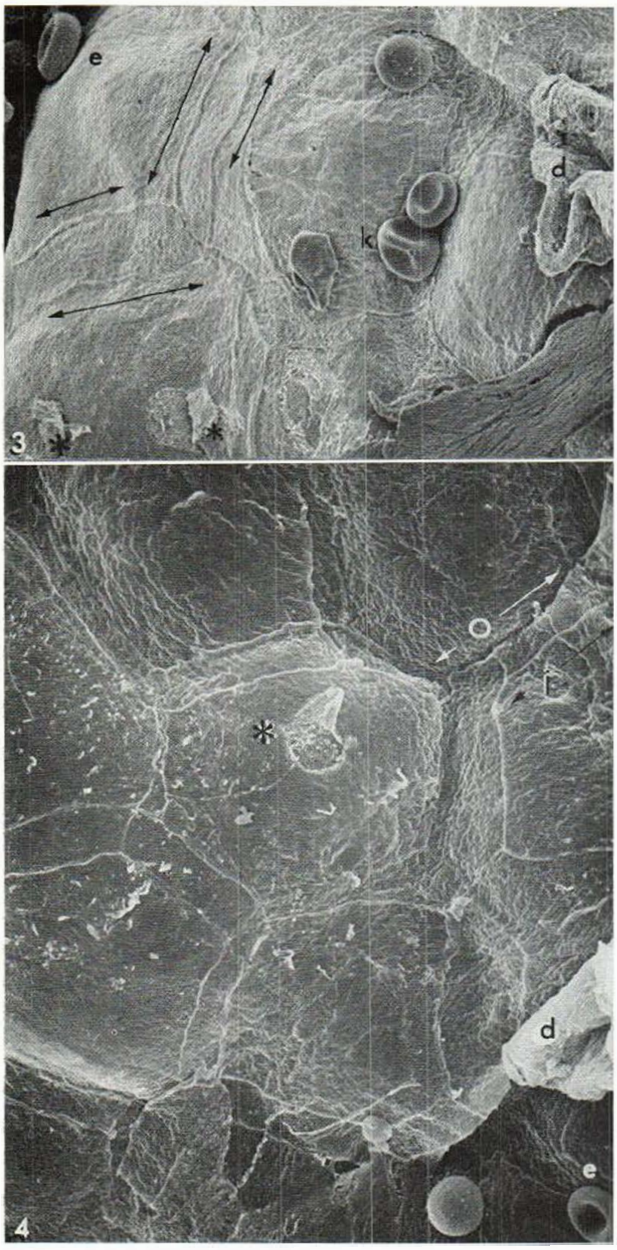
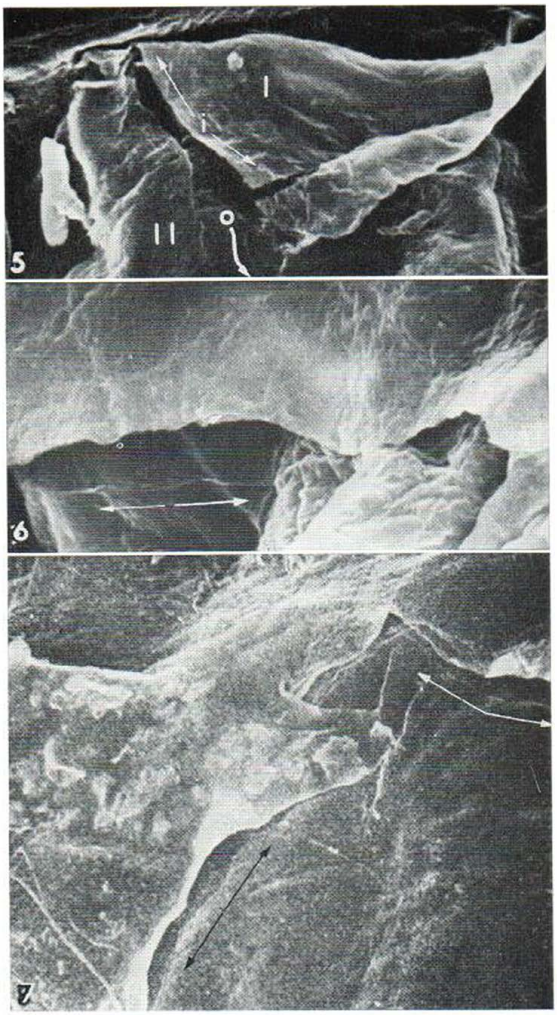



*Fig. 3.* A low voltage (5 kV) scan also reveals PPLs clearly (*arrows*). *d*, desquamated cell; *e*, erythrocyte; *K*, knizocyte *: erosion of surface. 5 kV, ×1500.

*Fig. 4.* PPLs are visible in most cells. In one cell, a gap is formed between the outer PPL ($o\rightarrow$) and the adjoining cell, suggesting that the outer PPL represents cell margin. $i\rightarrow$: inner PPL. *d*, desquamated cell; *e*, erythrocyte. *: erosion, 20 kV, ×1500.

face (Fig. 1A), whereas the specimens prepared without it appeared like the convoluted surface of the brain (Fig. 1B). Excessive wrinkling masked or distorted the primary skin lines (Fig. 1B). The CPD image demonstrated in Fig. 1A is, in fact, very similar to that of the normal skin surface as seen under the dissecting microscope at about ×75, except that the contour of individual surface horny cells cannot be visualized by the latter. Primary skin lines (6, 20) were clearly visible and converged upon or started from one area as previously described by the light microscopists (11, 19). These lines divided the skin surface into trianguar, rectangular or even polygonal fields (Fig. 1A). Individual cells had semi-dome-shaped surfaces and paved each field like cobblestones (Fig. 1A). In non-CPD-treated specimens, this pattern is completely lost (Fig. 1B).

The underlying dermis was not shrunken in CPD-




*Figs. 5–7.* In Fig. 5 a partial detachment of a surface horny cell (*I*) reveals a sharp imprint of its margin (*i*→) on the subjacent cell (*II*). For the cell II, the imprint of the cell margin of cell I (*i*→) is the inner PPL, since the outer PPL (the cell border) is visible (*o*→). In Figs. 6 and 7 only the imprint of the overlying cell margin (*arrows*), i.e. the inner PPL of the underlying cell, is visible. Fig. 5: ×2 900. Fig. 6: 6100, Fig. 7: 4 700. All taken at 25 kV.

prepared specimens (Fig. 2A). In control specimens prepared without CPD the dermis was usually more shrunken than the epidermis (Fig. 2B) and seemed to contribute to the formation of many gross folds and convolutions of the epidermal surface (Fig. 1B). Individual dermal collagen fibrils were clearly visible in CPD-prepared specimens (inset, Fig. 2A), whereas collagen appeared coagulated in the specimens prepared without CPD (Fig. 2B). Thus it was obvious that non-CPD-treated specimens were altered significantly by artefacts. In the following, all descriptions are therefore based upon the findings of CPD-prepared specimens, unless the preparation method is specifically mentioned.

