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The Barrier Function in Atopic Dry Skin

Disturbance of membrane-coating granule exocytosis and formation of epidermal lipids?

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Non-eczematous atopic dry skin (DS) shows an enhanced transepidermal water loss denoting an impaired water permeability barrier (WPB) function. The WPB is formed by intercellular lipid lamellae located between the horny cells of stratum corneum (SC). The lipids are provided via the exocytosis of membrane-coating granules (MCG). By differentiating two dynamic states of MCG, the ultrastructural morphometric comparison of atopic DS and healthy skin of controls revealed a retarded and incomplete extruding mechanism of these organelles. Additionally the structure and spacial organization of the epidermal lipids in DS and healthy skin were visualized and analysed by applying a special primary fixation (acrolein vapour) and postfixation with ruthenium tetroxide. The present findings suggest that some pathologic extruding mechanism of MCG in DS may be responsible, at least partly, for the recently detected biochemical alterations of epidemnal lipids and for the deficient WPB. ***Key words: Atopic dry skin; Transepidermal water loss; Ultrastructure; Membrane-coating granules; Epidermal lipids.***

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INTRODUCTION

Generalized non-eczematous dry skin (DS, xerosis), i.e. rough skin, is a characteristic feature of atopic eczema (AE) (1, 2) that seems to be principally independent of eczematous eruptions (1). DS may persist throughout life, without any additional symptoms of recurrent AE and is accompanied by changes in the biophysical properties of the stratum corneum (SC), especially of the epidermal water permeability barrier (WPB) revealed by an enhanced transepidermal water loss (TEWL) of the non-lesional dry skin (3–7).

At the ultrastructural level, the WPB consists of intercellular lipid lamellae located between the horny cells of the SC. These lipids contain approximately equal quantities of ceramides, cholesterol, and free fatty acids, as well as lesser amounts of non-polar lipids and cholesterol sulfate (8–10). Because of their intercellular localization and their distinctive composition, SC lipids are presumably important not only for the formation of a barrier to transcutaneous water transfer but probably also control desquamation and cohesiveness of corneocytes (11, 12) and the water-retaining function of SC (13). Current studies suggest that these lipids originate largely from polar lipid precursors (phospholipids and glycosphingolipids), presumably derived from membrane-coating granules (MCG, lamellar bodies, Odland bodies, keratinosomes) (12). These organelles fuse with the cell membrane in the upper stratum granulosum and extrude their lipid discs within the intercellular spaces of SC which are than transformed to the intercellular barrier lipids.

Recent studies on non-lesional atopic skin suggest that MCG in DS show a greater relative volume at the level of the granular-cornified layer interface, a finding which has been interpreted as denoting a disturbed maturation of MCG (14). Additionally, DS shows biochemical abnormalities in epidermal lipid metabolism (15), and a reduced ceramide content (16–18). Routine transmission electronmicroscopic techniques, though clearly showing the structure of the MCG, have not been effective in demonstrating the intercellular lamellae of SC and there is no knowledge of the spatial organization of the multilamellar lipids in DS which shows the above-mentioned biochemical alterations.

In the present study the structure of the lipid lamellae in DS was analysed via a modified ruthenium tetroxide (RuO_4) fixation and the role of MCG – particularly concerning the dynamics of the lipid extrusion via the MCG exocytosis – was studied.

MATERIAL AND METODS

Subjects

Non-lesional skin from 9 adult patients with either past or present history of AE and 7 non-atopic control subjects. Both groups had a similar age distribution (22–32 years). The diagnosis of AE was established according to the criteria of Hanifin & Rajka (19) and Diepgen et al. (2). Further requirements for inclusion in the study were a history of persistent dry skin in non-lesional regions, especially in eczematous-free periods, and the absence of eczematous lesions on the lateral aspect of the buttock (site of biopsy). Five patients were free from eczematous eruptions at the time of biopsy. The affected body surface in the other 4 patients ranged between 10 and 22% (chronic flexural eczema).

Skin vapour loss measurements

The measurements of TEWL were performed at the biopsy sites before punch biopsy. For TEWL measurements the Evaporimeter EP1 (Servomed, Stockholm, Sweden) was used under standardized conditions (20) at a constant room temperature of 21°C and a relative humidity of 47 ± 5%.

