The Role of the Corneocyte Lipid Envelopes in Cohesion of the Stratum Corneum

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Treatment of isolated stratum corneum with certain detergents results in complete disaggregation of the corneocytes within hours at 45°C without agitation. This is prevented by prior heating of the tissue to 80°C or by solvent extraction of the intercellular lipids. In the present study, electron microscopy revealed that the heated or solvent-extracted tissue was characterized by cell-to-cell contacts that appeared to involve the chemically bound hydroxyceramides which constitute the corneocyte lipid envelope. It is proposed that the irreversible bonding between corneocytes that results from heating or lipid extraction results from interdigitation of the sphingosine chains belonging to those hydroxyceramides that are bound to the corneocyte protein envelope by the ω-hydroxyl function of the 30- and 32-carbon hydroxyacid moieties. Similar interdigitaion of adjacent envelopes might be involved in natural stratum corneum cohesion, limited mostly to the periphery of corneocytes where the absence of intercellular lamellae allows the appropriate cell-to-cell contact. *J Invest Dermatol 93:169–172, 1989*

Despite numerous investigations, it is still unclear what provides the cohesion of stratum corneum and what changes allow the orderly exfoliation of individual corneocytes in the process of normal desquamation. A number of studies have implicated cholesteryl sulfate in the cohesion, possibly involving calcium bridges between lipid bilayers [1,2]. This is supported by the demonstration that removal of the sulfate group is the only change in lipid composition that occurs during desquamation [3–5]. More recent studies have indicated a role for proteins [6] or specific glycoproteins [7] in stratum corneum cohesion, possibly including the involvement of calcium [6].

These conclusions must be interpreted in conjunction with the demonstration that the corneocytes are separated by multiple intercellular lipid lamellae [8,9] and that the corneocyte itself is entirely enveloped in a continuous layer of chemically bound hydroxyceramide [10,11]. It also seems relevant that treatment with certain detergents results in complete disaggregation of isolated stratum corneum [6,12,13], whereas complete removal of the intercellular lipids by extraction with chloroform : methanol not only fails to disaggregate the tissue, but results in a compact structure which can no longer be disaggregated by detergents [12,13]. Extraction of isolated stratum corneum with ether does produce individual corneocytes, but apparently only during vigorous grinding [7,14].

In the present study we have used electron microscopy to examine the effects of heat, solvents, and detergents on stratum corneum. The results suggest that cohesion involves direct contact between the lipid envelopes of adjacent corneocytes, and that this close contact occurs in limited regions where intercellular lamelle are absent, mostly at the periphery of the cells.

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MATERIALS AND METHODS

Preparation of Isolated Stratum Corneum Young pigs weighing 20 to 30 kg were killed at a slaughter house and then brought to the laboratory intact. Most of the hair was removed with animal clippers followed by an electric shaver. Circular sheets of full thickness epidermis were removed by 60-sec application of a hot (60°C) aluminum cylinder 2.5 inches in diameter, followed by gentle scraping with a spatula. Fifty such sheets of epidermis were suspended in 0.5% trypsin (Type III, Sigma, St Louis, MO) in 500 ml of buffered isotonic saline at 4°C overnight. The sheets of tissue were collected on a sieve and resuspended in a large volume of water. From this the pieces of tissue were transferred individually to 500 ml of fresh trypsin in a 1 L flask which was then rotated at 100 rpm for 2 h at 25°C. The tissue was again collected and washed and then stored at 4°C in water containing 1 mM sodium azide.

Heat Treatment of Stratum Corneum Single pieces of isolated stratum corneum 2.5 inches in diameter were placed in 10 ml of distilled water in separate screw-capped culture tubes. Tubes were then heated at 80°C for 1, 4, or 22 h. The tubes were then cooled and samples of the tissue were taken for examination by electron microscopy.

Solvent Extraction of Stratum Corneum Pieces of isolated stratum corneum were blotted dry and then placed in 10 ml of chloroform : methanol (2 : 1) in capped culture tubes. After 4 h at room temperature, the solvent was decanted and replaced with 10 ml of methanol. After a further 2 h the methanol was poured off and, without being allowed to dry, the tissue specimens were subjected to detergent treatment.

Detergent Treatment of Stratum Corneum Pieces of untreated stratum corneum, or those that had been subjected to heating or solvent treatments as described, were submerged individually in 10 ml portions of a solution containing 8 mM N, N-dimethyldodecylamine oxide and 2 mM sodium dodecylsulfate in phosphate-buffered isotonic saline, and the tubes were heated at 45°C for 7 d.