Higher magnification revealed that there were two parallel lines along the periphery of many surface cells (Figs. 3, 4). These lines will be referred to as parallel peripheral lines (PPL). PPL's were seen in pictures taken at 5 kV (Fig. 3) as well as 20 kV (Fig. 4) and 25 kV (Fig. 4) of accelerating voltages. If one assumes that the electrons accelerated at 5 kV do not penetrate deeply into gold-coated horny cells, the peripheral parallel lines were surface structures. Desquamating cells had slightly elevated edges (Figs. 5, 6, 7); such edges often coincided with the outer line of the PPL (Fig. 4). When the lifted edge was viewed from the underside, the imprint of the edge of the subjacent cell could be detected on the surface (Fig. 8). These findings suggested that the outer line of the PPL is the true cell border and the inner line could be the imprint of the border of the cell which once overlapped.

Lines similar to PPL were occasionally seen running semi-parallel to PPL at the periphery or/and crossing at random the center of the cell (Fig. 9). The former were considered to be related to the peripheral interdigitation of the cell, whereas the latter could cross PPL and were interpreted as imprints of overlaid cell border which did not conform to the columnar stacking of horny cells (see below). At high magnification, the surfaces of individual cells were finely wrinkled (Figs. 4, 9), but showed neither specialized areas nor villi at the highest SEM resolution. In contrast, the underside of half-peeled cells showed numerous villi except at the periphery corresponding between presumptive outer and inner PPL of the underlying cell (Fig. 8), namely the peripheral zone of depression (PZD) of the underlying cell (see below, Replica Studies). The periphery covering a PZD had fewer villi than the center (Fig. 8). Desquamated cells appeared like scales and were loosely attached (Figs. 1A, 3, 4). Peeling of the cell membrane and formation of defect or erosion were evident in many cells (Figs. 3, 4).

Erythrocytes accidentally attached on the surface at the time of surgery were round to oval with concave centers (Figs. 3, 4, 9); they were seldom wrinkled except those damaged, which were either shriveled (Fig. 3) or appeared like knizocytes (Fig. 3) (2). The fact that the majority of the erythrocytes were in-

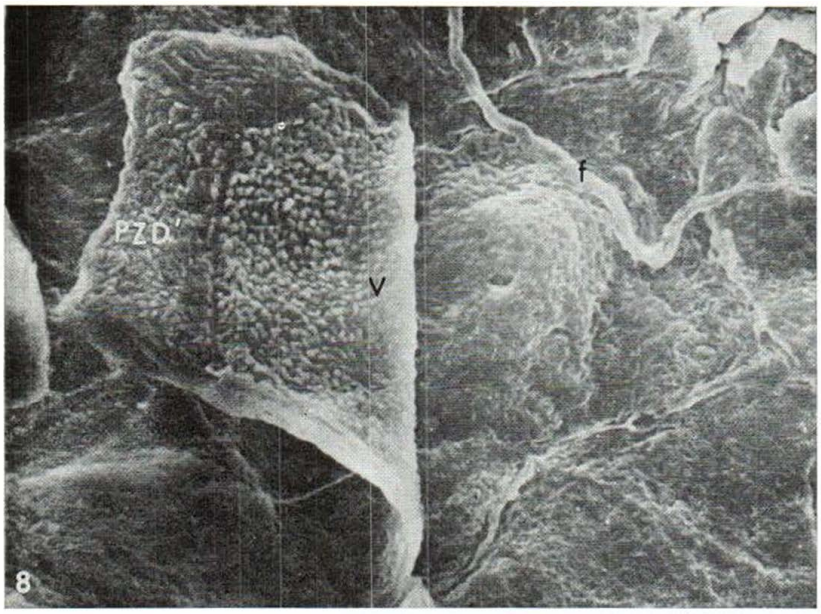



*Fig. 8.* A surface horny cell is half-peeled and shows its underside. It is seen that numerous fine villous projections (*V*) are present within a well-demarcated center, whereas the peripheral zone (*PZD'*) presumably facing PZD of the subjacent cell is relatively smooth. *f*, fibrin; × 9 000.

tact suggested that CPD operation was optimal and that fine wrinkles on the surface of the horny cells such as seen in Fig. 10 were less likely to be artefact.

II. *Replica studies*

The replicas of surface horny cells showed dome-shaped elevations (Fig. 10), although the stereographic effect of replicas was not as striking as in SEM pictures. PPL were seen in many cells (Figs. 10A, 10B, 11–15), whereas in some cells or in some parts of the cells three-lined PPL (Figs. 10A, 10B, 14) instead of the two-lined (Figs. 12, 13) or single-lined PPL (Fig. 15) were found. The cell border, namely the outer line of PPL (see SEM above), seemed to be tightly adhering to the surface of the subjacent cell. Except for detaching cells, puckered

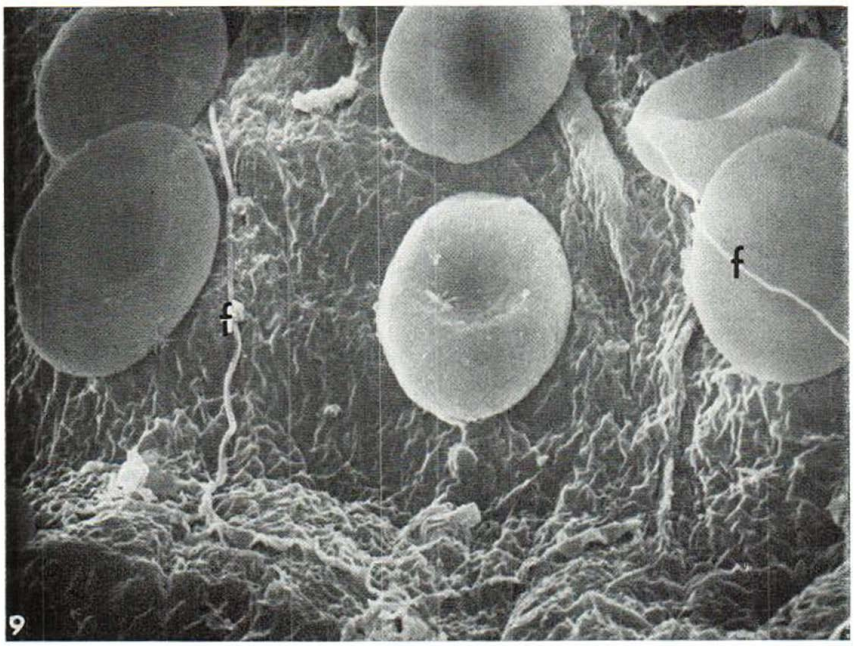



*Fig. 9.* A high magnification of the surface horny cells reveals fine wrinkles. Erythrocytes show concave centers and smooth cell surfaces. *f*, fibrin. × 5 000; 25 kV.


