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Lipids, proteins and corneocyte adhesion

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Summary. Three factors were examined for their relative contribution to corneocyte cohesion in normal adult pig ear: (1) extracellular lipids derived from membrane-coating granules (MCG); (2) corneosomes (modified stratum corneum desmosomes); and (3) corneocyte covalently bound lipid envelopes. Cohesion strength of the outer stratum corneum was measured directly by cohesometry, then altered by removing MCG lipids with solvents of varying potency. Cohesion changes were related to degree of lipid removal and ultrastructural alterations. Trypsin was also used to see if proteolysis of corneosomes promoted squame shedding. Potent solvents increased cohesion in relation to the amount of MCG lipid extracted. Tighter cohesion was due to fusion of the outer leaflets from covalently bound lipid envelopes on adjacent corneocytes. However, lipid envelopes are unlikely to mediate normal stratum corneum cohesion since MCG lipids play a significant anti-cohesive role preventing their apposition. Mild solvents partially removed MCG lipids causing a slight decrease in cohesion compared with untreated samples. This suggests a minor cohesive role for MCG lipids, consistent with maintaining their barrier function. We believe that corneosomes are the major determinant of stratum corneum cohesiveness because, in untreated skin, both cohesion and the number of corneosomes increased from the surface towards the granular layer. Furthermore, corneosome digestion with trypsin induced superficial squame shedding.

Key words: Stratum corneum – Corneosomes – Lipids – Proteins – Corneocyte cohesion

The mechanism by which corneocyte adhesion is maintained in the stratum corneum is still unclear although several hypotheses have been proposed. The problem has been studied in animal systems such as bovine and pig epidermis. In pig ear the stratum corneum is composed of two regions: the compactum comprising the lowest four to six layers, and the disjunctum containing around 14 corneocyte layers [2]. Within stratum corneum, adjacent cells are separated by extracellular lipids derived from membrane-coating granules (MCG). In the "bricks and mortar" theory the extracellular MCG lipids are believed to cement corneocytes together [7, 8].

As keratinocytes migrate from the granular layer into the stratum corneum, desmosomes are structurally transformed into corneosomes [2]. Our recent findings suggest that corneosomes may play a key role in corneocyte adhesion because corneocyte attachment and corneosome distribution correlated morphologically [2]. Compactum packing was relatively tight and corneosomes were dotted all over cell surfaces. In the disjunctum, cohesion was mainly peripheral but corneosomes were decreased in number and confined to overlapping corneocyte edges [2].

Finally, it has recently been observed that corneocytes are surrounded by a lipid monolayer, composed mainly of ceramides, covalently attached to the underlying cross-linked cell envelope by ester bonding. Corneocyte adhesion is proposed to occur via fusion of the outer leaflets of the lipid envelopes from adjacent cells [21].

In order to understand the nature of adhesion defects in desquamatory disorders, it is essential to know what mechanism(s) govern corneocyte attachment and dishesion in normal epidermis.

Our strategy was to measure cohesion strength of the outer stratum corneum directly by cohesometry, then to see how cohesion was affected by the removal of extracellular lipids with a range of organic solvents varying in their potency. Extracts were subjected to lipid analysis so that changes in cohesion could be related to the degree of lipid removal. Samples from each treatment were examined ultrastructurally to correlate morphological alterations with cohesion changes. To determine whether squame shedding could be induced by proteolysis of corneosomes, skin fragments were digested with trypsin.

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Materials and methods

Material

Pig ears were obtained fresh from a local abattoir, carefully rinsed under running tap water, patted with paper towel and air dried for 15 min.

Cohesometry

These experiments were undertaken using a cohesometer made by Unilever Research (Port Sunlight). At the start of each reading, one drop of cyanoacrylate glue was applied to a 6-mm-diameter cohesometer stub (area 28.3 mm^2). The stub was mounted at the end of a piston and pressed on the skin at a force of 50 g for 10 s, after which the cement was allowed to set for a further 10 s. The piston was then raised with a small motor and the force required to strip off the outer stratum corneum measured using a dial gauge calibrated in g. A minimum of three ears were used for each solvent treatment (described below), and at least six measurements were taken per ear.

The strength of cohesion at various depths within untreated stratum corneum was measured by performing ten serial cohesometric readings at the same site. A total of eight sites were repeatedly stripped, four on each of two ears. In addition, to determine both the number of corneocyte layers remaining, and whether cleavage was extra or intracellular, replicate sites were also stripped, excised and processed for electron microscopy. To establish the number of corneocyte layers removed with each strip from both treated and untreated specimens, glue-coated Epon blanks were attached to stubs, cohesometry performed and the blanks detached and examined ultrastructurally.