Morphologic preparations and morphometric analysis

Buttock skin punch biopsy specimens were processed for routine electron microscopy: 2.5% glutaraldehyde (primary fixative), postfixation with 1% osmium tetroxide, embedding in Epon 812. To visualize the structure of the epidermal lipids additionally, some tissue

Abbreviations: WPB, water permeability barrier; DS, dry skin; AE, atopic eczema; ICS, intercellular space; SC, stratum corneum; TEWL, transepidermal water loss; MCG, membrane-coating granules; RuO_4, ruthenium tetroxide; OsO_4, osmium tetroxide.

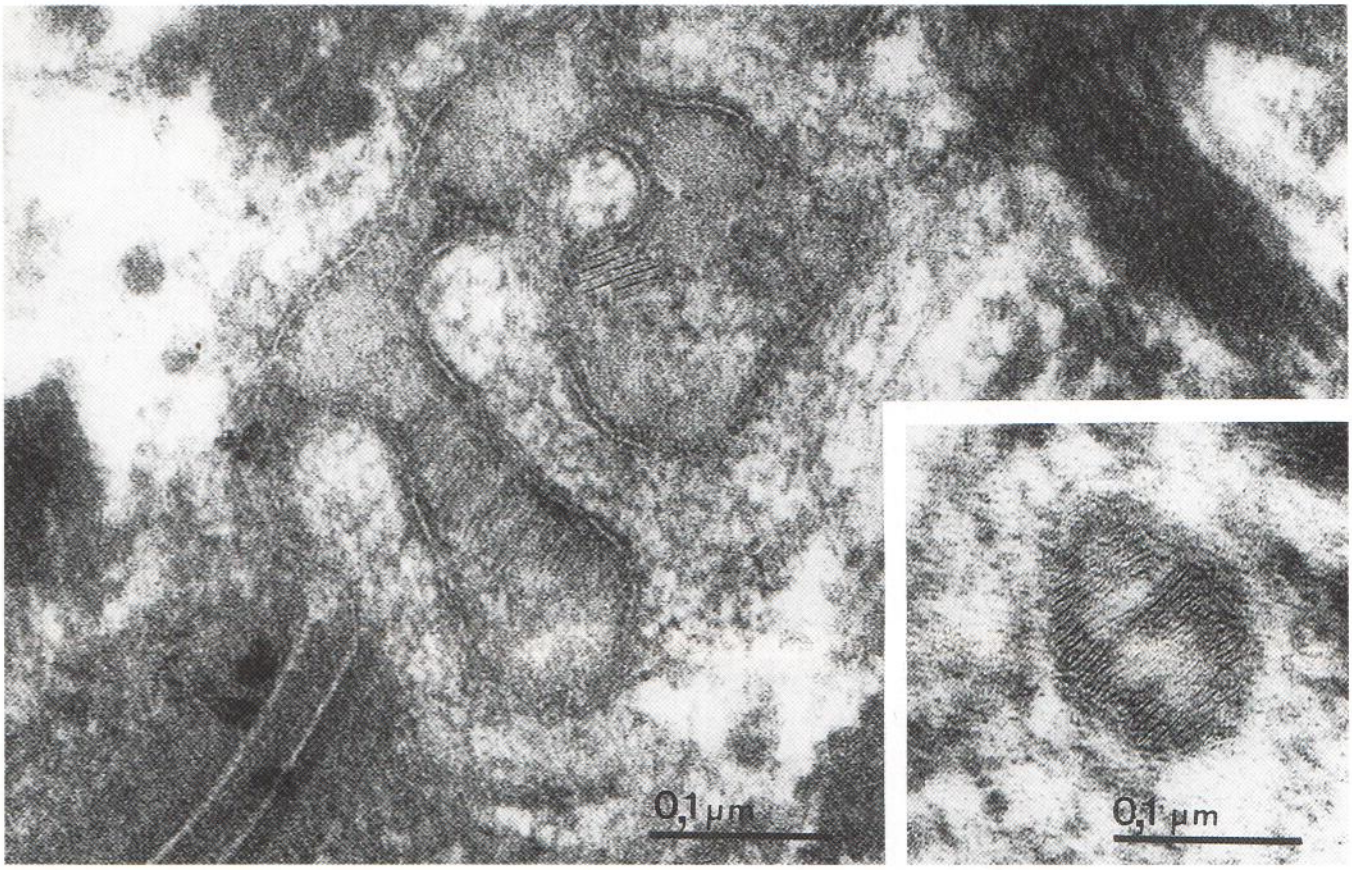

Fig. 1. "Extracytoplasmic" MCG which have already fused with the cell membrane of stratum granulosum cell. *Inset:* "intracytoplasmic" MCG. Glutaraldehyde OsO_4, ×200,000.