*Fig. 10A, 10B.* Dome-shaped bulging of the center of each cell. PPL (*arrows*), PZD, fine surface furrows and plications are demonstrated with a better resolution than by SEM in the replica of CPD-prepared skin. No surface villi are present. The area lettered PZD is magnified in Fig. 11. Tiny numbers (*12, 13, 14*) indicate figures in which the numbered areas are magnified, whereas large numbers (*1, 2, 3, 4*) are assigned to individual cells to be followed through Fig. 14. *: corresponding area in 10A and 10B. 10A: × 2 450; 10B: × 3 700.

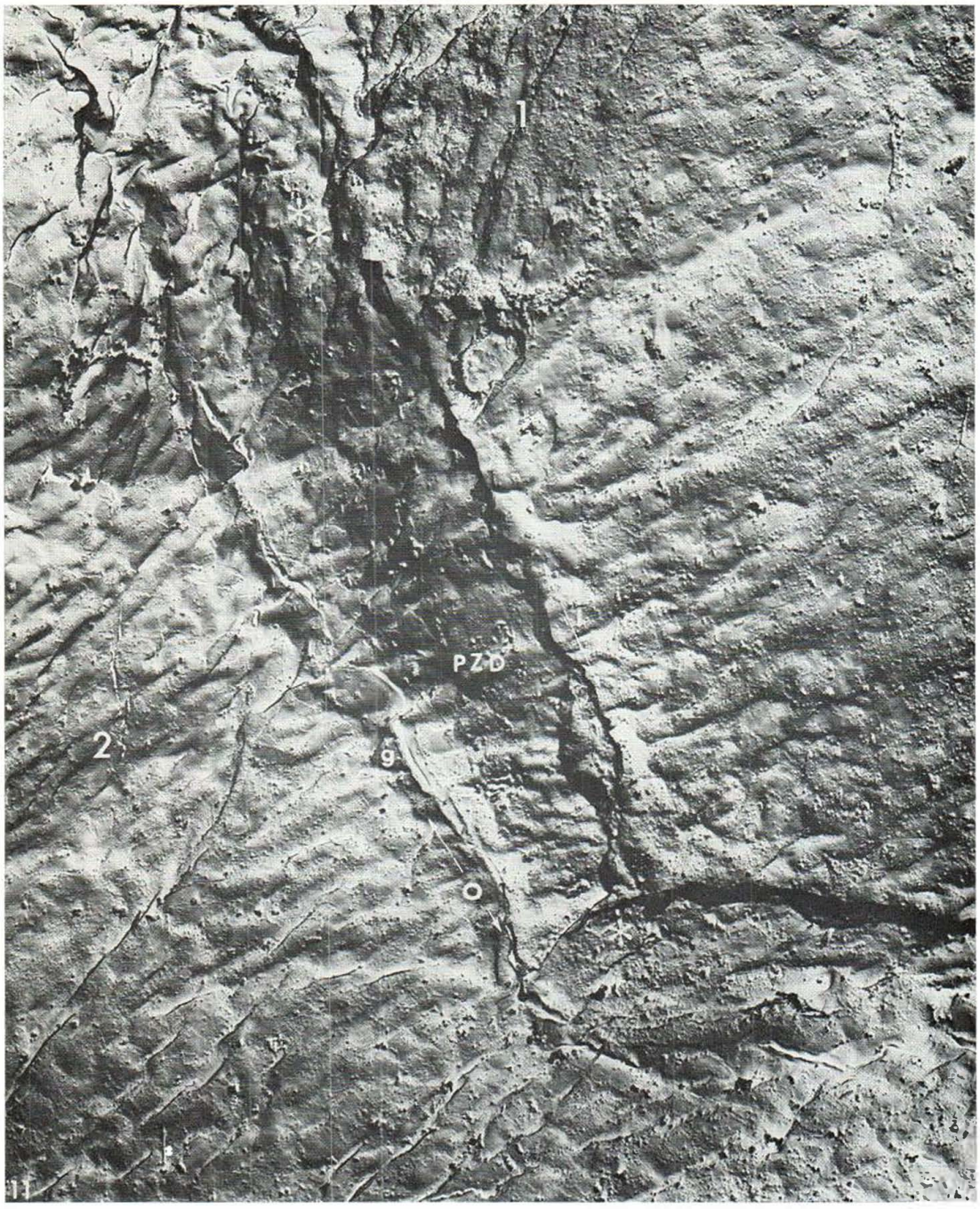



*Fig. 11.* A step-like depression is seen along the inner PPL (*i*→), producing a steep ridge. The depressed zone extending to the outer PPL (*o*→), namely the cell border, is the PZD. Continuity of PZD to cell surface no. 1 through the ridge (* and **) suggests, that PZD belongs to cell no. 1, whereas discontinuity of the cell surface by a slight gap formation (*g*) at the outer PPL suggests that the latter is the border of cell no. 1. The areas marked with * and *g* are magnified in Fig. 12 and the area marked with ** is enlarged in Fig. 13. ×9 000.


*Fig. 12.* The areas marked in Fig. 11 with * and *g* are enlarged. It is clearly seen that cell surface no. 1 is abruptly and sharply depressed at the ridge (*i*), i.e. the inner PPL, but nevertheless continues (←*PZD*→) to the outer PPL (*o*) where a gap (*g*) is formed between no. 1 and no. 2. It is obvious that in SEM both the ridge and the gap were observed as lines. At a corner of hexagonal cell no. 1, the ridge tapers off (*) toward the gap. The surfaces of cell no. 1 and no. 2 bear small particles and bizarre markings (cf. Fig. 18). ×15 200.

*Fig. 13.* Enlargement of the area marked with ** in Fig. 11. Continuity of cell surface no. 1 to PZD at ** and a narrow gap formation (*g*) between it and cell no. 2 is observed. Small particles and bizarre patterns are seen on these cells (cf. Fig. 18). ×9 600.