Electron Microscopy Small samples of each of the stratum cor-

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neum pieces were fixed in 2.5% glutaraldehyde and then postfixed in 0.2% ruthenium tetroxide [8]. The specimens were dehydrated in a series of acetone solutions before embedding in Spurr's resin. Ultrathin sections were floated onto carbon-stabilized Formvar-coated grids, stained with aqueous uranyl acetate and lead citrate, and examined in a Hitachi model 7000H electron microscope.

RESULTS

Untreated Stratum Corneum As in previous studies [8,9], electron microscopy showed that the untreated tissue consisted of layers of corneocytes separated by continuous multiple lipid lamellae (Fig 1).

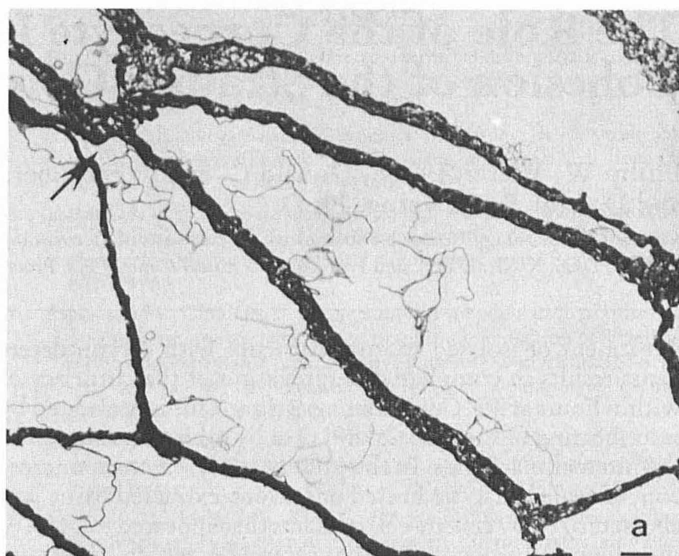
Detergent-Treated Stratum Corneum As in previous reports [6,12,13], detergent treatment of the isolated stratum corneum resulted in disintegration of the tissue into individual corneocytes in about 4 h without agitation. Electron microscopy revealed that the intercellular lamellae had been removed, but the cells retained their envelopes of bound lipid [10,11].

Heat-Treated Stratum Corneum Stratum corneum sheets remained intact after heating at 80°C for up to 22 h, but the tissue lost much of its tensile strength. Electron microscopy showed extensive disruption of the tissue (Fig 2*a*), resulting in greatly expanded intercellular spaces and dispersion of the intercellular lamellae. This disruption was more extensive with longer heating periods. Where lamellae were absent, the envelopes of adjacent cells were sometimes in contact; and these regions seemed to be the major remaining points of intercellular attachment (Fig 2*b*).

When the heat-treated tissue pieces were subjected to the detergent treatment at 45°C, the disaggregation seen with non-heat-treated stratum corneum was never observed. The tissue that had been heated for only 1 h broke up into a number of large flakes, but the tissue that had been heated for 4 h or 22 h did not disintegrate even after heating for 7 d in the detergent mixture. Very few individual corneocytes were produced even with vigorous vortexing.

Solvent-Treated Stratum Corneum As shown previously [11], stratum corneum that had been extracted with chloroform : methanol consisted of tightly compacted cells separated by uniform lucent bands (Fig 3). The lucent bands, representing the corneocyte lipid envelopes, were everywhere in close contact with adjacent cells and could often be seen to be demarcated from the lipid envelope of the adjacent cell by a faint electron-dense line. We have postulated that this electron-dense line represents the apposed polar surfaces of the respective lipid envelopes [11]. Splitting at this polar interface, which would result in cell separation, was infrequent.

Detergent treatment of the solvent-extracted tissue invariably failed to produce any disaggregation, even after heating for 7 d and occasional vigorous agitation on a vortexer.



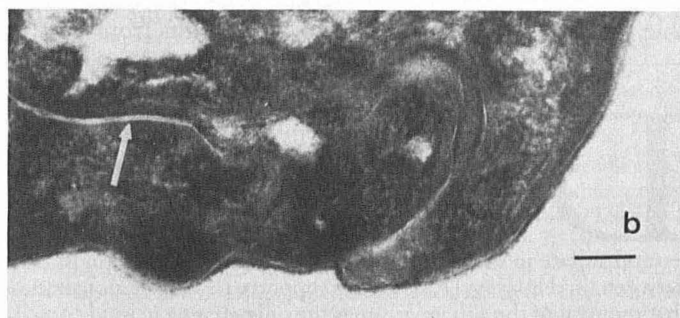


Figure 2. Transmission electron micrographs of isolated pig stratum corneum after heating in water at 80°C for 22 h. *a:* Survey micrograph (×4,000) showing expansion of the intercellular spaces and disruption of the intercellular lamellae. *Arrow* indicates remaining area of attachment. *Bar:* 1,000 nm. *b:* Enlargement (×85,000) of the indicated area from *a* showing remaining area of attachment *(arrow)* between corneocytes. *Bar:* 100 nm.