samples from the atopics and the control group were also fixed with acrolein vapour and then postfixed with a modified ruthenium tetroxide (0.5%) / potassium hexacyanoferrate (0.25%) staining protocol published elsewhere (21). Electron microscopy was performed with a Jeol 100 CX.

The relative volumes of MCG in the two uppermost subcorneal cell layers of the epidermis (stratum granulosum) were measured by applying standard ultrastructural stereological methods (22, 23). Two tissue blocks from each person were sectioned perpendicular to skin surface. In randomly selected areas of suprapapillar regions of interfollicular epidermis, thin sections were investigated. At least five cells of the two uppermost cell layers of the stratum granulosum were photographed consecutively in slightly overlapping fields at a primary magnification of ×6600. The negatives were printed to give a final magnification of ×66000. Altogether 140 micrographs were taken in each layer and 280 micrographs per patient or control. By fitting the slightly overlapping electron micrographs together, a complete picture of the two granulosum layers (at least 5 cells in each layer) was obtained. MCG were identified by their typical lamellar internal structure and their cell membrane-like coating membrane. Those MCG which were already fused with the cell membrane directly or indirectly via fusion with fused MCG, i.e. by building so-called 'MCG conglomerates', were defined as 'extracytoplasmic' MCG. Those that were still found in the cytoplasm of the cell were defined as 'intracytoplasmic' MCG (Fig. 1).

The planometric analyses were performed by using commercial computer software (Videoplan, Kontron, Bildanalyse GmbH, Munich, Germany).

Statistical analyses

All data are presented as median values. Comparisons between patients and controls were made by the Wilcoxon-Mann-Whitney test. All reported *p*-values are two-tailed.

RESULTS

Skin dryness and TEWL

The atopic group showed a significantly higher mean baseline TEWL value (8.6 g × m^{-2} × h^{-1}) than the control group (5.4 g × m^{-2} × h^{-1}) ($p < 0.01$).

Ultrastructure

In contrast to normal skin where MCG discs disappeared regularly, one to three cell layers above the SG, in the atopic skin MCG discs were focally clearly recognized in higher layers (up to five above SG) within the SC interstices. This was not a persistent but a focal finding. Additionally, undelivered MCG discs in the cytoplasm of some transit cells as well as MCG discs in the horny cell matrix were found, as shown by the modified RuO_4 staining. The intercellular multilamellar lipid sheets with alternating electron-dense and electron-lucent lamellae were visualized at all levels of SC. The different lamellae seemed to have similar thickness. In the lower parts of SC already secreted, MCG-discs and transformed epidermal lipids were visualized simultaneously (Fig. 2A, B). The majority of the extruded discs are arranged in parallel to the plasma membranes of adjacent keratinocytes and were in contact with the lower regions of the desmosomes (Fig. 2A). Especially in the second and third intercellular space (ICS) the MCG sheets formed spindle-like conglomerates. Here the MCG sheets showed an unfurling of their lamellar contents