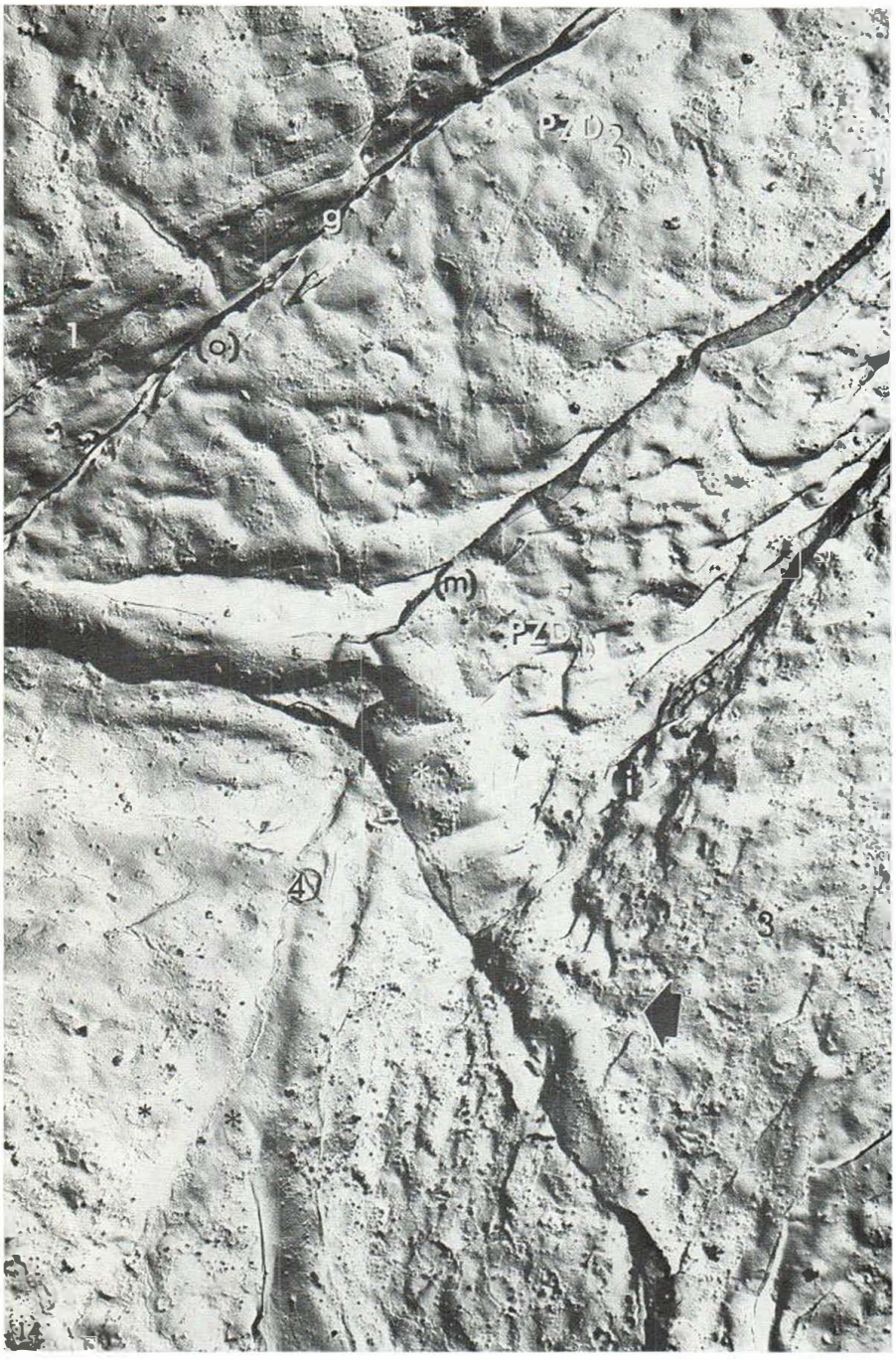
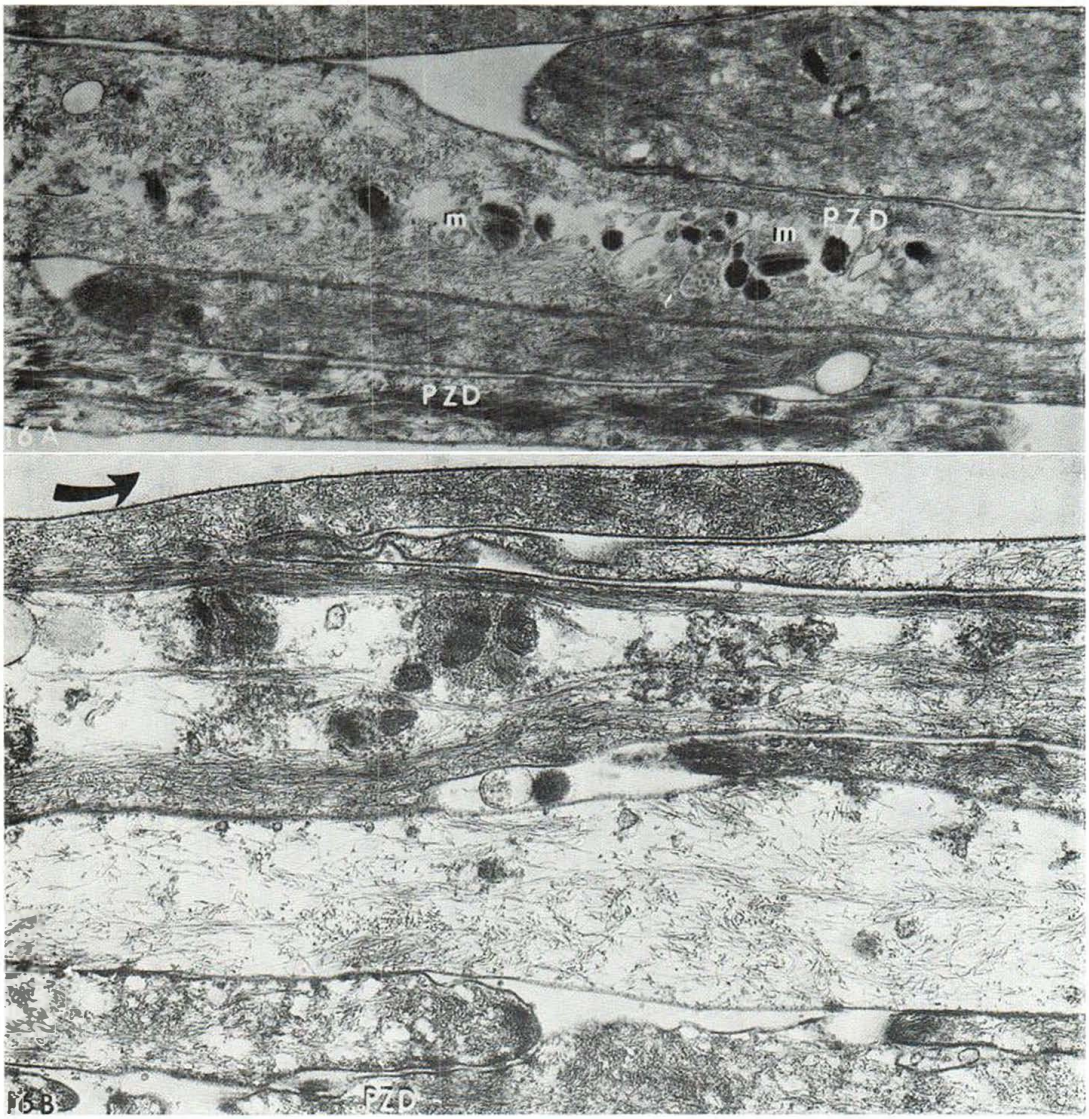



