review

Lipid and protein structures in the permeability barrier of mammalian epidermis

Donald T. Downing

The Marshall Dermatology Research Laboratories, The University of Iowa College of Medicine, Iowa City, IA 52242

The epidermis of terrestrial vertebrates has evolved to provide a barrier to water permeation that is essential for survival in a desiccating environment. It has been realized for decades that this barrier is provided by lipids, but it was only during the 1980s that the molecular structure and physical arrangement of these lipids were defined. During the past several years, it has become apparent that in the performance of their function, epidermal lipids interact with proteins, and in some instances are covalently linked to protein structures. It is the purpose of this review to inspect and evaluate those instances where lipids and proteins interact in providing the epidermal barrier. For the benefit of those whose experience does not include dermatology, it will be desirable to briefly review the macroscopic and microscopic anatomy of the skin, as well as the biochemistry of the epidermal lipids.

THE STRUCTURE OF SKIN

The skin consists of a thin sheet of epidermis layered upon a much thicker substrate, the dermis. The superficial cornified layer of the epidermis provides the barrier to water permeation, and some abrasion resistance, while the dermis provides most of the bulk and toughness of the skin. The dermis is highly vascularized and permeable, consisting predominantly of a fibrous collagen meshwork that is sparsely populated with cells. The epidermis is devoid of blood vessels, receiving all of its nutrients, and disposing of its waste products, by diffusional exchange with the dermis. Reviews on the structures of the epidermis and dermis of each of the classes of vertebrates are collected in a volume edited by Bereiter-Hahn et al. (1–3).

In mammalian skin, the epidermis is maintained by continuous cell division in the germinative basal layer. Daughter cells move outward, undergoing differentiation and the production of characteristic epidermal lipids and proteins. Ultimately, the keratinocytes cornify, producing the dead, flattened corneocytes of the horny layer, or stratum corneum. An accumulation of 20 to 30 layers of corneocytes are embedded in a matrix of lipid that is extruded from the cells immediately before cornification. Corneocytes continually exfoliate from the skin surface, at a rate of desquamation that maintains a constant thickness of the epidermis.

Study of the epidermal lipids of most mammals is complicated by the copious amounts of nonpolar lipids that are produced by the sebaceous glands and excreted onto the surface of the skin (4, 5). The sebaceous lipids probably have little effect on the physical properties of the epidermal lipid barrier because their low polarity should prevent their intrusion into lipid bilayers. However, lipid extracts of the epidermis will inevitably contain a large proportion of sebaceous lipids unless steps are taken to prevent this. One species, the pig, produces little or no sebum, and so provides a ready supply of uncontaminated epidermal lipid. The pig is therefore the animal of choice for the study of epidermal lipid composition and metabolism.

THE ULTRASTRUCTURE OF MAMMALIAN EPIDERMIS

In addition to the structures and organelles common to most mammalian cells, keratinocytes have several features specific to their function, as seen in **Fig. 1**.

Keratin filaments

Throughout the epidermis, the cells contain bundles of protein filaments that become more numerous as differentiation proceeds. After cornification, the flattened cells contain little more than densely packed keratin filaments running parallel to the surface.

