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Plasma Membranes in Psoriatic Cells. A Freeze-fracture Study

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A freeze-fracture study of affected and unaffected psoriatic skin has demonstrated the presence of marked modification of the plasma membrane in the psoriatic lesion. In the lower layers of the epidermis, an increase of membrane associated particles was observed in many keratinocytes, possibly representing the morphological intramembranous equivalent of changes in the outer cell membrane demonstrated with cytochemical techniques. Furthermore, in the malphighian layer, numerous gap junctions have been found, which may be interpreted as a phenomenon compensating the uncontrolled proliferation, and may represent a point of differentiation between cell proliferation in psoriasis and neoplasia. This technique confirmed the poor tendency to adhesion of keratinocytes in extrajunctional areas, which had already been shown by other morphological techniques.

Recent investigations on psoriasis [1–7] apparently suggest a membrane defect of keratinocytes as one of the most important pathogenetic events of the disease. Biochemically, this defect seems to consist of a deficiency of the adenylcyclase, ATPase and acid monophosphoesterases activities which are involved in membrane mediated growth control mechanisms [7]. The ultrastructural study of plasma membranes of psoriatic lesional keratinocytes [1,3,6,8] has up to now revealed an inability to produce a complete glycocalyx, a decreased adhesiveness of extradesmosomal membrane regions and their microvillous transformation. In order to acquire a deeper knowledge of the inner structure of cell plasma membranes and their specializations in this disease, we have used the freeze-fracture technique, which is especially suitable for obtaining this kind of information.

MATERIALS AND METHODS

Specimens of affected and unaffected skin were studied in 7 cases of untreated patch psoriasis in subjects aged 20 to 45 yr. In all the patients, biopsies were performed on trunk lesions, at the center of the patch. Control biopsies were carried out on trunk areas which had never been affected by the disease. Trunk skin bioptic fragments from healthy volunteers were also used as controls. The changes undergone by membranes in epidermal cells of psoriatic lesions were compared with unaffected epidermal cells of psoriatic subjects and with normal epidermal cells.

Punch biopsies of affected and unaffected skin of 7 cases of untreated psoriasis were fixed with 3% glutaraldehyde in phosphate buffer 0.12 M, pH 7.3, for 2 to 3 hr at 4°C and then infiltrated with increasing concentrations (10%, 20%, 30%) of glycerol buffered with phosphate 0.12 M, pH 7.3. The samples were frozen by immersion in Freon 22, cooled to −150°C in liquid nitrogen and freeze-fractured according to the method of Moor et al [9] in a Balzer freeze-etching device. Psoriatic skin specimens and unaffected psoriatic skin controls from a same patient were fractured during the same run. The fracturing temperature was −115°C. Platinum carbon replicas were washed in sodium hypochloride to remove the organic material and then in distilled water, and recovered on 200-mesh copper grids. The replicas were examined with Philips EM 200 and EM 300 electron microscopes.

Calculation of the Number of Membrane-associated Particles

The number of particles per μ^2 of membranes was calculated on photographs printed at constant magnification (× 54,000). 20 photographs of each type of membrane from the bioptic fragments of the 7 patients were chosen at random. In order to minimize errors due to curvatures of the various structures under study, only relatively plane portions not close to junctions were considered. The number of particles was determined by direct counting.*

RESULTS

Psoriatic Unaffected Skin

In the replicas obtained from psoriatic unaffected skin, no significative changes were found in plasma membranes and their specializations, as compared to normal skin. In particular, both the basal layer and the spinous layer cells exhibited a homogeneous density of membrane associated particles (map) on the P face of 728 ± 26 particles per μ^2 (Fig 1*a*). Cells in contact with dermal structures (collagen fibers and elastic fibers) containing filament particles (tonofilaments) in the cytoplasm, and exhibiting plasma membranes rich in pinocytotic vesicles were regarded as basal layer cells [10]. We regarded as spinous layer cells those located above the basal layer, characterized by a roundish nucleus, filament particles rich cytoplasm with lamellar bodies in the most superficial layers, and with plasma membranes rich in desmosomes but exhibiting no pinocytotic vesicles.