Statistical analysis of data was performed using one-way analysis of variance.

Lipid extraction for cohesometry

Ears were immersed to a depth of 6 cm in 150 ml of solvent for 2 h at room temperature in a fume cupboard. Cut ends remained above the fluids ensuring only topical exposure. This approach provided the maximum surface area for cohesometry. Eight Analar grade solvents were used either alone (chloroform, ethanol, methanol, butanol, isoamyl alcohol) or combined [butanol/chloroform (1:1), methanol/chloroform (1:1), and ethanol/chloroform (1:1)].

Lipid analysis

All glassware was soaked in detergent overnight and thoroughly rinsed in distilled deionized water. To relate the amount of lipid extracted to the treated site area, extractions were performed using 10 ml of each of the eight solvents (described above) applied in glass cylinders (area 9.62 cm^2) for 2 h. Evaporation was prevented by sealing the cylinders with polythene-lined rubber bungs.

Lipid standards

The following lipid classes were located by thin-layer chromatography (TLC), using standard markers (Sigma, Dorset, UK) which appear in parentheses: cholesterol esters (cholesterol oleate), triglycerides (triolein), free fatty acids (linoleic acid), cholesterol (cholesterol), ceramides (ceramides), glucosyl ceramides (glucosyl cerebrosides), cholesterol sulphate (cholesterol sulphate sodium salt), squalene (squalene), phospholipids (sphingomyelin, phosphatidylcholine, phosphatidylethanolamine).

Thin layer chromatography

Extracts were evaporated to dryness in a stream of dry nitrogen on a warm hot-plate and redissolved in 200 µl of 2:1 chloroform/methanol. Then 5-, 10-, 15- and 20-µl aliquots were applied to a 0.25-mm-thick layers of silica gel G precleaned by eluting with 2:1 chloroform/methanol. Experiments with the lipid standards revealed that all the major classes of skin lipids could be separated in a one-dimension two-phase solvent system. The first phase was chloroform/methanol/ammonia (2 *N*) (80:20:2) run three-quarters of the way up the plate, and then dried in a fume cupboard for 10 min. The second phase was diethyl ether/hexane/acetic acid (15:85:1) run to the top. Plates were dried at 110° C for 15 min, cooled, sprayed with 50% sulphuric acid and charred at 220° C for 15 min. Chromatograms were quantified using a Zeiss photodensitometer.

Gas chromatography

To 10 µg of *n*-heptadecane, the internal standard, was added 0.5 µl of the initial 10-ml lipid extracts. Following solvent removal, samples were derivatized using 10 µl *n*-O-bis(trimethylsilyltrifluoroacetamide) at 70° C for 5 min and redissolved in 50 µl hexane. A 1 µl sample was injected onto the column of a Hewlett Packard 5890 gas chromatograph equipped with an automatic integrator. A 12 m, 0.22 mm diameter BP-1 quartz column was used at a starting temperature of 50° C increasing at 10° per min until 350° C was reached. The start and end times were 2 and 5 min, respectively, with a total run time of 37 min. Response factors of cholesterol and *n*-heptadecane were shown to be identical. The presented data are the results from one experiment.

Sequential digestion with protease and solvent

To examine the effect of protease attack on the stratum corneum, 1×2 mm dermatome skin slices (0.5-mm-thick) were totally submerged in trypsin (1 mg/ml) in 0.9% saline at 37° C.

In an attempt to remove both extracellular lipid and protein, trypsinized skin slices were totally submerged in solvent (1:1 methanol/chloroform). Conversely methanol/chloroform treatment was followed by trypsinization. All incubations were for 2 h.

Electron microscopy

Treatment effects were monitored ultrastructurally using excised material. To determine whether surface cells were lost during extraction or processing for electron microscopy the skin surface of some specimens was marked with indian ink (a colloidal carbon source) before treatment. All specimens were fixed for 2 h with 2% formaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer pH 7.2, containing 0.05% calcium chloride, post-fixed with 1% osmium tetroxide (2 h), dehydrated in ethanol (70 – 100%), cleared in propylene oxide, and Epon embedded. Ultrathin sections (60 nm), stained with 5% uranyl acetate and Reynolds lead citrate [17], were viewed in an AEI Cora or Philips EM300 transmission electron microscope.

