Acta Derm Venereol (Stockh) 1991; 71: 369–372

Intercellular Lamellar Lipids in Plantar Stratum Corneum

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Plantar stratum corneum was examined by means of transmission electron microscopy after conventional osmium fixation and after fixation with ruthenium tetroxide. The latter fixative was used in order to reveal the possible existence of lamelarly ordered lipids in the intercellular space, as has previously been demonstrated for non-palmo-plantar stratum corneum. A major part of the plantar stratum corneum intercellular space was occupied by extracellular parts of desmosomes. In specimens fixed with ruthenium tetroxide the intercellular space not occupied by desmosomes was found to contain multiple alternating electron dense and electron lucid bands, suggestive of membraneous structures. This pattern appeared to be similar to that previously described for non-palmo-plantar stratum corneum.

It is suggested that the intercellular lipids of palmo-plantar stratum corneum may be qualitatively similar to the intercellular lipids of non-palmo-plantar stratum corneum. The lower lipid content, expressed as weight per unit weight of tissue, in palmo-plantar stratum corneum as compared to non-palmo-plantar stratum corneum may be related to the fact that a larger portion of the intercellular space of the former tissue is occupied by desmosomes. The relatively high water permeability of palmo-plantar stratum corneum implies that desmosomes, i.e. non-lipid regions of the intercellular space, may have a high water permeability and hence could establish a hydrophilic route through the stratum corneum. ***Key words: Stratum corneum lipids; Ruthenium tetroxide; Electron microscopy.***

(Accepted February 27, 1991.)

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The skin surface of palms and soles is subjected to more mechanical stress than skin on other parts of the body. Palmo-plantar stratum corneum is specially adapted to function as a barrier between body interior and exterior at these sites. Palmo-plantar stratum corneum differs from non-palmo-plantar stratum corneum as regards morphology, composition, and water permeability. The fact that some inherited disorders of cornification affect palmo-plantar skin almost exclusively while other diseases spare the palms and soles also suggests differences between the two types of epidermis as regards regulation of proliferation rate, cornification, corneocyte cohesion, and desquamation.

An inverse correlation has been found between the lipid content and water permeability of the stratum corneum of different body sites (1). The transepidermal water loss is highest in palms and soles (2), and the lipid content (expressed as weight lipid per unit weight of tissue) is lowest in palmo-plantar stratum corneum (3, 4). On the basis of findings in certain inherited and aquired ichthyoses it has also been suggested that stratum corneum lipids may have a role in corneocyte cohesion and desquamation (5–7).

We have recently presented evidence that desquamation in palmo-plantar (8–12) as well as non-palmo-plantar (13) stratum corneum involves degradation of intercellular cohesive protein structures. One difference between the two types of tissue, that may imply differences in the mechanisms of cell cohesion and/or desquamation, was also found. Whereas a unipolar, proteinase-dependent cell shedding could be observed when slices of palmo-plantar stratum corneum were incubated in a simple buffer (9), a similar phenomenon could be found with non-palmo-plantar stratum corneum only when the incubation medium also contained a detergent mixture (13). This difference may be related to the higher lipid content of non-palmo-plantar stratum corneum, and a relatively more important lipid contribution to cell cohesion in this tissue. It could also, however, imply a qualitative difference, for instance in the arrangement of intercellular lipids, between the two types of tissue.

In non-palmo-plantar stratum corneum the lipids occur as multiple, intercellular lamellae (14–17). In this report we present evidence of a similar arrangement of lipids in palmo-plantar stratum corneum. We also discuss possible explanations to the low lipid content and the relatively high permeability to water of palmo-plantar stratum corneum.

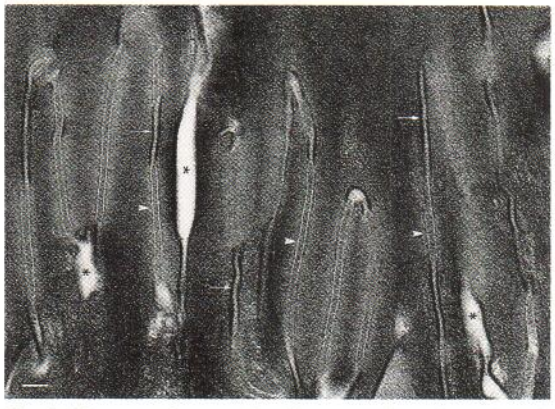

Fig. 1. Electron micrograph of the upper (but still cohesive) part of hyperplastic plantar stratum corneum, osmium fixation. The contact zone between two corneocytes (upper and lower part of the figure respectively) is shown. Note the interdigitating surface extensions. *Arrowheads:* desmosomes, *arrows:* narrow intercellular spaces with no desmosomes and apparently filled with amorphous material. Note the high electron density of the cell envelope at these sites as compared to the parts of desmosomes continuous with the cell envelope. *Asterisks:* wide extracellular space, apparently empty. Bar = 100 nm.

MATERIALS AND METHODS

Ruthenium tetroxide and epoxy resin (Poly/Bed 812) were purchased from Polysciences, Inc., Warrington, PA.

Hyperplastic plantar stratum corneum from the central, weight-bearing parts of heels of volunteers with normal skin was obtained by means of a skin transplantation knife (11).

Before being processed for electron microscopy the tissue was soaked for 1 h at room temperature in phosphate buffered saline, pH 7.5, 0.1% sodium azide. Loosely attached surface cells were scraped off (9). The tissue was prepared for transmission electron microscopy after osmium fixation as described earlier (8). The method for visualizing intercellular lipid lamellae was adopted from Madison et. al. (17). Small pieces of cohesive tissue were homogenized in 0.1 M Tris-HCl pH 8 in a glass homogenizer. The suspension obtained was fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate pH 7.2 for 48 h and then in 0.2% ruthenium tetroxide in the same buffer for 1 h at room temperature. After rinsing in buffer and distilled water the pelleted material was dehydrated with ethanol and embedded in epoxy resin. Ultrathin sections were contrasted with uranyl acetate and lead citrate, then viewed in a Jeol 1200 EX transmission electron microscope at 80 kV.