Psoriatic Affected Skin

In the basal and spinous layers, many cells with a significant increase of map on the P faces were observed (Fig 1*b*). On an average, the P faces of these psoriatic lesion keratinocytes have 2478 ± 125 particles per μ^2 whereas the P faces of the membranes of normal skin keratinocytes have 646 ± 40 particles per μ^2. The ratio is therefore about 4:1. In the spinous layer, gap junctions which in normal and unaffected psoriatic skin are quite small (max diameter 0.5 μ) and few (no more than one per cell), were numerous on the membrane of a same cell (Fig 2 and 3), even 6 within 10 μ^2 (Fig 3) and often fairly big (over 0.8μ in diameter, Fig 4). Cells rich in gap junctions were normally among those exhibiting a marked increase in map (Fig 1*b*). In the cytoplasm of some keratinocytes, we observed also vesicles whose membranes exhibited a map arrangement practically identical with that of gap junctions. There was no apparent continuity between these structures and the plasma membrane (Fig 5). The structure and number of desmosomes appeared normal, while intercellular spaces were often dilated in the extrajunctional areas: sometimes gap junctions could be observed in the sites corresponding to the areas of contact between membranes (Fig 6). In the transitional layers, the above phenomenon was even more evident, so that the plasma membrane acquired a villous appearance (Fig 7). The apex of villi, which is the contact area between adjacent cells, was always charac-

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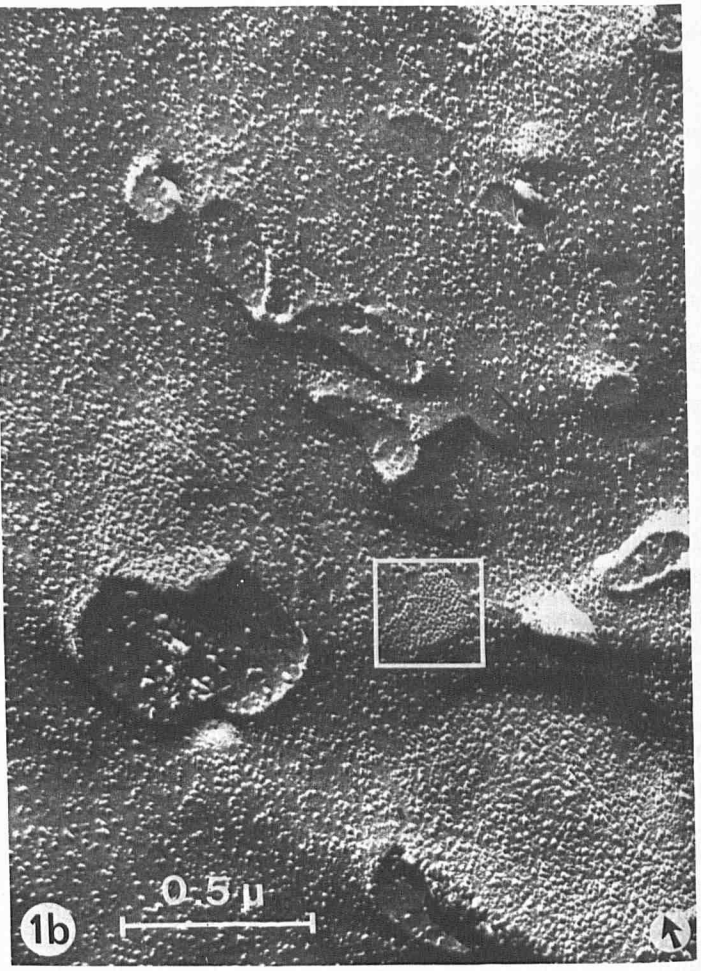
Abbreviation:
map: membrane associated particles

* The membrane faces exposed by fracturing are conventionally designed as P, the half membrane frozen to the cytoplasm, and E, the half membrane frozen to either the extracellular or endoplasmic space.