*Fig. 14.* Enlargement of the area marked in Fig. 10 A. A three-line PPL with double PZD ($PZD_1$, $PZD_2$) probably belongs to cell no. 3, because the inner (*i*), middle (*m*) and outer (*o*) PPL do not cross the junction between cells 3 and 4 (which is, by the way, a "non-stepped" or "single-line" PPL type) and cell surface no. 3 is continuous to PZD over the ridge (*arrow*), i.e. the inner PPL (*i*). The ridge between the $PZD_1$ and $PZD_2$ may be the middle line of PPL if the cell which once covered this area interdigitated with cell no. 3 by a three-step mode (cf. Fig. 17 A). $PZD_2$ ends at the cell border, i.e. the outer PPL (*o*) of cell no. 3, and is separated from cell no. 1 by a narrow gap (*g*). Surface particles and markings are numerous: Some of these appear to be grouped into macules (*) (cf. Fig. 18). × 12 250.


*Fig. 15.* Cell no. 1 is junctioned to cell no. 2 with the formation of two-lined PPL (*i, o*) and PZD. It is junctioned to cell no. 3 by one-line PPL with a rather steep lateral surface (*). The junction between cells 2 and 3 is one-line PPL. There are many desmosomal macules (*m*) composed of small particles. ×21 000.




*Figs. 16A, 16B.* Tissue cultured keratinocytes undergo keratinization by accumulation of filaments, loss of nucleus and cytoplasmic organelles and by becoming flat. The periphery of individual horny cells overlaps in layers, but PZD is not as markedly depressed as *in vivo* (cf. Fig. 17D). The junctional zone is therefore slightly swollen and the uppermost cell is slightly lifted up (curved arrow in Fig. 16B). The ends of individual cells, however, do not invaginate or interdigitate as in *in vivo*. *m*: phagocytosed melanosomes. ×17 500.

or rolled-up edges were not common. It was clearly seen that the inner line of PPL represented the edge of step-like, downward depressions of the surface at the cell periphery (Figs. 11, 12). The edge of the depression could be sharp (Fig. 12). This, of course, depended partly upon the shadowing angle. The depressed zone between the inner and outer PPL, therefore, was the area between the edge of the depression and the cell border. This zone will be referred to as the peripheral zone of depression (PZD). When three-lined PPL were present, the PZD was double (Figs. 13, 14). TEM observation of the cellular junction in tissue culture and in tissue revealed that the edges of horny cells pile in column and they do indeed overlap. In tissue cultured keratinocytes the overlapping was simple (Figs. 16A, 16B).

In the tissue, the horny cells in the lowest layers of the stratum corneum showed a simple overlapping, whereas in the upper layers the ends of horny cells frequently invaginated into the adjoining cell to produce a two-lipped edge in the invaginated cell (Figs. 17A, 17B, 17C). As mentioned above, PPL were not present in every cell junction; abrupt ending of the cell periphery with steep edge and without PZD was also encountered (Fig. 15). On reviewing TEM pictures, it was understood that a non-stepped ending could result from a non-depressing overlapping as in tissue cultured horny cells (Figs. 16A, 16B), but also from more complicated endings such as formation of two lips of equal length produced by a peg-like invagination of the adjoining cell (Figs. 17A, 17B, 17C).

The general surface of surface horny cells did not bear villi. This confirmed SEM observations. However, with a higher resolving power of the replica–TEM combination, various degrees of wrinkling, ridge formation and plication were seen on the surface (Fig. 15). In sone cells, erosions of the surface were clearly seen.