DISCUSSION

Consideration of the present findings leads us to infer that the tight cohesion between the corneocytes in solvent-extracted stratum corneum results from some kind of strong interaction between adjacent lipid envelopes. Figure 4 shows diagramatically how this contact might appear at the molecular level if only the polar surfaces of the lipid envelopes were in contact. In the absence of any covalent bonding, such an arrangement could be expected to be easily

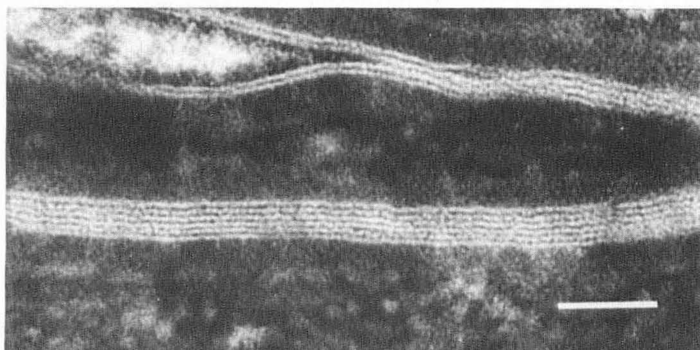


Figure 1. Transmission electron micrograph of isolated pig stratum corneum showing multiple intercellular lamellae (×240,000). *Bar:* 50 nm.

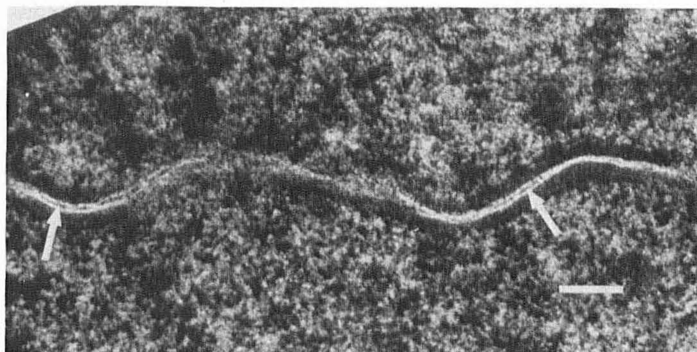


Figure 3. Transmission electron micrograph of pig stratum corneum after extraction with chloroform/methanol (2 : 1) showing closely apposed lipid envelopes separated by a fine dense band *(arrows)* (×85,000). *Bar:* 100 nm.

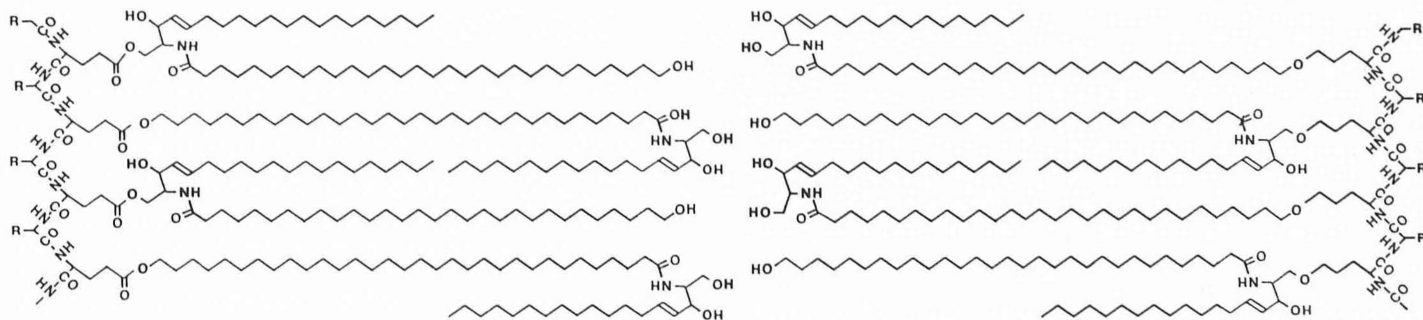


Figure 4. Proposed molecular structure of two protein-bound lipid envelopes in close apposition without lipid-lipid interdigitation.

disrupted by association of water molecules with the polar head groups of the lipids.