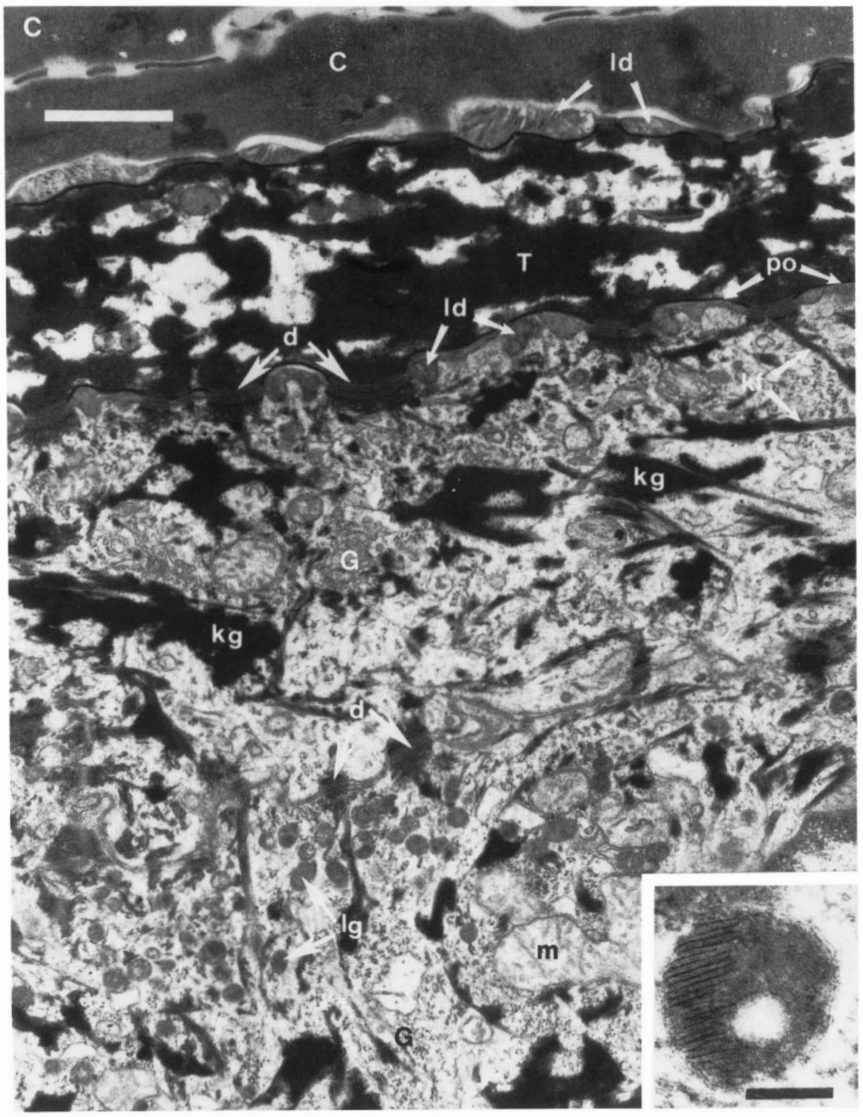


Fig. 1. Transmission electron micrograph of epidermis fixed with glutaraldehyde and post-fixed with osmium tetroxide. Five cell layers are shown: G: granular cells (two); T: transitional cell (one); C: corneocytes (two). Structures seen include: lg: lamellar granules; ld: lamellar disks in the intercellular space; d: desmosomes; po: cross-linked protein envelope; kf: keratin filaments; kg: keratohyalin granules. Inset: lamellar granule, showing a stack of lamellar disks and the bounding membrane. From micrographs kindly provided by Dr. D. C. Swartzendruber.

Keratohyalin

In the uppermost viable cells of the epidermis, large, densely staining irregular granules can be seen, even under a light microscope, giving rise to the term "granular cells" for this stage of differentiation of the keratinocytes. During cornification, keratohyalin appears to form the matrix between the keratin filaments, and may aid in the aggregation of the fibers.

Desmosomes

Throughout the epidermis, cell-to-cell contact is maintained and strengthened by button-like structures that have both an intercellular component and an intracellular plaque within each of the adjacent cells. In addition, the internal plaques are linked to intracellular filaments that extend throughout the cytoplasm and connect to other desmosomes. This system clearly provides strength to the entire epidermis by maintaining cell-to-cell mechanical contact. However, in the stratum corneum, the desmosomes over most of the cell surface appear to become degraded, and desmosomal adhesion is maintained only around the periphery of the corneocytes (6). Eventually this cohesion is lost also, allowing the cells to exfoliate from the skin surface.

Lamellar granules

As keratinocytes differentiate, they produce increasing numbers of small, membrane-bound ovoid bodies that contain one or more stacks of lamellar disks. Based on their appearance and dimensions, it has been proposed that the disks are formed by flattening of lipid vesicles, and each disk therefore consists of two lipid bilayers, joined at their periphery (7). Just prior to cornification, the bounding membrane of each lamellar granule becomes attached to the cell membrane and the contents of the granule are extruded into the intercellular space. The stacks of disks are then dispersed and the disks join edge-to-edge to form multiple, continuous extracellular sheets (7).