Results

Normal untreated ears: sequential stripping at the same site

The first strip required a mean force of 17.8 g to tear the 6-mm-diameter stub from the untreated skin surface

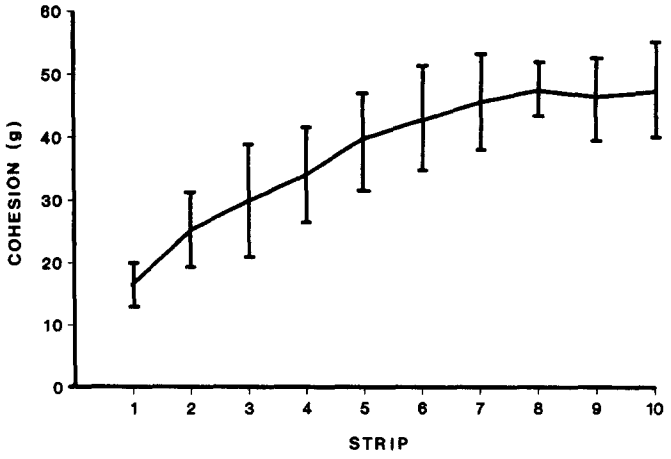

Fig. 1. Sequential stripping on the same site. Cohesion strength gradually increases from the skin surface downwards through the disjunctum (strips 1–7), plateauing in the compactum (strips 8–10)

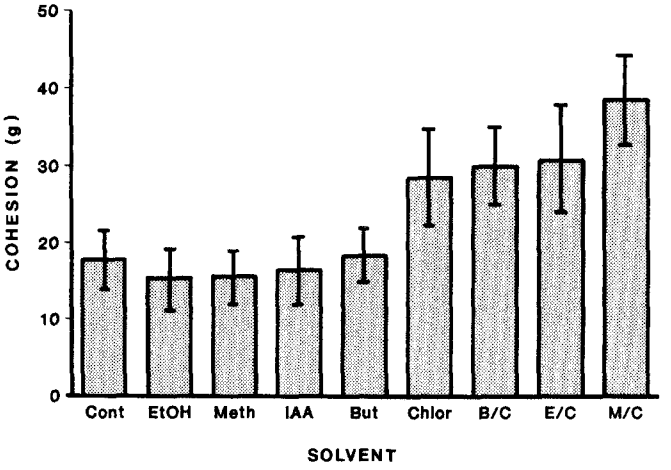

Fig. 2. Solvent effect on cohesion. Control data (*Cont*) is the mean and standard deviation of 59 readings from six untreated ears. For each solvent 26 readings were made on three ears. Solvent treatments fell into 4 groups: (1) ethanol (*EtOH*), methanol (*Meth*); (2) isoamyl alcohol (*IAA*), butanol (*But*); (3) chloroform (*Chlor*), butanol/chloroform (*B/C*), ethanol/chloroform (*E/C*); (4) methanol/chloroform (*M/C*). Within groups one to three there was no statistical difference. Compared with controls group 1 was slightly decreased ($p < 0.05$), but group 2 was statistically similar. Group 3 solvents significantly increased cohesion above controls and groups 1 and 2 ($p < 0.001$). Methanol/chloroform caused a marked increase compared with all other treatments ($p < 0.001$)

(Fig. 1). The initial strip detached two to four corneocyte layers whilst each subsequent stripping removed approximately two layers. Ultrastructural examination of the remaining stratum corneum revealed that strips 1–3 removed the upper half of the disjunctum, 4–7 the lower half of the disjunctum, and 8–10 removed the compactum. The force required to detach corneocytes gradually increased with stratum corneum depth, reaching a plateau in the compactum (Fig. 1).

Cohesometry following solvent extraction

Solvents were ranked in four groups in relation to their ability to increase cohesion. Group 1 solvents (ethanol and methanol), the mildest used, caused a slight decrease compared with controls ($p < 0.05$; Fig. 2). Group 2 (isoamyl alcohol, butanol) did not statistically alter cohesion from control values (Fig. 2). However, chloroform-containing solvents dramatically increased cohesion (Fig. 2). There was no statistical difference between the increased cohesion induced within group 3 solvents (chloroform, butanol/chloroform and ethanol/chloroform), but all were significantly increased when compared with chloroform-free extraction ($p < 0.001$; Fig. 2). Group 4 (methanol/chloroform) caused the largest increase (38.5 g), which was greater than all other treatments ($p < 0.001$; Fig. 2).