0·5 µ
1a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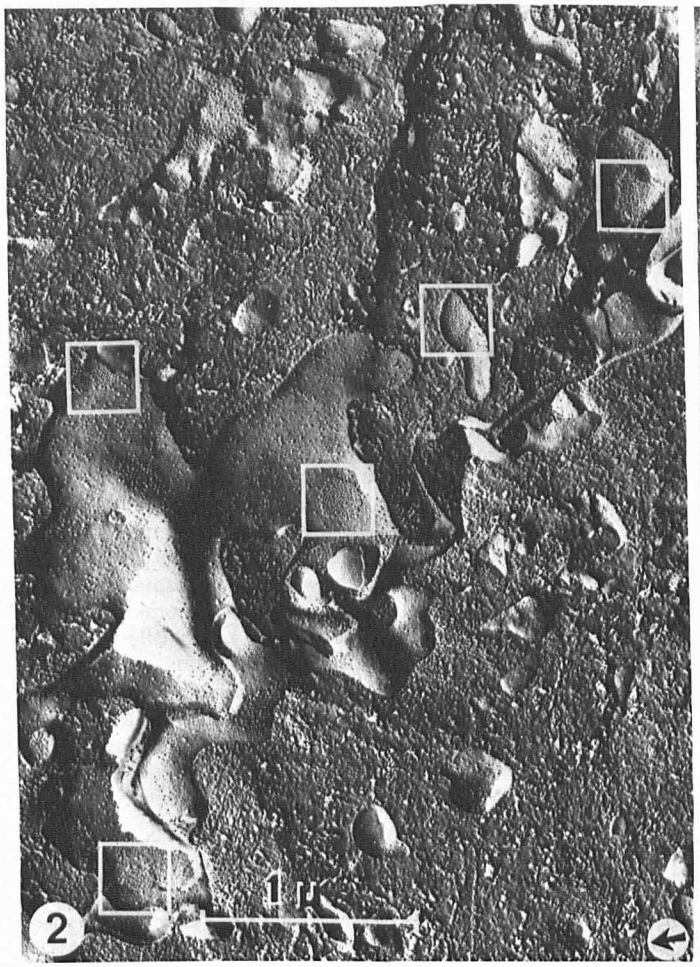
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