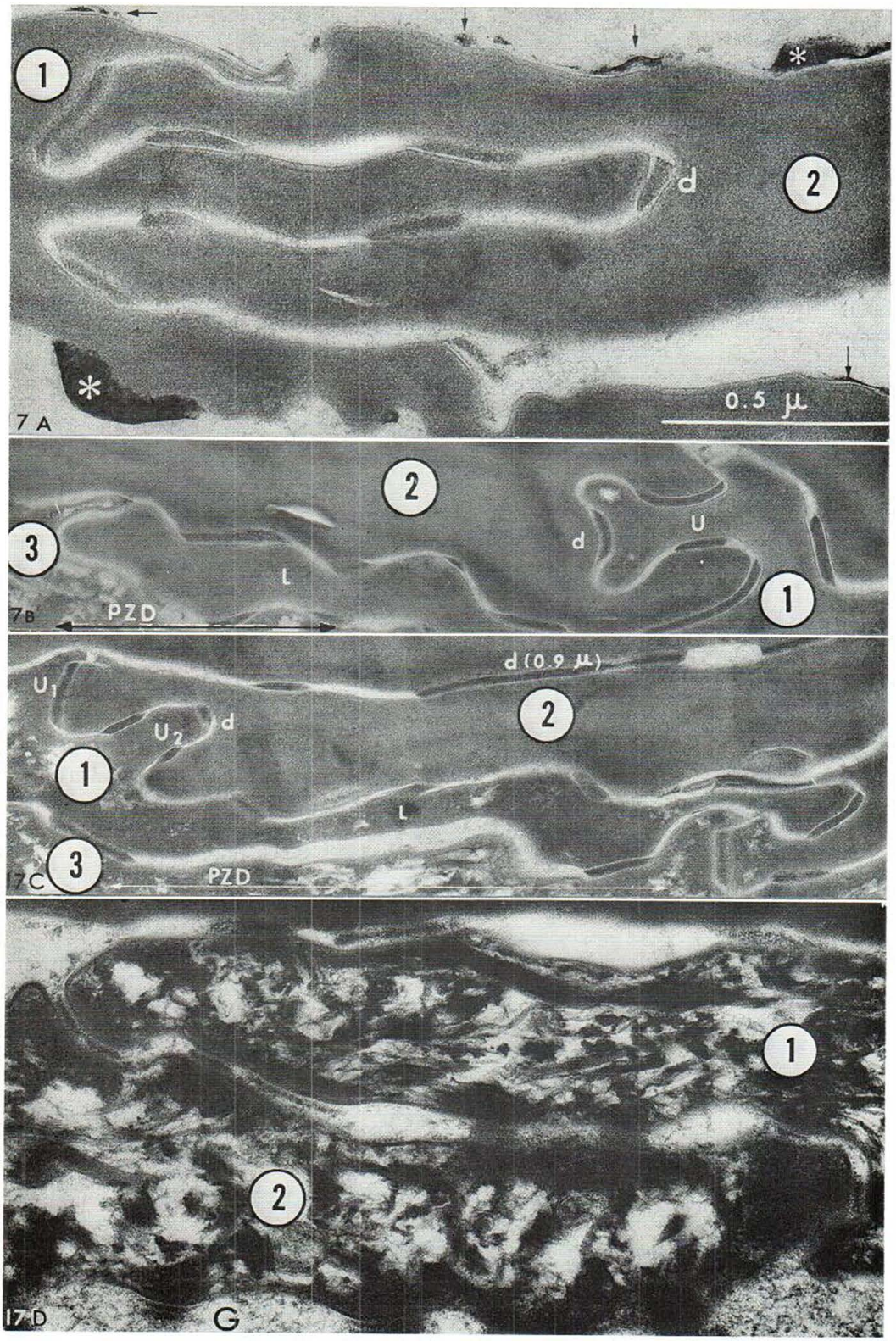
Specialized areas which might represent former desmosomal junctions or nexus were occasionally seen (Fig. 18). These areas were round-, oval- or bizarre-shaped macules and often elevated (Fig. 18). The dimensions of these macules on elevations were varied but, on average, measured 0.3–0.8 $\mu$m in long diameter (Fig. 18). These specialized areas often had a patch of small particles (Fig. 18). Freeze-fractured keratinocytes in the upper epidermis showed similar macules, usually in depressed recesses (Fig. 19). Aggregations of small particles were also seen in these macules which measured approximately 0.16–0.4 $\mu$m (Fig. 19). These macules on the fractured cell membranes were interpreted as desmosomal markings of horny cells as their morphology and dimensions are compatible with those described as freeze-fractured desmosomes (4 600 Å) of human stratum corneum (18). On comparison with freeze-fractured desmosomes (Fig. 19), the macules of surface horny cell (Fig. 18) could be interpreted as separated desmosomes due to their similar size, shape, distribution, and frequency: TEM observation of horny cells in the tissue and in cultures shows residues of desmosomes on the surface of separated horny cells (Fig. 18); they measure 0.3–0.8 $\mu$m. Bizarre-shaped surface patterns other than desmosomes were seen (Figs. 12, 13, 14). It was thought that the substance which produces these irregular surface marks could be an uneven remnant of intercellular or cell surface substances, detached nexus, etc.

## DISCUSSION

The horny cells in the uppermost layer of the stratum corneum (surface horny cells) are completely keratinized (dried). One might expect that such dried horny cells, which were in addition fixed with glutaraldehyde and dehydrated with acetone, would not change their surface ultrastructure very much whether or not CPD was used. Compared with previous works (6, 7, 12–14), CPD-treated skin preserved the dome-shaped elevations of individual surface horny cells. In addition, the surface was less wrinkled and specific marks such as PPL could be clearly differentiated from other lines caused by shrinkage artefacts. Villous projections were less prominent than previously reported. Excessive wrinkling or villi formation on the surface horny cells as reported in some of the non-CPD treated specimens might be caused by an accentuation of slight elevations and depressions related to the previous villi.

In the replica studies, artefact-free surface ultrastructure in a true sense could be revealed for the first time; the peripheral parallel lines as seen in SEM were clearly defined as the cell border (outer line) and the ridge of step-like peripheral depression (inner line); the peripheral zone of depression (PZD) had a relatively smooth surface; the cell margin was tightly adhering to the underlying cell surface; remnants of desmosomal attachment were seen as macules of small particle aggregation; and bizarre surface patterns probably representing the residue of cell surface substances were observed.

The true surface of desmosomes has never been demonstrated: The freeze-fracture procedure cleaves the cell membrane and only the internal structures are exposed for replication (Fig. 20) (3, 10). In desmosome-rich epithelial tissues other than the skin there may be little opportunity to see the natural surface of desmosomes because either the cells do not desquamate (liver, kidney, etc.) or the surface becomes differentiated into villi or other structures (mucous membranes, intestines, etc.). Even if the exfoliated cells were recovered, it is most likely that the desmosomes would have undergone degenerative changes due to enzyme-containing fluids or would be contaminated by debris. Also, the development of desmosomes is most prominent in the epidermis.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For these reasons, the surface of the skin is the most logical subject for the study of the surface structure of detached desmosomes. The separated surface of epidermal desmosomes appeared like cobblestones: No substructures corresponding to several layers of desmosomes were found such as are seen in thin section. If, for example, the intermediate dense layer, which is straight, were a structural unit, some of the separated surface covered by this layer partially or entirely would be flat. It may be concluded that the cobblestone surface of the separated desmosomes is a reflection of the aggregated particles within the cell membrane at desmosomes and that several layers as seen in thin sections represent non-structural substances, probably condensed intercellular or cell surface materials which upon separation lose their integrity and passively adhere to the cobblestone surface.

The surface horny cells have lost their nuclei; the cell body is flattened; and the cell contour appears in TEM like corrugated French fried potatoes (Figs. 16, 17). The dome-shaped convex surface of individual horny cells as shown in the present study should reflect the nuclear elevation of the underlying granular cells. The fact that the center of each surface horny cell was elevated as if its own nucleus was present suggests that nucleus-containing granular cells and overlying horny cells are stacked up in a columnar fashion with their centers oriented around the common axis and with their margins lining up equi-distant from the center. The columnar arrangement of upper epidermal keratinocytes has been demonstrated previously by the light microscopic method (4) as well as by SEM (12, 13).