Intercellular lamellae

Broad, continuous lipid sheets between the cells of the stratum corneum were first visualized by freeze-fracture electron microscopy (8). These lamellae are rarely seen in conventional thin-section electron microscopy, apparently because their constituent lipids are not stained by the usual osmium tetroxide fixative. It was found, however, that fixation with the more reactive ruthenium tetroxide provides clear visualization of the extracellular lipids (9) and reveals a unique pattern of lamellae (**Fig. 2**) that has now been interpreted in terms of the specific lipids present and their interaction with each other and with the protein structures of the corneocytes (10, 11).

The corneocyte protein envelope

Following extrusion of the epidermal lamellar granules into the intercellular space, the keratinocyte produces a thick protein envelope beneath the cell membrane (12). In thin-section electron micrographs, the protein envelope is seen as a uniform, 11-nm-thick band around the entire cell periphery. Several proteins are believed to become cross-linked into the envelope in this way, the most fully investigated being involucrin, the structure and evolutionary development of which have been studied extensively (13–17).

The corneocyte lipid envelope

In thin-section electron micrographs, a lucent band is visible (Fig. 2), on the outside of the thickened protein envelope, that traditionally has been referred to as the corneocyte plasma membrane (12). However, in stratum corneum that has been exhaustively extracted with chloroform–methanol the "plasma membrane" is still seen, so that between adjacent corneocytes (which are no longer separated by any free lipid) there are always two closely apposed lucent bands. Recently it was shown that these remaining lucent bands represent lipid that is covalently bound on the outer surface of the corneocyte protein envelope (18, 19). After mild alkaline hydrolysis, this lipid can be extracted with chloroform–methanol, and consists almost exclusively of hydroxyceramides, in which very-long-chain (C_{30-34}) ω-hydroxyacids are amide-linked to sphingosine (18). It is clear that a closely packed monolayer of such molecules would have the appearance and dimensions of a lipid bilayer. However, a close-packed structure would require that the hydroxyceramide molecules alternate in their orientation, such that half of the molecules were bound to the protein surface by their ω-hydroxyl group and half by their sphingosine head group, as shown in **Fig. 3**.

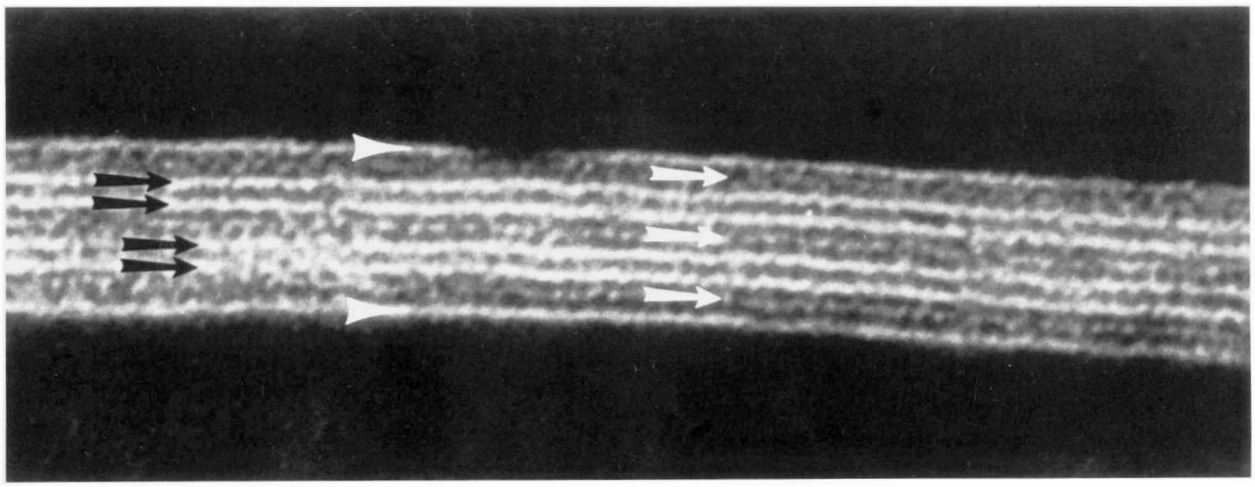


Fig. 2. Transmission electron micrograph of multiple intercellular lamellae between two corneocytes in pig epidermis post-fixed with ruthenium tetroxide. In this instance the lamellae include: black arrows: paired lipid bilayers; white arrows: lipid monolayers; white arrowheads: lipid envelopes. From a micrograph kindly provided by Dr. D. C. Swartzendruber.