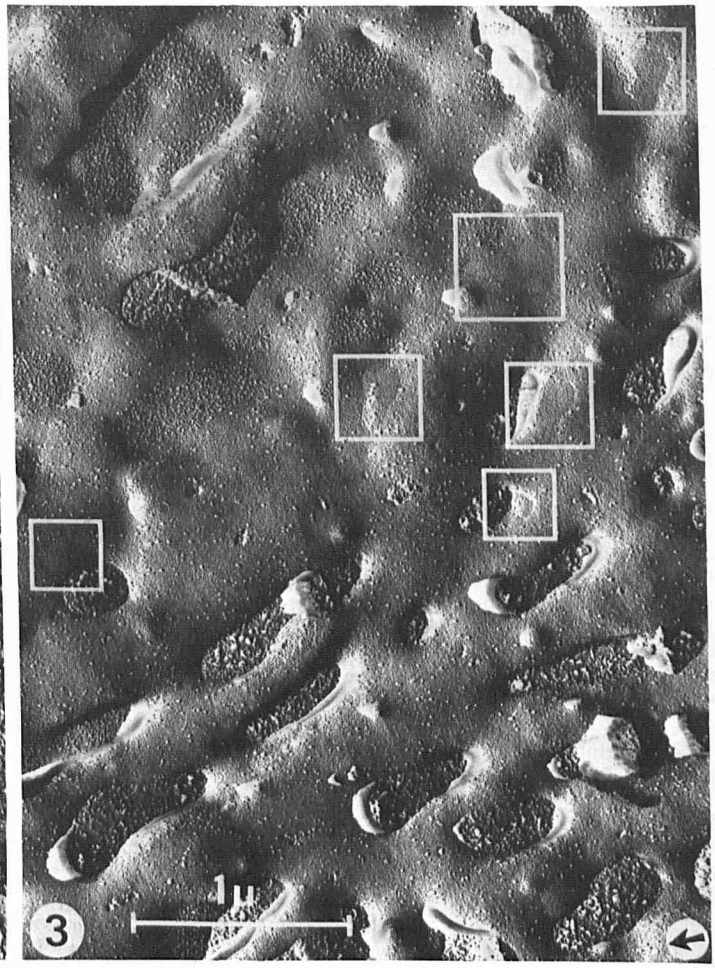
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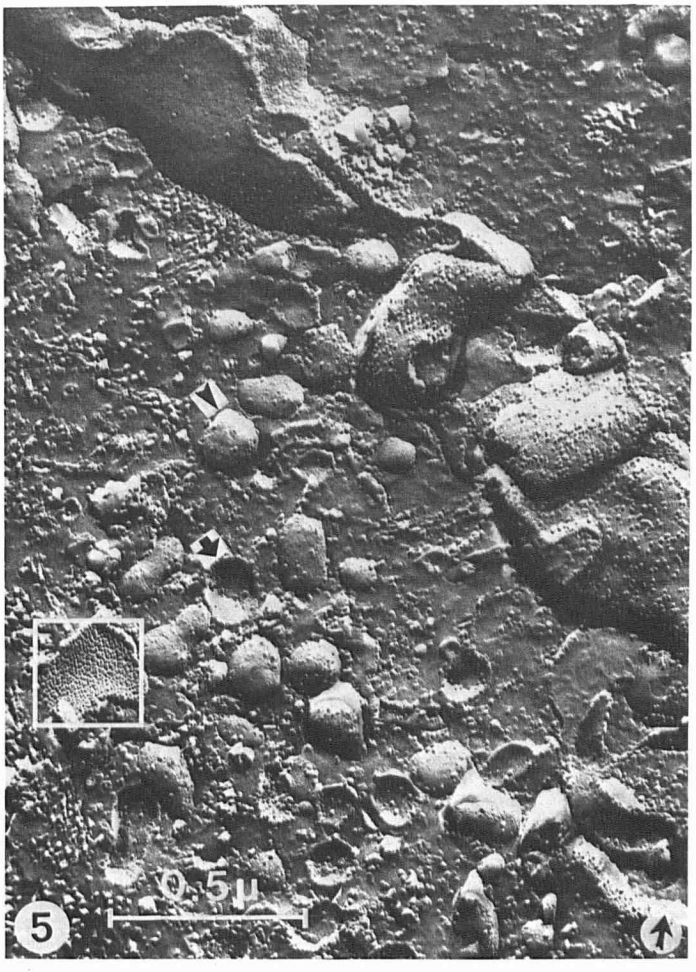