Likewise, there is no apparent reason why polar organic solvents should not similarly result in separation of the contacting cells. However, the lipid envelopes possess a number of features that should promote strong mutual attraction, based on principles of membrane interaction that have recently been summarized [15]. According to these principles, membrane interactions are governed by a balance between repulsive and attractive forces. Only the relatively weak van der Waals forces are available to provide attraction between the corneocyte lipid envelopes, but this attraction will be maximized, and the repulsive forces resulting from thermal motions will be minimized, by the known physical properties of the corneocyte lipid envelopes. Essentially, the more smooth and the more rigid the surfaces of the membranes are, the less the repulsive fluctuational force will be. Rigidity of the lipid envelopes is promoted by the high melting, very long chain ω-hydroxyceramides of which they are predominantly composed. In addition, the hydroxyceramides span the full thickness of each envelope, minimizing the ripple effect which results from fluctuations in membrane thickness. Moreover, the corneocyte lipid envelope appears to be covalently bound to a β-pleated sheet of protein on the outside of a thick, cross-linked protein envelope [11,16], providing a relatively rigid substrate to which the lipid layer is bound. As a result, the repulsive forces might be sufficiently small to allow the attractive van der Waals force to dominate the interaction between corneocyte lipid envelopes.

However, the remarkable cohesion that exists between corneocyte envelopes that are in direct contact leads us to propose an additional mechanism based on interdigitation of lipid chains between adjacent envelopes, as shown in Fig 5. This molecular arrangement is based on the recent demonstration that the bound lipids consist almost exclusively of ω-hydroxyceramides, in which the hydroxyacid moieties are 30 to 32 carbons in length and the sphingosine moieties to which they are attached have 16 to 22 carbons [10,11]. Pig stratum corneum contains 2% of the bound hydroxyceramide, which is just sufficient to provide a monomolecular layer of bound lipid over the surface of each cell [10,11]. About half of the hydroxyceramides are bound by esterification of the ω-hydroxyl function and half by one of the hydroxyls of the sphingosine [10].

Detergent removal of the unbound intercellular lipids should allow cell-to-cell contact and might be expected to produce the envelope interdigitation that results in irreversible bonding after solvent extraction of the lipids. However, lipid chains would be unlikely to fold out into this configuration as long as an aqueous medium surrounds the cells. In preliminary studies we have found that irreversible cohesion does indeed occur when dispersions of corneocytes that have been delipidized by detergents are allowed to dry.

Our hypotheses can be extended to the inability of detergent treatment to disaggregate stratum corneum that has been heated to 80°C. Dispersion of the intercellular lamellae presumably resulted from melting of the lipid domains, which occurs at about 75°C [17,19]. As a result, the tissue was seen by electron microscopy to be extensively disrupted, with large spaces between the cells (Fig 2). However, where there was contact between cells, the apposed cell envelopes were clearly in intimate association, presumably involving the stable chain interdigitation that we propose as the basis for irreversible corneocyte cohesion. Most of this contact appeared to exist at the periphery of the cells and might represent persistence of limited points of envelope-to-envelope cohesion in the original stratum corneum. These limited points of contact might explain the basketweave appearance that is typical of standard histologic sections and is now seen also in the heat-treated stratum corneum.

It remains necessary to explain, however, why native stratum corneum can be disaggregated by detergents but not heat-treated stratum corneum. One possibility is that a less intimate form of envelope interdigitation exists in the native tissue than in the heated material. This might be the basis for the somewhat different appearance of the envelope contact points in the two tissues. In the untreated tissue, the lipid envelopes appear to be only partially interdigitated, forming a lipid monolayer between them [9], whereas in the heated tissue, only two lucent bands are seen (Fig 2*b*), which might result from more intimate interdigitation. This could only result from expulsion of any lipid molecules that were not cova-