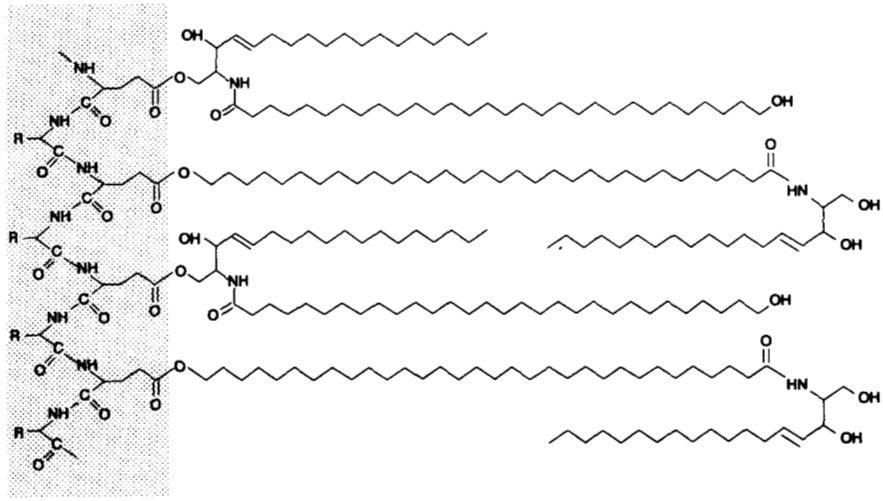


Fig. 3. Proposed arrangement of hydroxyceramide molecules in the corneocyte lipid envelope. The lipid molecules alternate in being attached by their fatty acid hydroxyl group or one of the sphingosine hydroxyl groups. Attachment is to glutamate side chains exposed on one side of a protein in the β-sheet conformation.

The thickness of the corneocyte lipid envelope (4 nm on each side of the cell) and the cell thickness (average 300 nm) indicate that the corneocyte lipid envelope should constitute 1.8% of the dry weight of lipid-extracted stratum corneum, allowing for the densities of lipid (0.9) and protein (1.3). This is the amount of hydroxyceramides actually liberated by alkaline hydrolysis (18), showing that all of this probably originated from the lipid envelope, and little or none from within the corneocytes.

Chemical evidence in support of the proposed lipid envelope structure was obtained by treatment of solvent-extracted stratum corneum with acetone–HCl, to form the acetonides of the 1,3-diol of those ceramides having free head groups. The tissue was then subjected to mild alkaline hydrolysis and the liberated lipids were extracted. Chromatographic analysis showed that about half of the head groups formed acetonides, confirming that those molecules were esterified to protein through the hydroxyl group on the acyl moiety (18). The ceramide molecules that did not form an acetonide presumably were bound to protein through one of the hydroxyl groups of the sphingosine moiety.

EPIDERMAL LIPID COMPOSITION

By isolation of epidermal cells from different layers of the epidermis, followed by analysis of the extracted lipids, it was shown that the amount and composition of the epidermal lipids change dramatically during differentiation (20). The basal cells of the epidermal germinative layer have a lipid composition that consists predominantly of conventional phospholipids. As the keratinocytes differentiate, a series of unusual lipids are added that then undergo profound changes in amount and composition as the cells mature. Even after conversion to the dead, flattened cells of the stratum corneum, enzymically controlled changes in lipid composition continue to occur that may influence both the exfoliation of cells from the surface and the rate of their replacement by cell division in the basal layer.

Glucosylceramides

The suprabasal cells accumulate a set of glucosylceramides that vary in structure according to their content of sphingosine, phytosphingosine, and fatty acids. The structures and relative amounts of these glycolipids are shown in **Fig. 4**. The glucosylceramides are thought to be present in the disks that are packaged in the lamellar granules and then extruded into the intercellular space.

Ceramides

In stratum corneum, almost half of the lipid consists of a mixture of ceramides, the structures of which are shown in Fig. 4. The ceramides are thought to be produced by deglycosylation of the glucosylceramides shortly after their extrusion into the intercellular space. As a result, the uncornified cells of the epidermis contain almost all of the glucosylceramides, while the cornified layer contains the ceramides (21).