FIG 4. Freeze-fracture replica of the spinous layer of psoriatic skin (P face). Note 5 gap junctions, 3 of which are quite large. *Arrow* indicates the direction of the platinum shadowing (reduced from × 56000).

FIG 5. Freeze-fracture replica of the most superficial layers of the spinous layer of psoriatic skin. In the *square*, an intracytoplasmic gap junction can be observed. The *arrows* indicate lamellar bodies (their P face is concave, while the E face is convex). *Arrow* in the lower right corner indicates the direction of the platinum shadowing (reduced from × 56000).

terized by the presence of a desmosome having a structure similar to that of lower layers. Gap junctions could still be found, while there were no tight junctions. Furthermore, map progressively decreased. In the horny layer, map and gap junctions disappeared: desmosomes had undergone changes similar to those observed in normal epidermis [10], i.e. they appeared as an elevated plaque on the E face and as a cluster of particles on the P face (Fig 8). There was an increase of intercellular material, and several fracture jumps could be seen between the membranes of adjacent cells in the most superficial layers (Fig 9).

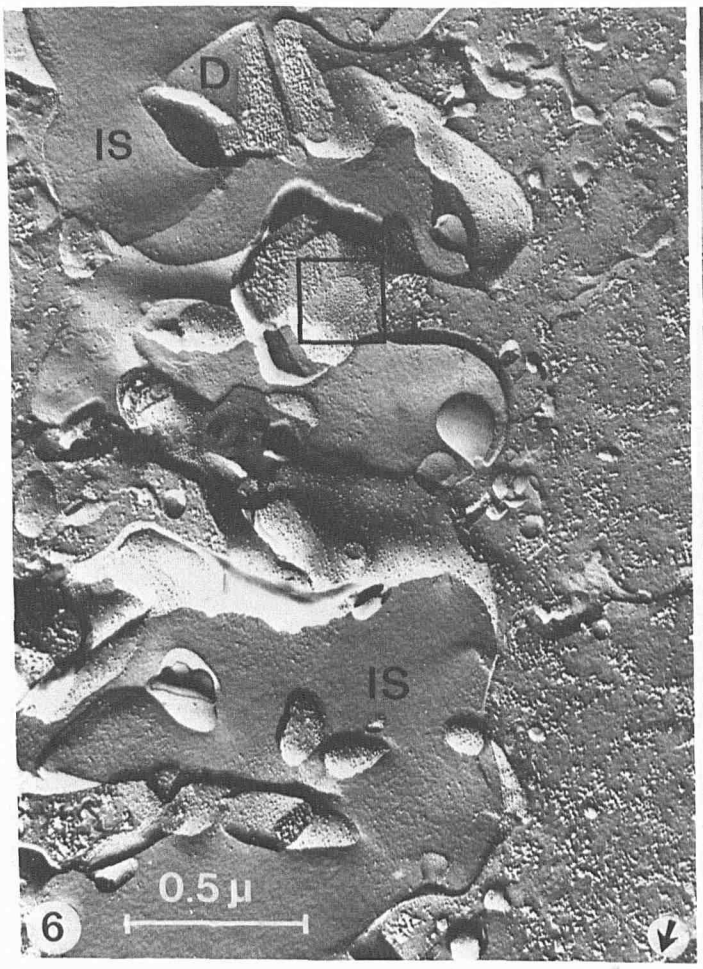
DISCUSSION

The plasma membrane changes revealed by the freeze-fracture technique in psoriatic epidermis appeared to involve mainly the lower layers, where many keratinocytes differed from those of normal epidermis and of unaffected psoriatic skin because of the density of map on the P face. The increase in the number of map of these keratinocytes might be the result of an increased synthesis of membrane proteins or, alternatively, the expression of different aggregation conditions of the protein components of the membranes, correlated to the changes taking place in the outer cell membrane, which have been demonstrated with cytochemical techniques [3,7]. This latter hypothesis, in our view, has more ground since in other diseases, such as lamellar ichthyosis or epidermolytic hyperkeratosis (where the mitotic index is increased [11], no increase in map has been observed (unpublished data). The other important finding was the dramatic increase in gap junctions observed in the membranes of spinous layer cells of the psoriatic lesion. Quantitatively, this increase was shown by a remarkable amount of gap junctions in limited membrane areas of a single psoriatic cell lesion, while in unaffected psoriatic epidermis and in normal epidermis [10] these junctions are rarely found and

FIG 1. *a*, Freeze-fracture replica of the plasma membrane of a keratinocyte of the spinous layer of psoriatic unaffected skin (P face). *Arrow* indicates the direction of the platinum shadowing (reduced from × 54000). *b*, Freeze-fracture replica of the plasma membrane of a keratinocyte of the spinous layer of psoriatic skin (P face). The number of membrane-associated particles is clearly increased. Note a gap junction (*square*) (reduced from × 54000).

FIG 2. Freeze-fracture replica of the spinous layer of psoriatic skin. Note 5 gap junctions (*squares*) in a small area of plasma membrane (P face). *Arrow* indicates the direction of the platinum shadowing (reduced from × 32000).

FIG 3. Freeze-fracture replica of the spinous layer of psoriatic skin. Note the great number of gap junctions (*squares*) on the E face. *Arrow* indicates the direction of the platinum shadowing (reduced from × 32000).