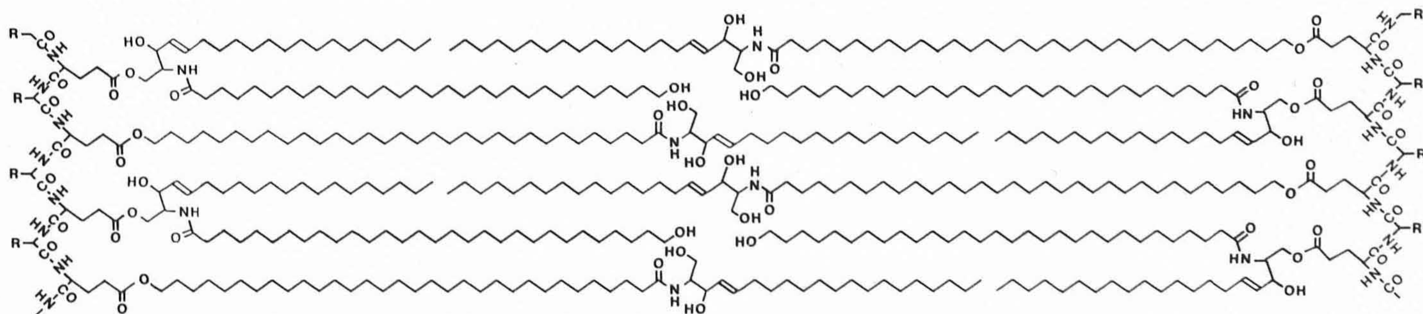


Figure 5. Proposed molecular structure of two protein-bound lipid envelopes in close apposition with interdigitation of their sphingosine chains.

lently bound, a circumstance that could be promoted by chain melting. Similarly, solvent extraction might promote the more intimate form of interdigitation by removal of all nonbound lipid molecules.

Whether additional mechanisms of cohesion such as protein-protein interactions are needed to explain the properties of the stratum corneum remains to be determined. However, because corneocytes are widely separated from each other by intercellular lipids over most of their surfaces, additional interactions might also be limited to the few points of envelope contact at the cell peripheries.

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REFERENCES

1. Epstein EH Jr, Williams ML, Elias PM: Steroid sulfatase, X-linked ichthyosis, and stratum corneum cell cohesion. Arch Dermatol 117:761–763, 1981
2. Elias PM: Epidermal lipids, barrier function, and desquamation. J Invest Dermatol 80(suppl):44s–49s, 1983
3. Long SA, Wertz PW, Strauss JS, Downing DT: Human stratum corneum polar lipids and desquamation. Arch Dermatol Res 277:284–287, 1985
4. Wertz PW, Swartzendruber DC, Madison KC, Downing DT: Compostion and morphology of epidermal cyst lipids. J Invest Dermatol 89:419–425, 1987
5. Ranasinghe AW, Wertz PW, Downing DT, Mackenzie IC: Lipid composition of cohesive and desquamated corneocytes from mouse ear skin. J Invest Dermatol 86:187–190, 1986
6. Bissett DL, McBride JF, Patrick LF: Role of protein and calcium in stratum corneum cell adhesion. Arch Dermatol Res 279:184–189, 1987
7. Brysk MM, Rajaraman S, Penn P, Barlow E: Glycoproteins modulate adhesion in terminally differentiated keratinocytes. Cell Tissue Res 253:657–663, 1988
8. Madison KC, Swartzendruber DC, Wertz PW, Downing DT: Presence of intact intercellular lamellae in the upper layers of the stratum corneum. J Invest Dermatol 88:714–718, 1987
9. Swartzendruber DC, Wertz PW, Kitko DJ, Madison KC, Downing DT: Molecular models of the intercellular lamellae in mammalian stratum corneum. J Invest Dermatol 92:251–257, 1989
10. Wertz PW, Downing DT: Covalently bound ω-hydroxyacylsphingosine in the stratum corneum. Biochim Biophys Acta 917:108–111, 1987
11. Swartzendruber DC, Wertz PW, Madison KC, Downing DT: Evidence that the corneocyte has a chemically bound lipid envelope. J Invest Dermatol 88:709–713, 1987
12. Takahashi M, Masami A, Miyazawa K, Machida Y: Effects of surface active agents on stratum corneum cell cohesion. J Soc Cosmet Chem 38:21–28, 1987
13. Swartzendruber DC, Kitko DJ, Wertz PW, Madison KC, Downing DT: Isolation of corneocyte envelopes from porcine epidermis. Arch Dermatol Res 280:424–429, 1988
14. Smith WP, Christensen MS, Nacht S, Gans EH: Effect of lipids on the agreggation and permeability of human stratum corneum. J Invest Dermatol 78:7–11, 1982
15. Israelachvili JN, McGuiggan PM: Forces between surfaces in liquids. Science 241:795–800, 1988
16. White SH, Mirejovsky D, King GI: Structure of lamellar lipid domains and corneocyte envelopes of murine stratum corneum. An X-ray diffraction study. Biochem 27:3725–3732, 1988
17. Van Duzee BF: Thermal analysis of human stratum corneum. J Invest Dermatol 65:404–408, 1975
18. Golden GM, Guzek DB, Kennedy AH, McKie JE, Potts RO: Stratum corneum lipid phase transitions and water barrier properties. Biochem 26:2382–2388, 1987
19. Wertz P, Abraham W, Landmann L, Downing DT: Preparation of liposomes from stratum corneum lipids. J Invest Dermatol 87:582–584, 1986