Cholesterol

Free cholesterol is the second most abundant lipid in the stratum corneum, amounting to 25% of the ex-

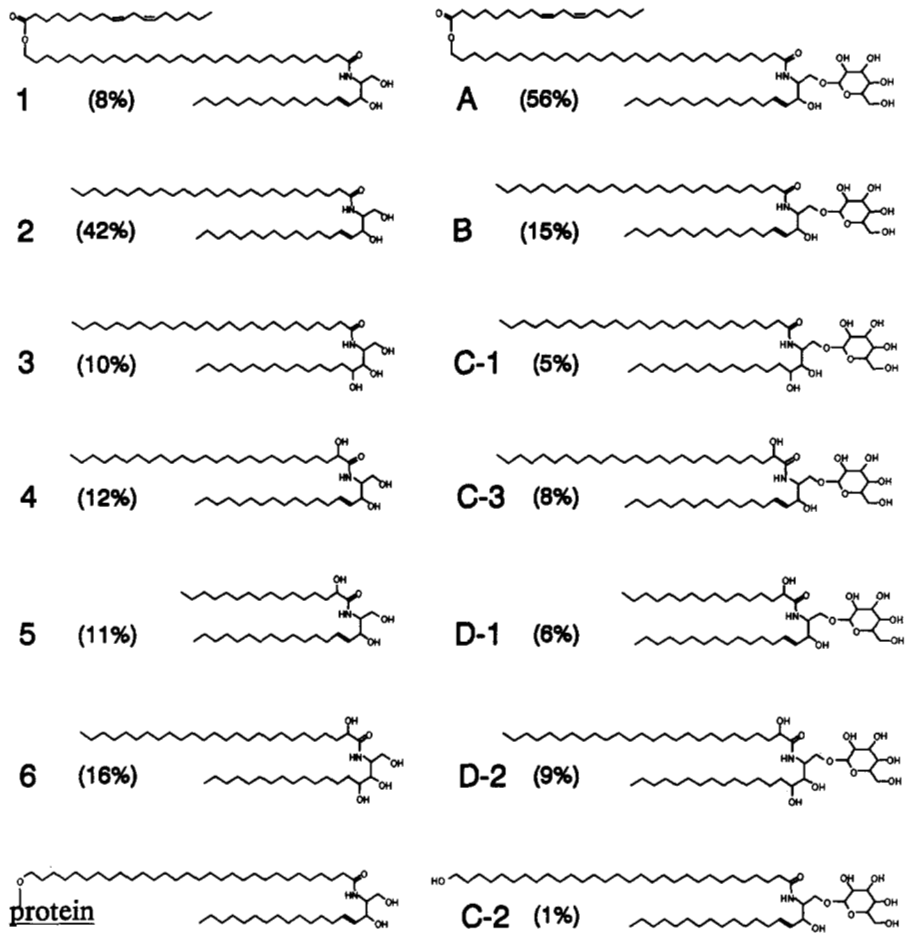


Fig. 4. Molecular structures of the ceramides (left column) from the stratum corneum and the glucosylceramides (right column) from the viable cells of porcine epidermis (45–47).

tractable lipid. In addition, the stratum corneum lipids contain about 5% of cholesteryl sulfate and 2% of fatty acid esters of cholesterol. Desquamation of corneocytes from the skin surface is accompanied by enzymic hydrolysis of cholesteryl sulfate to free cholesterol (22). It has been proposed that this allows the separation of the surface cells by destroying linkages based on calcium bridges between cholesteryl sulfate molecules in adjacent lipid bilayers (23). This proposal arose from the discovery that in the genetic disease recessive X-linked ichthyosis, in which the sulfatase is inactive, cholesteryl sulfate accumulates in the epidermis and the stratum corneum becomes thick and scaly.

Free fatty acids

The stratum corneum lipids contain 10–12% of a free fatty acid fraction, the origin of which is uncertain, although some at least is produced by the hydrolysis of ceramides. The composition of the mixture of fatty acids is unusual in consisting predominantly of very long chain (C_{20}–C_{28}) saturated acids, with only 6% of monounsaturated and 1% of diunsaturated acids (24).

Triacylglycerols

Epidermal lipids have often been reported to contain 25–30% of triacylglycerols, but when the epidermis is removed from animals without incision into the subcutaneous fat, the extracted lipids contain as little as 1–2% of triacylglycerols (25, 26).