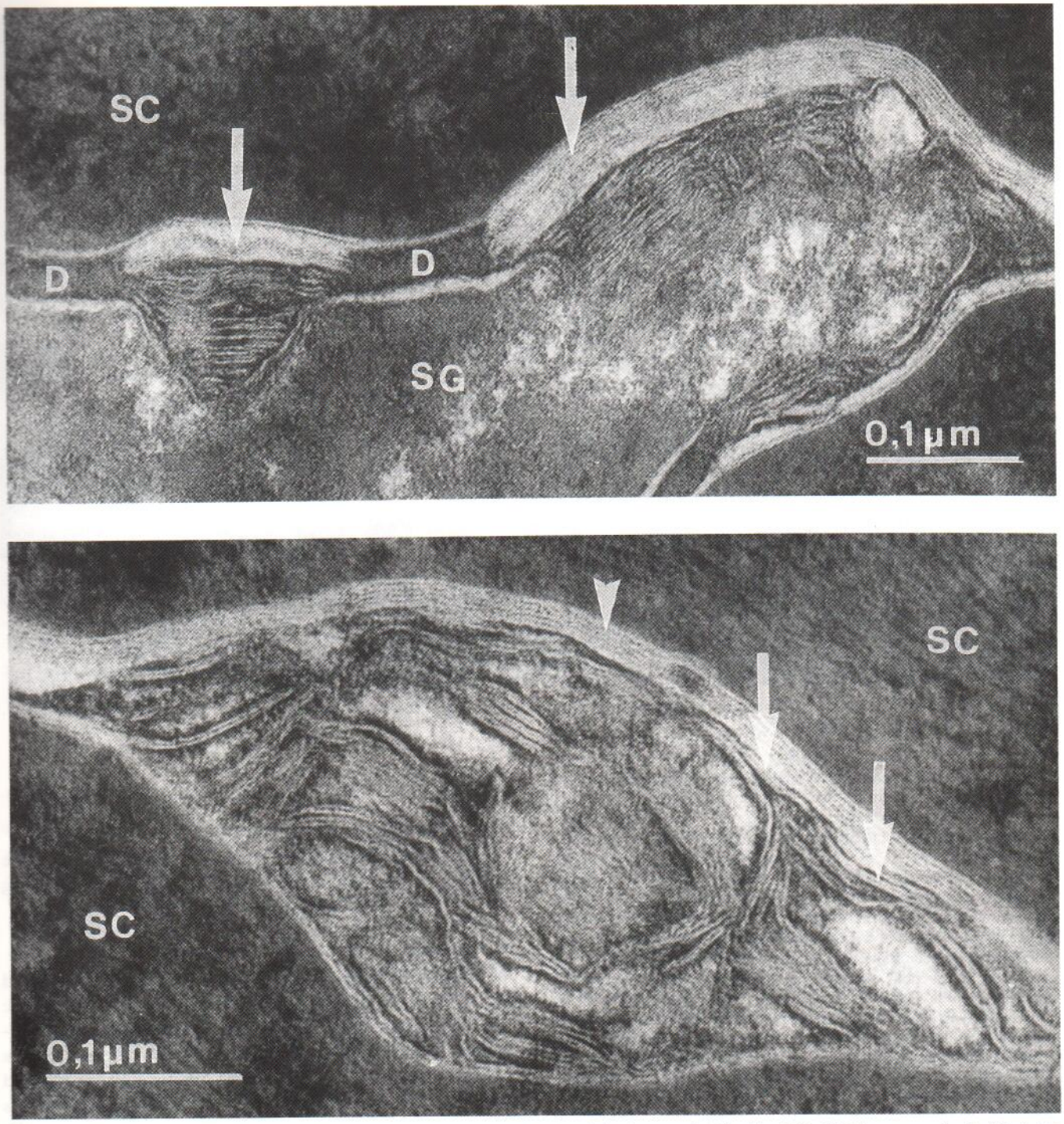

Fig. 2A. Secreted MCG sheets and already formed epidermal lipids are depicted simultaneously. The first ICS of SC is not completely filled with lipid lamellae. The lipid sheets first form triplets with three electron-dense lamellae separated by two electron-lucent lamellae (−>) and are linked to the upper region of the desmosomes (*D*) while the MCG sheets are in contact with the lower part. Acrolein vapour/RuO_4, ×180,000. *Fig. 2B.* Post-secretory, extracellular processing of MCG-derived sheets into the narrow lipid lamellae structures of the SC interstices. Unfurling of MCG sheets. Acrolein vapour/RuO_4, × 230,000.

(Fig. 2B). In the first ICS (interface of stratum granulosum and SC) the epidermal lipids apparently formed triplets with three electron-dense lamellae separated by two electron-lucent lamellae (Fig. 2A). The ICSs of SC were not uniformly filled with lipid layers and the number of the lipid layers varied from place to place and even within one intercellular space. In the lower parts of SC there was a tendency to form 6 to 9 electron-dense lipid layers. In the outer parts, 12 to more than 30 layers were present in one space (Fig.3A, B, C). In the upper stratum compactum (4–5 SC layers) the desmosomes seemed to degrade into less opaque bodies and there was no longer insertion of the lipid layers but the lamellae bulge around these structures (Fig. 3A). Further degradation of the electron-dense portion of desmosomes generated a powdery,