A step-like peripheral depression was reported by Menton & Eisen in their SEM studies of normal and abnormal skin (12, 13). Although their observation was limited because of the resolution of the SEM, their conclusion that this zone (PZD) is an imprint of the periphery of the overlapped horny cell is supported by the present study using replica and TEM. In the present study, however, it was shown that a step-like overlapping is just one of several methods of lateral junctioning; more complicated invagination and interdigitation producing double PZD or no PZD were also observed. Such complex interdigitation was encountered more frequently in the upper stratum corneum than in the lower strata: Infolding of the cellular envelope may take place when it becomes imperative for keratinocytes to reduce their circumference because of dehydration and condensation as keratinization proceeds.

The horny cell surface of the uppermost layer of the epidermis was free from villi: Surface tension and stretching may expand the surface area which was reserved as villi. The under surface covering the PZD was also smooth. It seems likely that the outside pressure applied to the skin is doubled in PZD where the overlapping of thickened horny cell envelope and widened intercellular spaces is to be accommodated; without the flattening of the surface, the overlapped zone of PZD might have been seen as an elevated peripheral band. In loosely layered keratinocytes in tissue culture, such pressure does not seem to exist and, consequently perhaps, an elevated peripheral band instead of PZD is indeed produced (Fig. 16B) (10). The flattening of PZD seems to be conducive to the tight sealing of the cellular junction. Many horny cells in the upper stratum corneum have a lipped border which further

*Fig. 17A.* The horny cells in the uppermost stratum corneum have residual desmosomes between cells (*D*) as well as on the surfaces (*). These measure, on average, 0.3 $\mu$m and are distributed approximately 0.3 $\mu$m apart from each other. Irregular remnants of cell surface or intercellular substances (*arrows*) are also visible. Intercellular junctions are generally loose and there is no indication that nexus persists at this level. The border of cell no. 1 invaginates into the end of cell no. 2. Suppose that cell no. 1 remained and cell no. 2 exfoliated, three-lined PPL with double PZD such as shown in Fig. 14 (*i, m, o*: and $PZD_1$ and $PZD_2$) might result. If cell no. 2 remained and cell no. 1 desquamated, a single-edged cell border such as shown in Fig. 14 (between 3 and 4) might well occur. ×75 000.

*Fig. 17B, 17C.* Horny cells in the mid-layer stratum corneum are still junctioned tightly with desmosomes (*d*), these measure between 0.3-0.8 $\mu$m and are on average 0.3 $\mu$m apart. As in Fig. 17A, these desmosomes are often located at the tip of villi or corresponding depressions. Cell no. 1 is bifurcated at the end and the upper lip (*U*) invaginates into cell no. 2, while the lower lip (*L*) interdigitates with the less keratinized underlying cell no. 3 and produces a step-like depression (*PZD*). If cell no. 1 were shed and cell no. 2 remained, the replica might show a sharp-edged false junction (one-lined PPL) of cell no. 2 with cell no. 3; if cell no. 2 is shed and cell no. 1 remained, the surface of cell no. 1 would show two-lined PPL. In Fig. 17C, cell no. 1 has two upper lips ($U_1$, $U_2$); if cell no. 2 were shed and cell no. 1 remained, the surface of cell no. 1 would show three-lined PPL and two PZD's. ×45 000.

*Fig. 17D.* Incompletely keratinized horny cells in the lowest layers of the stratum corneum are overlapped at their periphery to form a simple one-step interdigitation. If cell no. 1 were shed, cell no. 2 would show two-lined PPL in the surface replica. *G*: granular cell. ×45 000.