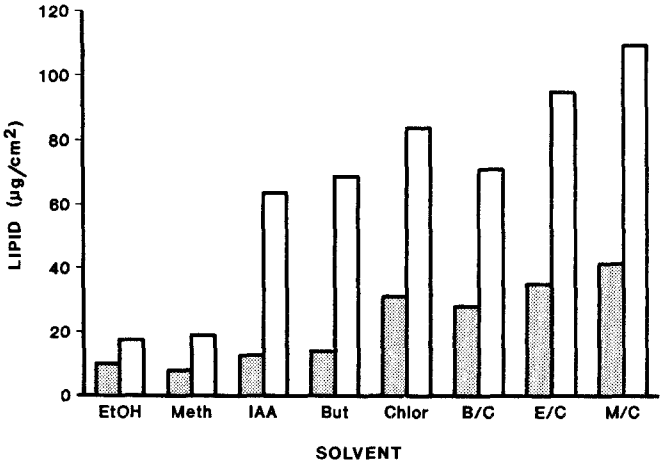

Fig. 3. Gas chromatographic lipid analysis of solvent extracts. Solvents (key as Fig. 2) are ranked in order of their ability to increase cohesion. Both the amount of total lipid extracted (*open bars*) and cholesterol extracted (*shaded bars*) follow a similar pattern to cohesion increase

Lipid analysis

Lipid analysis demonstrated that the strength of cohesion increased with the amount of lipid extracted (Fig. 3). There were no obvious qualitative differences in the types of lipid removed by solvents using either gas chromatography or TLC. However, the quantity of all lipid species increased with the potency of the solvent used (Fig. 3). TLC data indicate that contamination from the viable epidermis and sebum, using phospholipids and squalene as respective markers, was relatively minor but enough to make total lipid analysis an unreliable indicator of stratum corneum lipid extraction. Cholesterol is a more reliable indicator of lipid loss within the stratum corneum since it elutes as a single peak and is a major component of stratum corneum lipid (Fig. 3).

Morphology of untreated stratum corneum

Adhesion, within and between strata, had a tongue-and-groove appearance, with corneosomes "riveting" the cor-

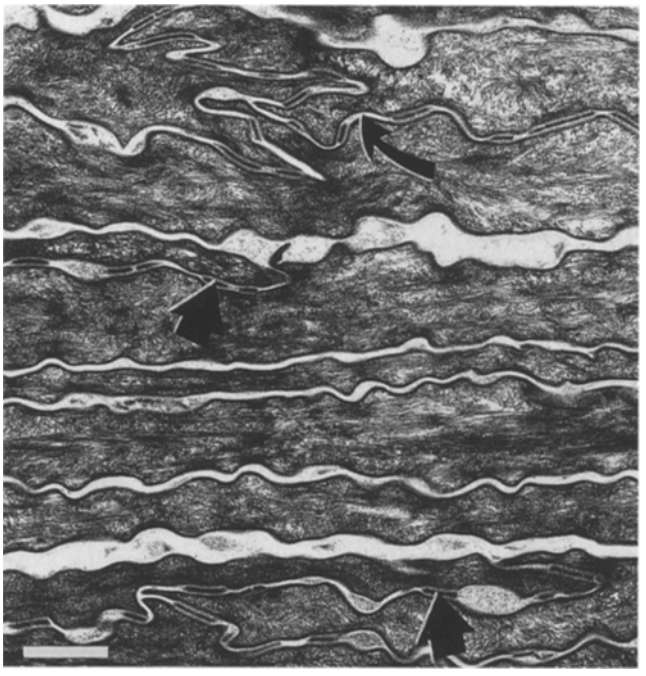

Fig. 4. Mid stratum corneum from untreated epidermis. Corneosomes (*arrows*) are confined to the overlapping edges of corneocytes. Non-peripheral corneosomes are absent. Between corneosomes, cells are separated by a definite space (*curved arrow*). This space is not empty, but contains lipid lamellae which are not visible due to limitations of the staining technique employed. (×22100; *bar*, 0.5 μm)

neocyte periphery into a lipped groove on adjoined cells (Fig. 4). Cells appeared to shed by peeling radially towards the lipped groove. Shedding correlated with the decreased corneosome frequency between the lower and upper disjunctum. MCG-derived lipid lamellae separate neighbouring corneocytes either side of corneosomes. However, in our study the extracellular lipid appeared as clear spaces approximately 10–60 nm wide (Fig. 4), although this material appears lamellate by electron microscopy following ruthenium tetroxide exposure [14].

Morphology of solvent-treated stratum corneum

The morphological effects of the solvents correlated with their potency, which corresponded to the four groups described above. Ethanol and methanol (group 1) caused little change or even a slight widening of the extracellular space (Fig. 5A). However, methanol/chloroform (group 4) extracted virtually all of the MCG-derived lipids leaving a mosaic of very tightly packed corneocytes (Fig. 5C). Tighter packing was due to fusion of the outer leaflets of the covalently bound lipid layers from neighbouring corneocytes, giving a single faint line midway between the corneocyte peripheral envelopes (Fig. 5C, inset). The inner leaflet to inner leaflet spacing of the covalently bound lipid layers was 7.9 nm following the total removal of extracellular MCG lipids (Fig. 5C, inset).