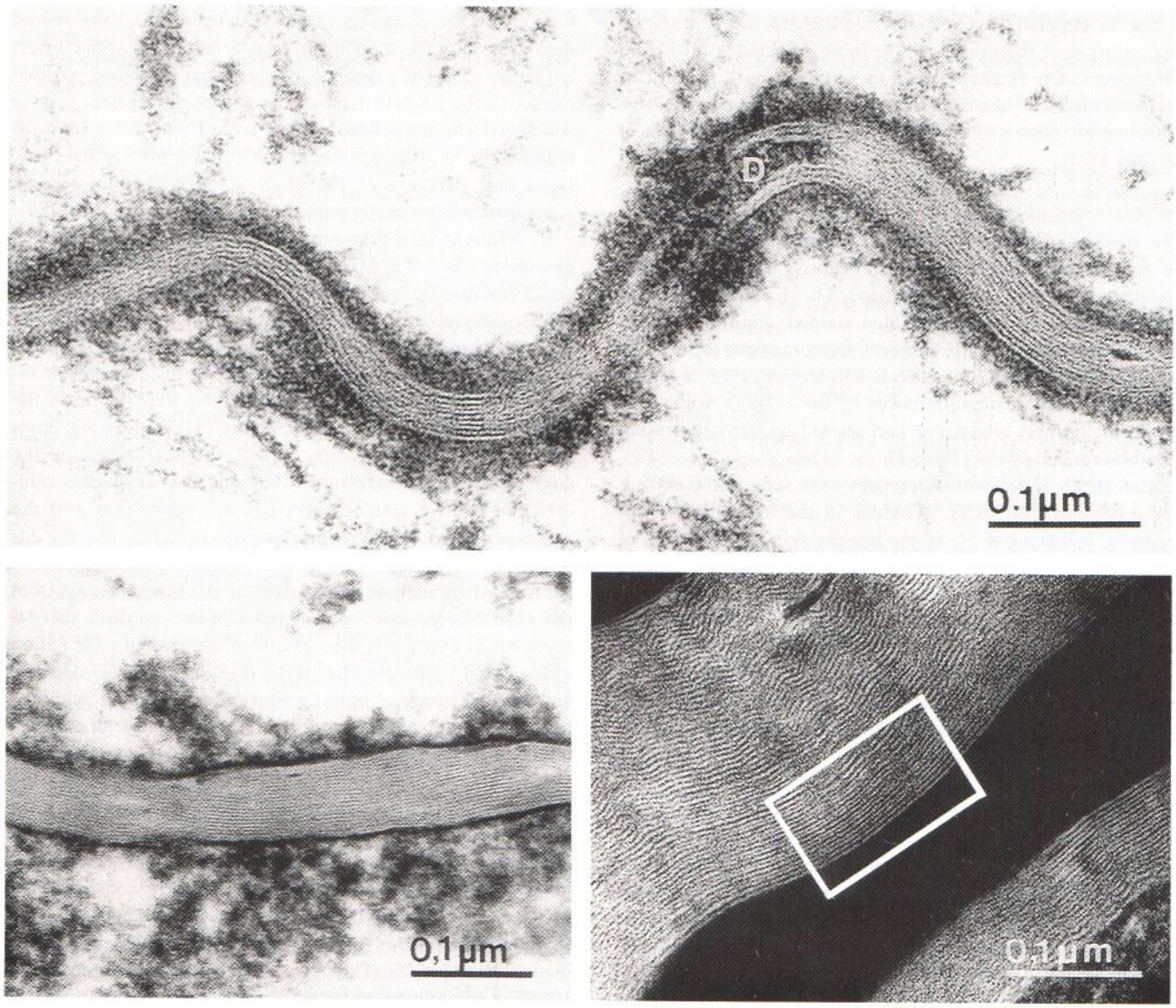

Fig. 3A. In the middle to upper parts of SC most of the desmosomal structures (*D*) are no longer inserted by the lipid lamellae, but the lamellae bulge around. Acrolein vapour/RuO_4, ×185,000. *Fig. 3B.* Numerous electron-dense and electron-lucent lipid layers in the upper SC. Acrolein vapour/RuO_4, ×153,000. *Fig. 3C.* More than 30 layers with alternating electron-dense and electron-lucent lamellae. The corneocytes are surrounded by an electron-lucent lamella complexed to the cornified envelope followed by an electron-dense lamella and a fenestrate-appearing electron-lucent lamella. Then two electron-lucent lamella are divided by an electron-dense lamella. After this triplet, another electron-dense lamella completes the basic unit which is repeated several times. Acrolein vapour/RuO_4, ×170,000.