RESULTS

Fig. 1 shows an electron micrograph of a part of the contact zone between two corneocytes of the upper part of hypertrophic plantar stratum corneum. The interdigitation of the villous extensions of the nearby cells gives rise to an undulating intercellular space which appears to a significant extent to be occupied by the intercellular parts of desmosomes. In this osmium-fixed preparation the parts of the intercellular space that contain no desmosomes appear empty or filled with amorphous material.

Since ruthenium tetroxide penetrates only a few micrometers into plantar stratum corneum it was found necessary to use this fixative on a suspension of homogenized tissue. In this way preservation of intercellular spaces close to the surface of the tissue

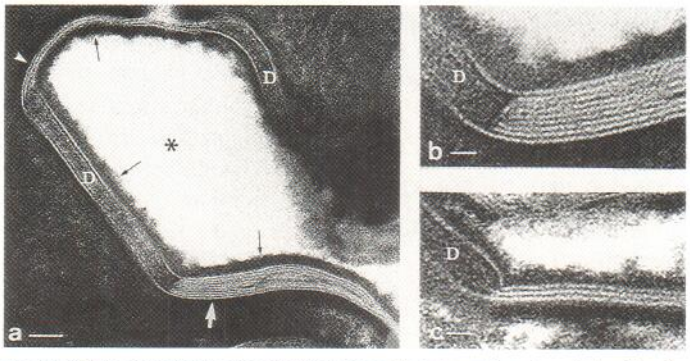

Fig. 2. Electron micrographs of homogenized plantar stratum corneum, ruthenium tetroxide fixation. The micrographs were taken at sites where the tissue had ruptured close to intercellular spaces. *Fig. 2a* shows an area where the intracellular contents of a villous extension of one corneocyte had been removed during the homogenization. *Fig. 2b* shows an intercellular space with multiple alternating electron lucent and electron dense bands (detail from Fig. 2a). *Fig. 2c* shows an intercellular space with two electron dense and three electron lucent bands; the apparent minimum number of membrane-like structures separating two cells at non-desmosomal sites. *D* = desmosome, *white arrow* = wide intercellular space without desmosomes, *white arrowhead* = narrow intercellular space without desmosomes, *asterisk* = empty space due to removal of intracellular material, *black arrows* = periphery of cell with intracellular contents removed. *Bars:* 50 nm (Fig. 2a), 25 nm (Fig. 2b-c).

fragments was made possible. As shown in Fig. 2 ruthenium fixation revealed lamellar structures in those parts of the intercellular space where there were no desmosomes. The number of lamellae appeared to be a function of the width of the intercellular space (Fig. 2b-c). There seemed to be a minimum distance between two contiguous cells however, where the intercellular space was divided into three electron lucent and two electron dense zones (Fig. 2c).

DISCUSSION

The results presented are compatible with the presence of the same type of ordered lipid structures in the intercellular space of hypertrophic plantar stratum corneum as has previously been found in non-palmo-plantar stratum corneum (17). This is corroborated by data presented earlier on stratum corneum lipid compositon (3, 4). Whereas the stratum corneum of the face contained nearly 4 times the amount of lipids per gram of tissue as plantar stratum corneum, the relative contributions of cholesterol, free fatty acids, and ceramides (i.e. the lipid classes believed to be major constituents of the intercellular lamellae (7)) to the total lipids were very similar in the two tissues (4).

We would like to suggest that the smaller amounts of lipids, as expressed in weight per weight of tissue, in palmo-plantar stratum corneum as compared to non-palmo-plantar stratum corneum, may merely reflect the fact that there is less space availabe for intercellular lipids in the former tissue. This in turn may be related to the number of desmosomes remaining in the upper layers of the stratum corneum. In upper non-palmo-plantar stratum corneum about 6% of the cell periphery is occupied by desmosomes; the corresponding number for hypertrophic plantar stratum corneum is about 50% (18). It seems reasonable to assume that a high number of intact desmosomes per unit volume of intercellular space will reduce the volume available for lipid deposition. In addition the mean width of the intercellular space may be smaller in palmo-plantar stratum corneum due to the presence of a high number of intact desmosomes regularly distributed over the corneocyte surfaces. The volume of individual corneocytes may be larger in palmo-plantar than in non-palmo-plantar stratum corneum (19, 20), which would also contribute to a smaller relative volume of the tissue being accounted for by intercellular space.

This way of reasoning leads to the conclusion that the difference in lipid content between palmo-plantar and non-palmo-plantar stratum corneum may be quantitative rather than qualitative. It could explain the sparse symptoms from palms and soles in diseases such as X-linked ichthyosis (21), and also the differences between the conditions under which corneocyte dissociation can be induced in vitro in plantar and non-palmo-plantar stratum corneum (9, 13). Any influence on cell cohesion and desquamation by intercellular lipids could be expected to be more pronounced in non-palmo-plantar stratum corneum.

The higher water permeability of palmo-plantar stratum corneum (2) could thus be a corollary to the presence in the intercellular space of a high number of desmosomes, which could possibly function as "hydrophilic pores".

ACKNOWLEDGEMENTS

The skilful technical assistance of Ann-Louise Green is gratefully acknowledged. This project was supported by grants from the Finsen Foundation, the Edvard Welander Foundation, the Swedish Psoriasis Association, and the Faculty of Medicine, University of Umeå.

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