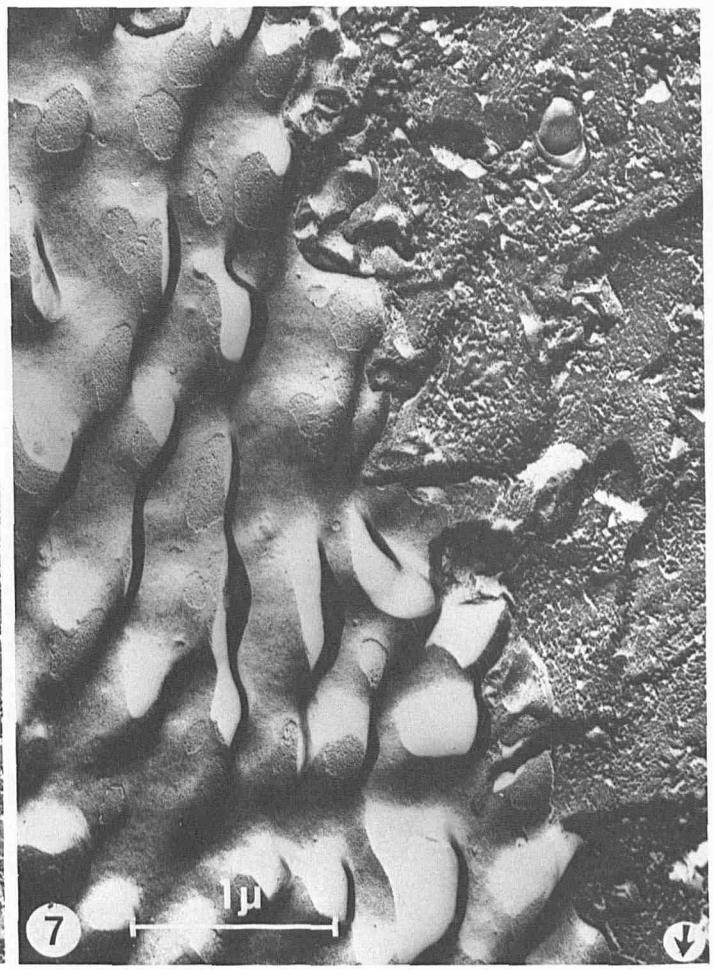


FIG 6. Freeze-fracture replica of the most superficial layer of the spinous layer of psoriatic skin. The intercellular space (*IS*) is dilated. Note a desmosome (*D*) and a gap junction (*square*) in correspondence with the areas of contact between opposite membranes. *Arrow* indicates the direction of the platinum shadowing (reduced from × 54000).

FIG 7. Freeze-fracture replica of the transitional layer of psoriatic skin. The cell membrane becomes irregular because of the presence of villi. *Arrow* indicates the direction of the platinum shadowing (reduced from × 30000).

there is never more than one per cell. Similar findings have been obtained by Mahrle (unpublished data) using extracellular markers (Lanthan Alcian blue method). This gap junction increase leads to intriguing speculations. Many authors [12–16] believe that these junctions may be instrumental in exchanging substances that control cellular growth and differentiation. However, they would merely represent an example of how the cell membrane may be involved in cell control, since other phenomena such as cell recognition and adhesion are potentially important with regard to this function [16]. In malignant epithelial tissue where uncontrolled cell growth takes place, a decrease in the number of gap junctions has been observed [17] although no definitive relationship has yet been demonstrated between deficiency of gap junctions and proliferative activity. Since with traditional techniques it has been suggested [1] that the number of gap junctions was also reduced between the keratinocytes of psoriatic lesions, some authors [1,7] believe that an analogy exists between epidermal proliferation in psoriasis and neoplasia. In contrast, our freeze-fracture investigations have shown a marked increase of gap junctions among the keratinocytes of the spinous layer of psoriatic lesions. This increase might be interpreted as a phenomenon compensating the uncontrolled proliferation observed, and might represent a point of differentiation between cell proliferation in psoriasis and neoplasia. The intracytoplasmic vesicles with gap-junction-like particle aggregation were quite similar to those observed in normal rat keratinized oral epithelium [18], which in replicas represent the equivalent of annular gap junctions of thin sections. On the basis of our images, it is impossible to decide whether these structures are really intracytoplasmic, or whether they are unfoldings of the plasma membrane. The widening of intercellular spaces in extrajunctional areas, and the microvillous transformation of the plasma membranes, confirm the data obtained with traditional techniques [1,8] and with the scanning microscope [1,19,20], thus supporting the hypothesis that the membranes of keratinocytes of psoriatic lesions show little tendency to adhesion. The behavior of membrane specializations in the horny layer apparently resembles that observed in normal epidermis [10]. The finding of numerous membrane jumps in the most superficial layers might be due to the fact that, in these areas, there was an increase of lipidic intercellular material organized in bilayers, presumably originating from lamellar bodies [21–23] so that the fracture plane was not confined to the cell membrane, but could involve the lipidic layers of intercellular spaces. Recent ultrastructural investigations [1,3,6,7,8] seem to support the concept that, in psoriatic lesions, there is a deficiency of contact inhibition of growth, probably as a consequence of a defect of plasma membranes. Actually, with the freeze-fracture technique, we could demonstrate a structural modification of the plasma membrane in many keratinocytes of the basal and spinous layers (increase in map). However, at this moment it is not yet possible to establish the relationship between this membrane modification and the mechanisms controlling cell proliferation. Furthermore, the finding of a marked increase in gap junctions among the keratinocytes of the spinous layer suggests that in this disease