Table I. *Morphometric analysis: comparison of relative volumes of the two upper stratum granulosum layers in atopics and controls*

Stratum granulosum	Subjects	% volume[a] extracytoplasm. MCGs	% volume[a] intracytoplasm. MCGs	% volume[a] of total MCGs	Percentage[b] of extracytoplasm. MCGs
1st cell layer	*Atopics*	1.34[n.s.]	0.41*	1.75[n.s.]	73.7**
	(*n*=9)	(0.91–2.14)	(0.13–0.70)	(1.07–2.52)	(66.0–88.1)
	Controls	1.94	0.17	2.11	91.6
	(*n*=7)	(0.98–3.56)	(0.04–0.53)	(1.04–3.61)	(84.3–98.7)
2nd cell layer	*Atopics*	0.08*	0.54[n.s.]	0.78[n.s.]	12.9*
	(*n*=9)	(0.00–0.66)	(0.52–1.58)	(0.54–1.58)	(0.0–57.6)
	Controls	0.32	0.57	1.13	41.9
	(*n*=7)	(0.14–1.90)	(0.29–1.36)	(0.69–2.87)	(9.3–74.3)

[a] MCG volume as a percentage of total volume of cell layer: median (range).
[b] Extracytoplasmatic MCG volume as a percentage of total MCG volume.
U-Test of Mann and Whitney: $*p < 0.05$; $**p < 0.01$; n.s.: not significant.

electron-dense debris seen especially between the lipid lamellae of the upper ICSs. On comparing the structure and spatial organization of the lipid lamellae in atopics and controls, no differences were found between those two groups. In contrast to the findings in healthy skin the MCG sheets persisted focally in higher levels of the SC (5–7 ICSs instead of 2–3 ICSs in healthy skin).

Morphometric analyses

The biopsies of atopic skin and of the controls showed no signs of eczema, i.e. parakeratosis, spongiosis, acanthosis or inflammatory dermal cellular infiltrate. The thickness of the SG as determined on methylene blue stained semithin sections was 2–3 cell layers in all 16 subjects. On comparing the relative total volume of MCG in controls and atopics, there were no statistically significant differences in the relative volume of MCG in the first uppermost and second uppermost stratum granulosum cell layers (Table I).

Statistically significant differences were seen in the extruding mechanism of MCG. Whereas in the 'beneath the uppermost' cell layer of SG of the healthy skin, already 42% of the MCG had fused with the apical part of the cell membrane, in the atopic skin only 13% were found adjoining the cell membrane ($p < 0.05$). The difference was even more obvious in the uppermost layer of str. granulosum: In healthy skin, already 92% of MCG had fused with cell membrane as opposed to 74% in the atopic skin ($p < 0.01$). Thus significantly more non-extruded MCG remained undelivered within the uppermost SG cells in the atopic skin.

DISCUSSION

Changes in biophysical properties of the SC, especially of the water-permeability barrier and water-retaining function, seem to be a characteristic feature in the dry non-eczematous skin of patients with atopic eczema (1–7, 17, 24). DS is also seen in chronic irritant dermatitis and in normal subjects without AE (7, 25). It is not known whether the functional aspects of barrier function are similar in these conditions. In the present study, the subjects with AE (5 of them only with a past history of AE) had a higher mean base TEWL than the control subjects. This is in agreement with other studies (3, 6, 7, 17).