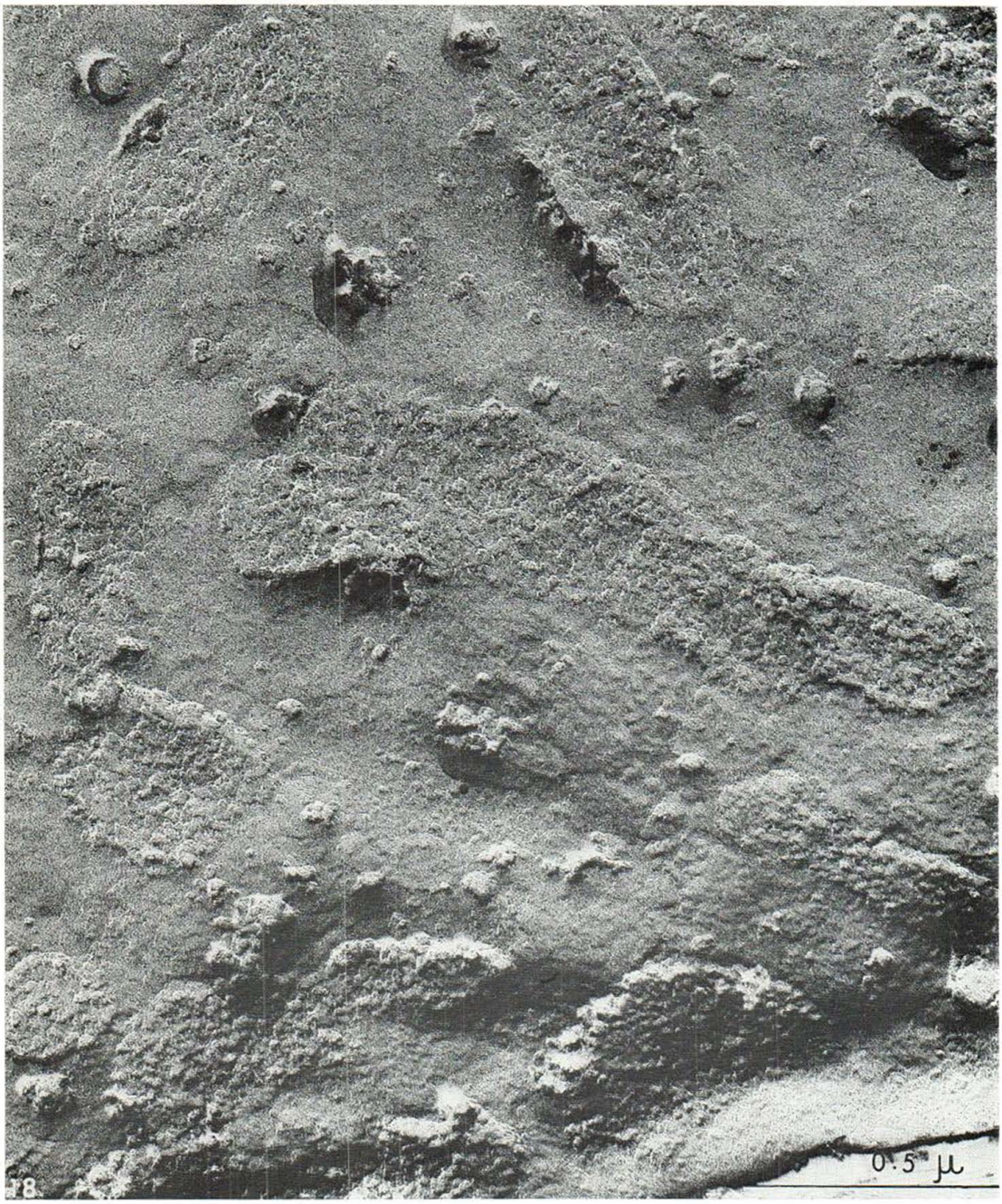
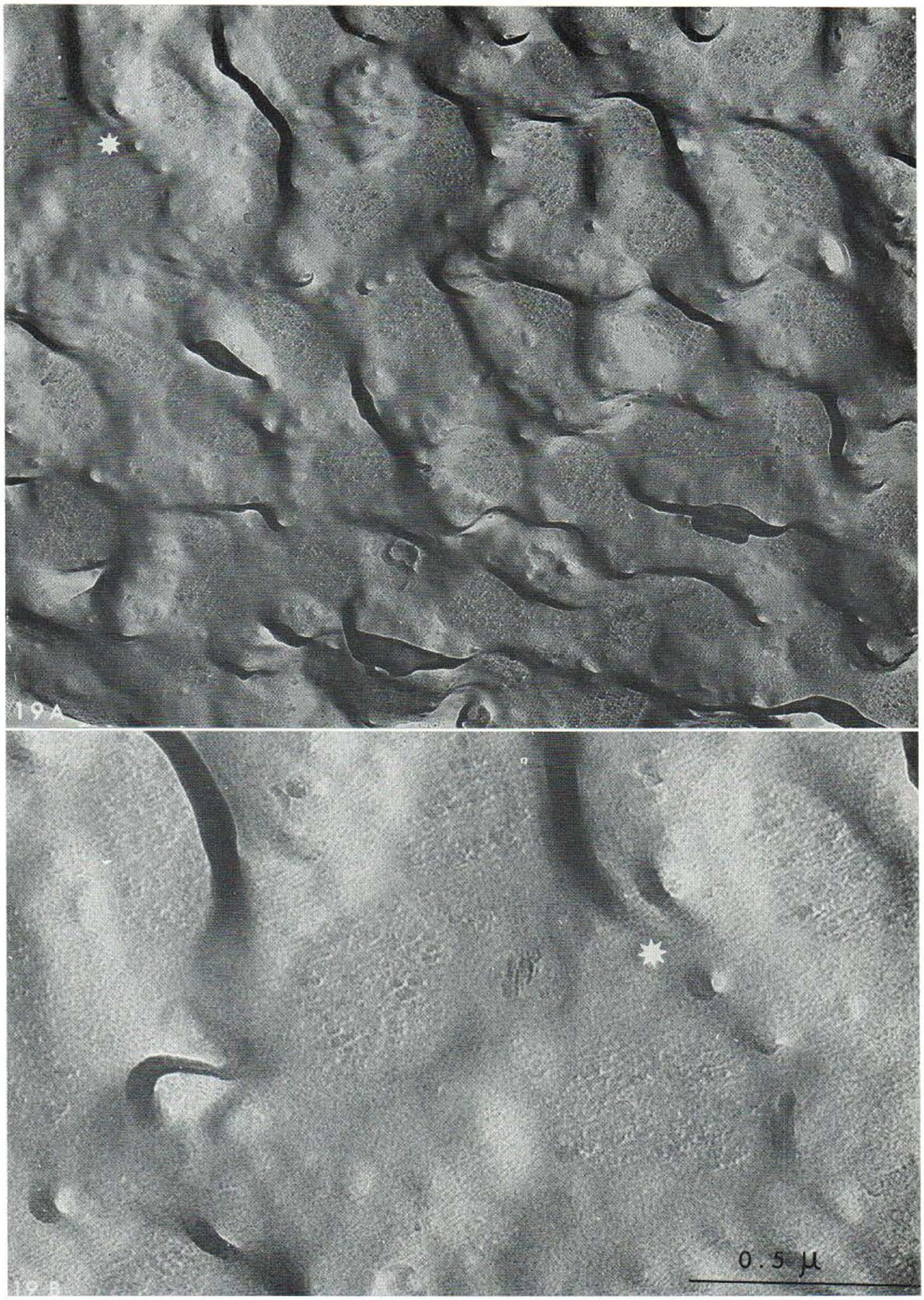



*Fig. 18.* High magnification of a replica of a surface horny cell shows round, oval or elongated macules. Individual macules consist of cobblestone-like particles. These macules measure on average 0.4–0.8 μm in long diameter and are distributed rather uniformly at a distance of 0.3–0.45 μm Reviewing thin sections of upper horny cells of the same specimen (Figs. 17A, 17B, 17C), the size and frequency is quite compatible with residual desmosomes. × 75 600.




For caption to Fig. 19 A, B, see overleaf.

increases the areas of the cell-to-cell junction or sealing. Permeation via intercellular spaces alone, therefore, is a long pathway in which residual desmosomes and intercellular substances might also interfere with the passage. Thus, the peripheral overlapping seems to be an inherent characteristic of keratinocytes in vivo as well as in vitro but the formation of PZD seems to be subject to environmental factors.

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*Fig. 19 A.* Freeze-fractured Malpighian layer of the normal human epidermis reveals desmosomes as oval to elongated macules composed of small particles. Comparing them with the residual desmosomes on the surface (Fig. 18) or between horny cells in upper layers of stratum corneum (Figs. 17 A, 17 B, 17 C), the sizes (0.3–0.8 $\mu$m in long diameter) and frequency (0.3–0.5 $\mu$m distant from each other) of these macules are quite similar. Unlike nexus particles, these desmosomal particles are not in orderly register. × 30 000.

*Fig. 19 B.* Enlargement of a part of Fig. 19 A, the corresponding area was marked by * in both pictures. Figs. 17 A, 18, 19 B are of the same magnification: compare the average dimensions of desmosomes and confirm the similarity. General configurations and particle sizes of desmosomal macules are strikingly similar between freeze-fractured desmosomes (Fig. 19 B) and separated desmosomes on the surface horny cell (Fig. 18). × 75 000.

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