In group 2 (isoamyl alcohol, butanol) approximately 10% of the corneocyte surfaces were tightly bound (7.9 nm spacing). Following exposure to group 3 solvents (chloroform, butanol/chloroform and ethanol/chloroform) approximately 65–75% of corneocyte surfaces were tightly apposed (Fig. 5B).

Regardless of whether or not the epidermis had been exposed to solvent, or regardless of solvent type, the initial strip removed from two to four corneocyte layers. Furthermore, the cleavage plane of corneocyte separation was always confined to the extracellular space as indicated by the presence of the intact extracellular element of corneosomes at the underside of detached cells (Fig. 6). No corneocytes were lost due to processing for electron microscopy or solvent treatment as evidenced by the retention of colloidal carbon at the skin surface in preliminary experiments.

Trypsin

Exposure to trypsin resulted in the patchy loss of superficial corneocytes, visible macroscopically due to the partial removal of colloidal carbon. Ultrastructurally, cell loss varied from none to two layers, and corneosomes had been digested. Below the surface, away from cut edges, corneosomes were intact (Fig. 7A, inset). However, at the cut edges exposed to trypsin all corneosomes were digested (Fig. 7A).

Trypsin followed by methanol/chloroform

The result of this regimen was almost the superimposition of the methanol/chloroform effect on the trypsin digested epidermis. There was partial loss of the outer one or two corneocyte layers, but the remaining cells were tightly bound via interaction of the covalently bound lipid envelopes. Corneosomes were intact below the outer one or two layers and away from cut edges.

Methanol/chloroform followed by trypsin

This treatment had the most dramatic effect. No corneocytes were lost and colloidal carbon remained at the surface, but only cell ghosts existed throughout the stratum corneum (Fig. 7B). Corneosomes were totally absent. Corneocyte ghosts contained nothing but an occasional faint granular deposit, presumably the breakdown remnants of tonofilaments, surrounded by the peripheral envelope. Despite its rudimentary structure the stratum corneum did not disintegrate, cohesion was maintained by the covalent lipid layer visible midway between the peripheral envelopes of adjoined cells (Fig. 7B).

Discussion

The major finding was that removal of the greater proportion of extracellular lipid was followed by a highly