There are conflicting data as to the cause of DS in non-eczematous skin of atopics. In earlier studies, some coincidence of AE with the autosomal dominant ichthyosis were discussed (26, 27) as the cause of DS in 30–40% of AE. However, we could show previously that only a few atopics, simultaneously have autosomal dominant ichthyosis (4–6%) and that the dry skin condition in AE is structurally distinguishable from DS in autosomal dominant ichthyosis (28). Moreover we could not confirm an earlier suggestion, that the persistent dry skin of atopics results from subclinical eczema in the majority of the cases. We did find, however, some evidence of disturbed maturation of WPB in atopic non-eczematous dry skin. Whereas most recent studies on the non-eczematous dry skin of atopics have focused on morphological ultrastructural quantification (14), light microscopic description of the epidermis (27) or quantitative biochemical analysis of the lipid contents of the DS (16–18) in the present study, we focused on the dynamics of MCG extrusion and the spatial organization of the transformed epidermal lipids at the different layers of the SC. Analysis of epidermal lipids was possible thanks to the primary fixation chosen, using acrolein vapour which gives better cohesion of SC cells. By post-fixation with modified RuO_4, visualization of epidermal lipids was achieved. Basic unit structure of the lipids and distribution were in accordance with recently published findings in murine SC (29).

By differentiating between MCG already fused with the cell membrane and still MCG intracytoplasmically located, we could visualize both a delayed and an incomplete extrusion of MCG, probably resulting in diminished and delayed delivery of their 'pro-barrier' polar lipids in the intercellular space. Such undelivered keratinized MCG (26% of the total MCG-volume in atopics versus 8% in controls) were found in the horny matrix of DS, while the structure of the epidermal lipids showed no difference compared with the healthy skin of the control group. In contrast to earlier studies (14) which considered the relative volumes of MCG in a narrow area at the granular-corneocyte layer interface we could not find any differences in the relative volumes of MCG between atopics and controls when analysing two adjacent cell layers. Apart from the probable reduction of the polar lipids supplied, the delayed MCG exocytosis may additionally impair the formation of the WPB by further disturbing presumably MCG-dependent processes such as lipid transformation (12) and acidification of the intercellular spaces of the granular-cornified layer interface to the pH optimum of the simultaneously extruded acid hydrolases. Thus the activity of these enzymes might be influenced too (30). Additionally the persistence of MCG-derived discs at higher levels within atopic SC is further evidence of the fact that MCG contents are not processed normally in DS. Such alteration in the orderly conversion of MCG-derived discs to lipid lamellae in SC also could explain the abnormal barrier function and/or the pathologic desquamation of atopic DS. The persistence of MCG contents in the ICSs has recently been described in psoriatic SC too (31).

In conclusion, the modified extruding mechanism of MCG may by responsible, at least partly, for the deficient WPB and the resulting susceptibility of the atopic skin to irritants and infections. It is still not known whether the described MCG alterations are also found in other pathological conditions leading to dry skin. The healthy skin and SC has the ability to regulate disturbed barrier function by increasing lipid synthesis (positive feedback regular mechanism of the TEWL) (32, 33). The unsuccessful attempts of the atopic epidermis to recover and compensate for the disturbed barrier function – especially after additional alteration – may elicit immunological and inflammatory mediators in the epidermis, ultimately leading to clinically visible atopic eczema lesions.

REFERENCES

1. Rajka G. Essential aspects of atopic dermatitis. Berlin, Heidelberg, New York: Springer Verlag, 1989.
2. Diepgen TL, Fartasch M, Hornstein OP. Evaluation and relevance of atopic basic and minor features in patients with atopic