Sphingosine

Stratum corneum has been shown recently to contain up to 0.5% of free sphingosine (27, 28), a very high concentration in view of the potent biological activity of this lipid, and much higher than in any other tissue. The sphingosine appears to be produced within the stratum corneum by a ceramidase that is active in this nonviable tissue (29). It has been proposed that production of this inhibitor of protein kinase C and cell division might represent the mechanism by which

epidermal cell proliferation is modulated in order to balance the rate at which cells are lost from skin surface.

THE TIME COURSE OF EPIDERMAL LIPID METABOLISM

Lipid metabolism in the epidermis is unique in that, once formed, the characteristic lipids are not in equilibrium with the circulation, and are not subject to continued acyl chain metabolism. As a result, time-course studies may be conducted over periods of weeks, during which time the tracers introduced do not become diminished or randomized between lipid classes. Therefore, the specific lipid transformations that do occur can easily be studied in vivo by radiotracer techniques, using intradermal injection of labeled substrates, at separate sites and different times before recovering the epidermis, and examining the extracted lipids.

With the use of [1-^{14}C]acetate (26), it was found that for several days after labeling, most of the lipid radioactivity was confined to the phospholipids, from which it was then transferred to glucosylceramides and, after 4–7 days, to ceramides. The amount of radioactivity in free fatty acids increased continuously between 1 and 7 days. Cholesterol became maximally labeled within 2 days. After 10 days, each lipid maintained its relative level of radioactivity for the 21-day duration of the experiment. The total amount of lipid radioactivity remained constant between 3 h and 10 days, indicating that once incorporated into the epidermal cells, the lipid was not in equilibrium with the circulation, and was only lost by the eventual exfoliation of cells from the skin surface, after the known cell cycle time of about 3 weeks.

A similar time course was obtained when the epidermal lipids were labeled by intradermal injection of [U-^{14}C]linoleate (30), although in that case the label was confined to the phospholipids and to the one acylglucosylceramide and the one acylceramide that are known to contain linoleate. The absence of transfer of label to other lipids, especially the nonappearance of label in cholesterol, showed the absence of catabolism of fatty acids in the epidermal cells over the extended time course of the experiment. In unpublished studies in this laboratory (A. H. Vaz and D. T. Downing), it was found that after labeling by intradermal injection of [1-^{14}C]palmitate a similar time course was obtained, but the saturated fatty acid was excluded from the two acylceramide locations for which linoleate was selected, and was incorporated into all of the saturated chains from which linoleate label was excluded.

MORPHOLOGY OF THE STRATUM CORNEUM INTERCELLULAR LAMELLAE

The adoption of ruthenium tetroxide fixation for the visualization of stratum corneum intercellular lipids for electron microscopy (9) has led to the realization that nonrandom lipid orientation must be involved in producing several aspects of the unique patterns of lipid lamellae seen in Fig. 2.

Paired lipid bilayers

Thin-section electron micrographs of stratum corneum show lucent bands in the intercellular spaces having the 4-nm width characteristic of lipid bilayers (Fig. 2). These lucent bands are always found in pairs, which may be explained in terms of the theory of Landmann (7) that the disks that are stacked in the epidermal lamellar granules are formed by flattening of membrane-bound vesicles, and therefore consist of pairs of bilayers (**Fig. 5**). The force that causes the original lipid vesicles to flatten seems to persist after fusion of the disks into sheets of paired bilayers, because after dispersal of the intercellular lamellae by heating, the distorted lipid sheets remain paired (11). The effect might be produced by those ceramide molecules that contain 30-carbon hydroxyacids, which are capable of spanning a bilayer while at the same time inserting their ester-linked fatty acid moiety into an adjacent bilayer. The effect was reproduced in vitro with liposomes prepared either from phospholipids or from stratum corneum lipids: addition of either acylceramide or acylglucosylceramide caused the liposomes to flatten and stack like coins (31, 32). Apposition of the internal surfaces of the liposomes apparently persists after the resulting disks have fused to form sheets, and accounts for the pairs of bilayers that are seen in the stratum corneum intercellular spaces.