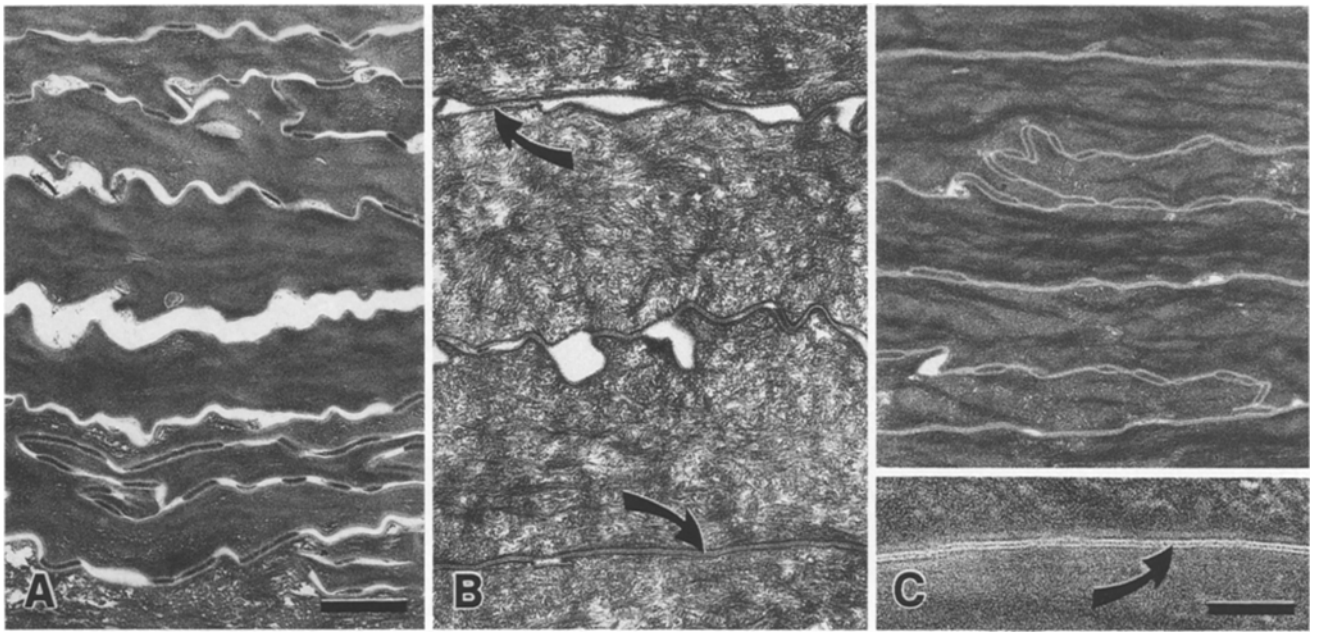

Fig. 5A – C. Mid stratum corneum from solvent-treated epidermis. When treated with a mild solvent, such as ethanol (**A**), the extracellular spaces remain unaltered or are slightly enlarged. However methanol/chloroform (**C**) is a potent solvent system and, in contrast to untreated skin, lipid-filled spaces no longer separate cells. Instead, the outer leaflets of the covalently bound lipid envelopes have fused, forming a single electron-dense line midway between adjoined corneocytes (*inset, arrow*). Extracting with an intermediate solvent system, such as ethanol/chloroform (**B**), causes tight cohesion throughout most of the stratum corneum (*arrows*) but some extracellular gaps are still evident. (×22100; *bar*, 0.5 μm. *Inset*: ×113000; *bar*, 0.1 μm)

significant increase in intercorneocyte cohesion. Moreover, the increase in cohesion following exposure to solvents was found to be related to the amount of lipid extracted from the stratum corneum. Stronger cohesion appeared to be due to the interaction of the covalently bound lipid layers from neighbouring corneocytes [21]. Both were maximal following methanol/chloroform extraction. However, the other chloroform-containing solvents extracted less lipid, and consequently both cohesion and the degree of lipid envelope interactions were correspondingly lower.

The suggestion that the MCG-derived lipid lamellae provide the major cohesive mechanism [3, 19] was not supported by our findings. We found that the MCG lipids played an important anti-cohesive role by acting as a spacer, preventing the interaction of the more adhesive covalent lipid envelopes.

Ethanol and methanol alone caused a small decrease in cohesion and extracted less cholesterol than chloroform-containing solvents. This indicates that MCG-derived lipids do play a minor cohesive role in normal epidermis since their partial loss causes a slight decrease in corneocyte cohesion. By analogy, a piece of bread is more easily stuck to a wall with a generous coating of butter than by a thin layer. This weak MCG-derived lipid cohesion helps maintain the permeability barrier. If the lipid had no cohesive property then adjacent corneocytes would only be attached at sites of corneosomes, ballooning between and offering only a leaky barrier. The minor lipid cohesion prevents ballooning and affords a coherent barrier.

Compactum cohesion is essentially corneosome dependent in untreated epidermis since MCG lipids are only weakly adhesive. However, increased cohesion in the outer stratum corneum, following the removal of MCG lipids with methanol/chloroform, is due to fusion of the covalently bound lipid envelopes. Serial stripping revealed cohesion to be greater within untreated compactum than in the outer stratum corneum following methanol/chloroform extraction. Therefore, the covalent lipid layers are less cohesive than corneosomes.

The proposition that the corneocyte lipid envelopes are a major cohesive element in normal stratum corneum was not supported by our results, since the distance between neighbouring corneocytes is generally too great for interactions to occur between lipid envelopes of adjacent cells [2]. Conceivably, lipid envelope cohesion may be important in pathological situations characterized by abnormal corneocyte retention such as harlequin ichthyosis, in which MCG-derived lamellae are focally [9] or totally absent [4].

Results from the present study indicate that the corneosome is the major cohesive structure within the stratum corneum. Two lines of evidence support this:

(1) Data from sequential strippings at the same site confirm a previous report [12] that the strength of cohesion gradually decreases from the compactum towards the skin surface. This fall-off in cohesion is mirrored by a decrease in the number of corneosomes across the stratum corneum [2].
(2) Exposure to trypsin results in the proteolysis of corneosomes with loss of superficial squames, as reported previously for plantar stratum corneum [6]. Furthermore, in the absence of exogenous protease, cell shedding from stratum corneum in vitro is dependent on the endogenous proteolysis of corneosomal