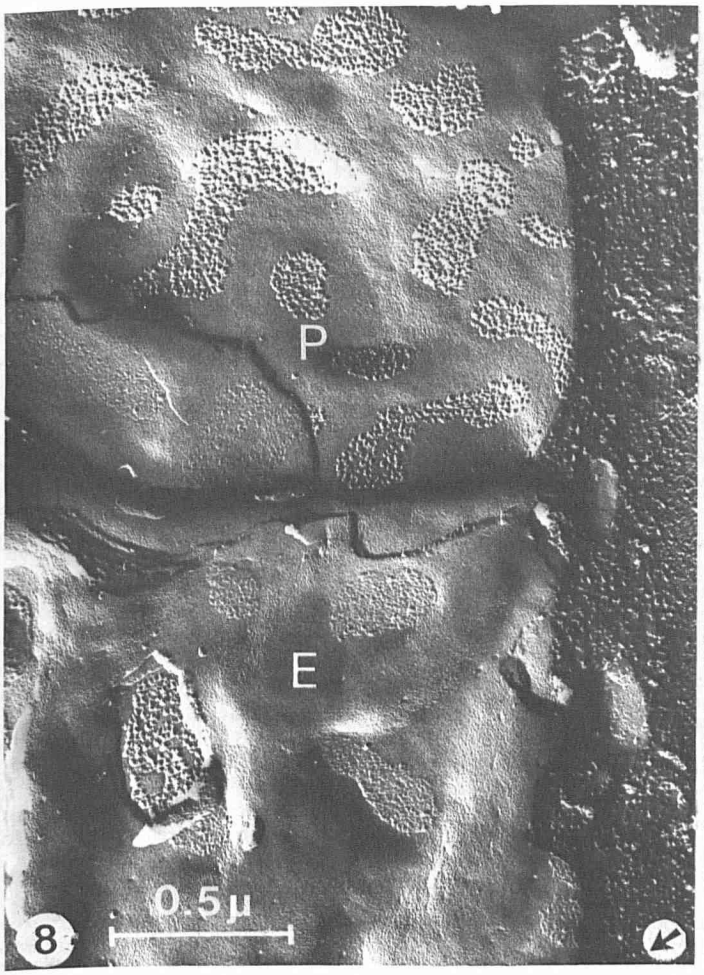


FIG 8. Freeze-fracture replica of the horny layer of psoriatic skin. The desmosomes appear as an elevated plaque on the E face and as a cluster of particles on the P face. *Arrow* indicates the direction of the platinum shadowing (reduced from × 54000).

FIG 9. Freeze-fracture of the most superficial layers of the horny layer of psoriatic skin. Note many fracture jumps (*arrows*) between the membranes of adjacent cells. *Arrow* in the lower right corner indicates the direction of the platinum shadowing (reduced from × 40000).

there are mechanisms able to check the uncontrolled cell growth, contrary to neoplasia where these junctions are usually reduced in number.

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