dermatitis and in the general population. Acta Derm Venereol (Stockh) 1989; Suppl. 144: 50–54.
3. Valk van der PGM, Nater JP, Bleumink E. Vulnerability of the skin to surfactants in different groups of eczema patients and controls as measured by water vapour loss. Clin Exp Dermatol 1985; 10: 98–103.
4. Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. Acta Derm Venereol (Stockh) 1985; 65: 102–105.
5. Werner Y. The water content of the stratum corneum in patients with atopic dermatitis. Acta Derm Venereol (Stockh) 1986; 66: 281–284.
6. Thune P. Evaluation of the hydration and the water-holding capacity in atopic skin and so-called dry skin. Acta Derm Venereol (Stockh) 1989; 144: 133–135.
7. Tupker RA, Pinnagoda J, Coenraads PJ, Nater JP. Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis. Br J Dermatol 1990; 123: 199–205.
8. Elias PM, Goerke J, Friend DS. Mammalian epidermal barrier layer lipids: composition and influence on structure. J Invest Dermatol 1977; 69: 535–546.
9. Gray GM, Elias PM. Isolation and lipid biochemical characterzation of stratum corneum cell membrane complexes: irnplications for the cutaneous permeability barrier. J Invest Dermatol 1982; 78: 128–135.
10. Abraham W, Wertz PW, Downing DT. Linoleate-rich acylglucosyl ceramides of pig epidermis: structure determination by proton magnetic resonance. J Lipid Res 1985; 26: 761–765.
11. Elias PM, Williams ML. Neutral lipid storage disease with ichthyoses. Arch Dermatol 1985; 121: 1000–1008.
12. Elias PM, Menon GK, Grayson St, Brown BE. Membrane structural alterations in murine stratum corneum: relationship to the localization of polar lipids and phospholipases. J Invest Dermatol 1988; 91: 3–10.
13. Imokawa G, Kuno H, Kawai M. Stratum corneum lipids serve as a bound-water modulator. J Invest Dermatol 1991; 96: 845–85 1.
14. Werner Y, Lindberg M, Forslind B. Membrane-coating granules in "dry" non-eczematous skin of patients with atopic dermatitis. Acta Derm Venereol (Stockh) 1987; 67: 385–390.
15. Schäfer L, Kragballe K. Abnormalities in epidermal lipid metabolism in patients with atopic dermatitis. J Invest Dermatol 1991; 96: 10–15.
16. Melnik B, Hollmann J, Hofmann U, Yuh M-S, Plewig G. Lipid composition of outer stratum corneum and nails in atopic and control subjects. Arch Dermatol Res 1990; 282: 549–551.
17. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A. Decreased levels of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin? J Invest Dermatol 1991; 96: 523–526
18. Yamamoto A, Serizawa M, Ito M, Sato Y. Stratum corneum lipid abnormalities in atopic dermatitis. Arch Dermatol Res 1991; 283: 219–223.
19. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980; 92: 44–47.
20. Pinnagoda J, Tupker RA, Agner T, Serup J. Guidelines for transepidermal water loss (TEWL) measurement. Contact Dermatitis 1990; 22: 164–178.
21. Fartasch M, Diepgen TL, Hornstein OP. Morphological changes of epidermal lipid layers of stratum corneum in sodium lauryl sulfute induced dry skin: a functional and ultrastructural study. J Invest Dermatol 1991; 96: 6 17–617 [Abstract].
22. Elias H, Hyde DM. A guide to practical stereology. Basel, New York: Karger, 1983.
23. Myking, AO. Studies on the volumetric composition of lymph nodes: problems of efficient sampling and the use of point counting versus digitizer tablets. In: Reith A, Mayhew TM, eds. Stereology and morphometry in electron microscopy. New York, Washington, Philadelphia, London: Ohemisphere Publishing Corporation, 1988.
24. Finlay AY, Nicholls S, King CS, Marks R. The dry non-eczematous skin associated with atopic eczema. Br J Dermatol 1980; 102: 249–256.
25. Piérard GE. What do you mean by dry skin? Dermatologica 1989; 179: 1–2.
26. Uehara M, Hayashi S. Hyperlinear palms. Arch Dermatol 1981; 117: 490–491.
27. Uehara M, Miyauchi H. The morphologic characteristics of dry skin in atopic dermatitis. Arch Dermatol 1984; 120: 1186–1190.
28. Fartasch M, Diepgen TL, Hornstein OP. Atopic Dermatitis – Ichthyosis vulgaris – Hyperlinear Palms – an ultrastructural Study. Dermatologica 1989; 178: 202–205.
29. Hou SYE, Mitra AK, White SH, Menon GK, Ghadially R, Elias PM. Membrane structures in normal and essential fatty acid-deficient stratum corneum: characterization by ruthenium tetroxide staining and X-ray diffraction. J Invest Dermatol 1991; 96: 215–223.
30. Chapman SJ, Walsh A. Membrane-coating granules are acidic organelles which posses proton pumps. J Invest Dermatol 1989; 93: 466–470.
31. Menon GK, Elias PM. Ultrastructural localization of calcium in psoriatic and normal human epidermis. Arch Dermatol 1991; 127: 57–63.
32. Proksch E, Elias PM, Feingold KR. regulation of 3-hydroxy-3-methylglutaryl-coenzyme a reductase activity in murine epidermis. J Clin Invest 1990; 85: 874–882.
33. Grubauer G, Elias PM, Feingold KR. Transepidermal water loss: the signal for recovery of barrier structure and function. J Lipid Res 1989; 30: 323–333.