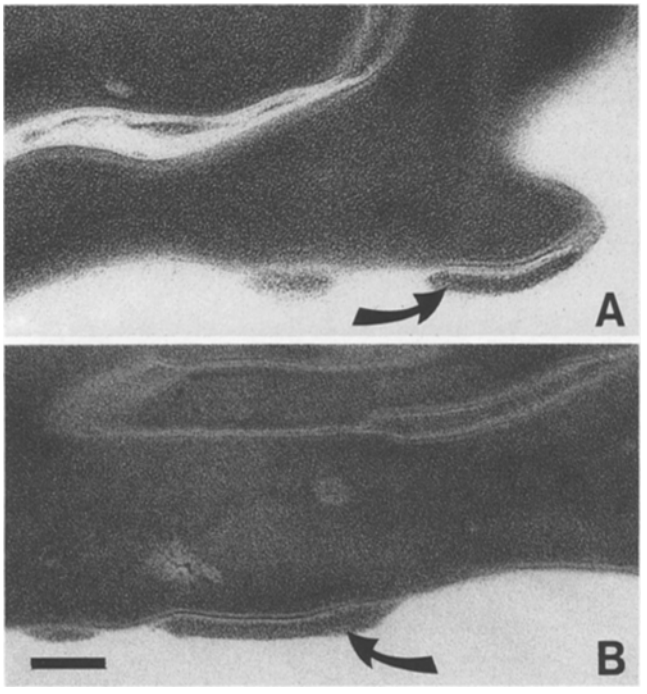

Fig. 6A, B. Cleavage plane following stripping. The split is always extracellular whether the epidermis is untreated (**A**) or treated with a solvent system such as methanol/chloroform (**B**). Arrows indicate the intact extracellular element of corneosomes on the undersurface of cells. ($\times 97000$; *bar*, 0.1 μm)

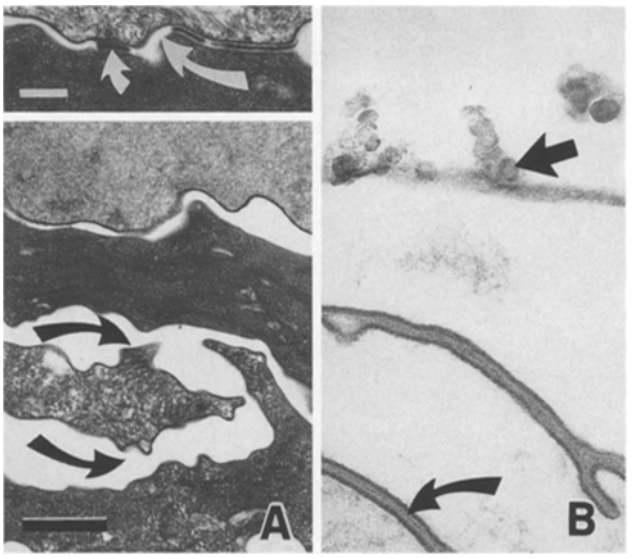

Fig. 7. Trypsin-treated stratum corneum (**A**). At the specimen edges exposed to trypsin corneosomes have been digested (*arrows*). Deeper in (*inset*) corneosomes remain intact (*small arrow*) and, as in untreated material, lipid-filled spaces separate corneocytes (*large arrow*). **B** Exposure to methanol/chloroform, then trypsin. Only cell ghosts remain, cell borders being demarcated by peripheral envelopes. Corneosomes are absent. Carbon particles (*arrow*) at the surface indicate that corneocytes were not lost. Ghost cohesion is maintained via fusion of the outer leaflets of their lipid envelopes (*curved arrow*). ($\times 22500$; *bar*, 0.5 μm. *Inset*: $\times 26200$; *bar*, 0.25 μm)

rotein, a step which is, therefore, potentially important in desquamation in vivo [5, 13].

The involvement of additional cell-surface proteins in corneocyte attachment [1] seems unlikely since freeze-fracture images of the stratum corneum extracellular space reveal smooth surfaces, devoid of characteristic protein projections, except at corneosomes [8, 18].

The trypsin/solvent digestion studies give insight into the possible role of the MCG-derived lipids in corneocyte cohesion and desquamation. Corneosomes are trypsin sensitive since they were digested at the edges exposed to trypsin and superficially where the barrier was imperfect. Retention of corneosomes deeper in the stratum corneum may be due to the MCG-derived lipids affording a physical barrier to the ingress of trypsin. This is supported by the observation that corneosomes are lost when trypsin follows lipid extraction, but unaffected by the reverse procedure. The presence of this physical barrier in vivo may protect corneosomes from the battery of MCG-derived acid hydrolases, including proteases [10, 11], and thus prevent premature desquamation.

In addition to their anti-cohesive role, and providing minor cohesion to maintain the permeability barrier, MCG-derived lipids may also have a third role, namely, modulating desquamation. The degradation of non-peripheral corneosomes in the outer compactum [2] coincides with biochemical modifications of the extracellular lipids [8]. Desquamation may, therefore, be initiated via lipid modulations which expose corneosomes to MCG-derived proteases [8, 16]. In the disjunctum, only edge corneosomes remain [2], perhaps protected by MCG-derived lipids and/or increased glycosylation, reducing susceptibility to proteolysis [15, 20].

The present study indicates that the most important determinant of stratum corneum cohesion is the corneosome. Covalently bound lipid envelopes do not appear to be essential in normal adhesion since their strong cohesion is only evident following lipid extraction. The extracellular MCG-derived lipids may have three roles in adhesion/desquamation: (a) an anti-cohesive function preventing the interaction of the lipid envelopes; (b) to provide weak cohesion to fulfil their barrier property; and (c) the potential to modulate desquamation.

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References

1. Brysk MM, Rajaraman S, Penn P, Barlow E, Bell T (1989) Cohesive properties of terminally differentiated keratinocytes. Exp Cell Biol 57:60–66
2. Chapman SJ, Walsh A (1990) Desmosomes, corneosomes, and desquamation: An ultrastructural study of adult pig epidermis. Arch Dermatol Res 282:304–310
3. Cox P, Squier CA (1986) Variations in lipids in different layers of porcine epidermis. J Invest Dermatol 87:741–744
4. Dale BA, Holbrook KA, Fleckman P, Kimball JR, Brumbaugh S, Sybert VP (1990) Heterogeneity in harlequin ichthyosis, an inborn error of epidermal keratinization: variable morphology and structural protein expression and a defect in lamellar granules. J Invest Dermatol 94:6–18

5. Egelrud T, Lundstrom A (1990) The dependence of detergent-induced cell dissociation in non-palmo-plantar stratum corneum on endogenous proteolysis. J Invest Dermatol 95:456–459
6. Egelrud T, Hofer PA, Lundstrom A (1988) Proteolytic degradation of desmosomes in plantar stratum corneum leads to cell dissociation in vitro. Acta Derm Venereol (Stockh) 68:93–97
7. Elias PM (1983) Epidermal lipids, barrier function and desquamation. J Invest Dermatol 80:44s–49s
8. Elias PM, Menon GK, Grayson S, Brown BE (1988) Membrane structural alterations in murine stratum corneum: relationship to the localization of polar lipids and phospholipases. J Invest Dermatol 91:3–10
9. Fleck RM, Barnadas M, Schulz WW, Roberts LJ, Freeman RG (1989) Harlequin ichthyosis: an ultrastructural study. Dermatopathol 21:999–1006
10. Freinkel RK, Traczyk TN (1985) Lipid composition and acid hydrolase content of lamellar granules of fetal epidermis. J Invest Dermatol 85:295–298
11. Grayson S, Johnson-Winegar AG, Wintroub BU, Isseroff RR, Epstein EW, Elias PM (1985) Lamellar body-enriched fractions from neonatal mice: Preparative techniques and partial characterization. J Invest Dermatol 85:289–294
12. King CS, Barton SP, Nicholls S, Marks R (1979) The change in properties of the stratum corneum as a function of depth. Br J Dermatol 100:165–172
13. Lundstrom A, Egelrud T (1990) Evidence that cell shedding from plantar stratum corneum in vitro involves endogenous proteolysis of the desmosomal protein desmoglein I. J Invest Dermatol 94:216–220
14. Madison KC, Swartzendruber DC, Wertz PW, Downing DT (1988) Lamellar granule extrusion and stratum corneum intercellular lamellae in murine keratinocyte culture. J Invest Dermatol 90:110–116
15. Overton J (1982) Inhibition of desmosome formation with tunicamycin and with lectin in corneal cell aggregates. Dev Biol 92:66–72
16. Rehfeld SJ, Plachy WZ, Hou SY, Elias PM (1990) Localization of lipid microdomains and thermal phenomena in murine stratum corneum and isolated membrane complexes: an electron spin resonance study. J Invest Dermatol 95:217–223
17. Reynolds ES (1963) The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Sci 17:208–212
18. Shimono M, Clementi F (1976) Intercellular junctions of oral epithelium. I. Studies with freeze-fracture and tracing methods of normal rat keratinized oral epithelium. J Ultrastruct Res 56:121–136
19. Smith WP, Christensen MS, Nacht S, Gans EH (1982) Effect of lipids on the aggregation and permeability of human stratum corneum. J Invest Dermatol 78:7–11
20. Walsh A, Chapman SJ (1990) Sugars protect desmosomal glycoproteins from proteolysis. Br J Dermatol 122:289
21. Wertz PW, Swartzendruber DC, Kitko DJ, Madison KC, Downing DT (1989) The role of the corneocyte lipid envelopes in cohesion of the stratum corneum. J Invest Dermatol 93:169–172