Lipid monolayers

Another remarkable feature of the stratum corneum intercellular lamellae that was revealed by ruthenium fixation is the presence of a narrow lucent band between each set of paired bilayers (Fig. 2). This has been interpreted as a lipid monolayer, formed by acyl or sphingosine chains everted from both adjacent bilayers (10). Additional chains required to replace those that are everted to produce the mutual monolayer could be provided by the free fatty acids that are abundant in the stratum corneum lipids. Additional members of the monolayer could be provided by ceramides which insert one hydrocarbon chain into a bilayer and the other into the monolayer. Monolayers also intervene, throughout the stratum corneum, between the paired bilayers and the cor-

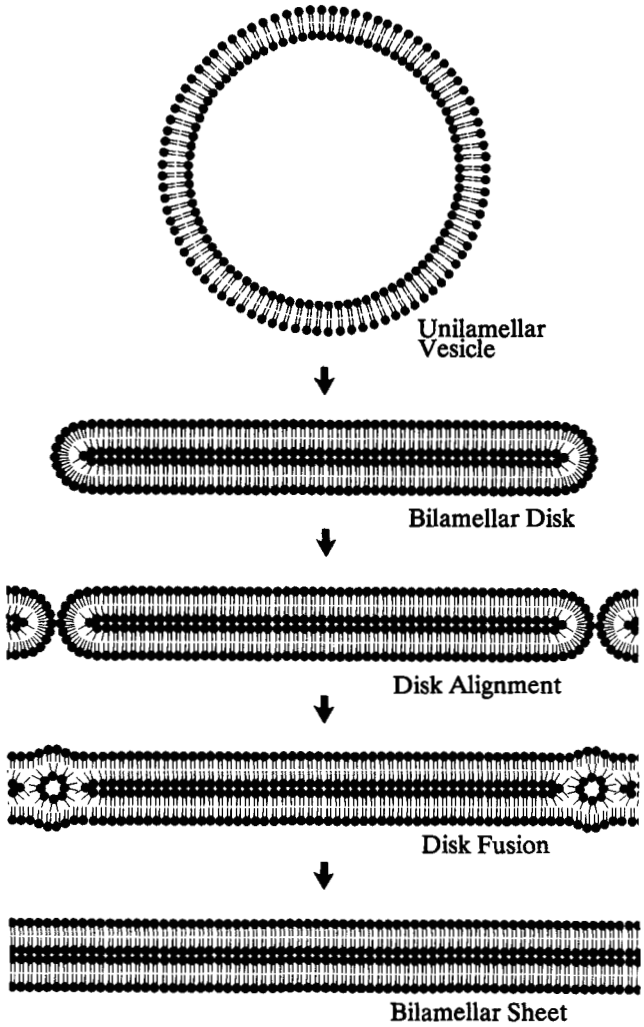


Fig. 5. Proposed explanation for the formation of epidermal lamellar disks and their fusion into pairs of lipid bilayers (1).

neocyte lipid envelopes, and may similarly be explained by the sharing of chains from adjacent lamellar structures (**Fig. 6**).

In some regions of the stratum corneum, intercellular lamellae are absent. This most often occurs at the periphery of the corneocytes, in the regions where cell-to-cell cohesion is maintained by desmosomes. However, where the paired bilayers are absent, the lipid envelopes of the apposing corneocytes are not in direct contact but are separated from each other by a narrow lucent band that again has the dimensions of a lipid monolayer. It has been proposed that this also represents sphingosine chains everted from both of the adjacent cell membranes to form an intervening mutual monolayer (10). Again, this can only happen if additional chains are provided to take the place of those chains that are everted from the lipid envelopes, and could consist of free fatty acids, cholesterol, or ceramides (**Fig. 7**).

As a result of the dimensions and locations of the corneocyte lipid envelopes, the paired bilayers, and the intervening monolayers, the broad and narrow lucent bands in the stratum corneum (as seen in ruthenium-fixed sections) always present the pattern:

$$(\text{broad/narrow/broad})_{n+1}$$

where n is the number of bilayer pairs (Landmann units) in the intercellular space.

ATTACHMENT OF THE CORNEOCYTE LIPID ENVELOPE

Chemical evidence indicates that the hydroxyceramides that constitute the corneocyte lipid envelope are attached through ester bonds (Fig. 3), since they are easily liberated by mild alkali (18). The hydroxyl groups are presumed to be esterified to carboxylic side chains of amino acid residues in the surface protein of the corneocyte cross-linked envelope. The protein would need to have a relatively smooth surface, as might be provided by a long sequence capable of being folded into a β-pleated sheet. Also, the sheet region of the protein would need to be extremely rich in carboxylic side chains, so that every second amino acid in the sequence might be glutamate or aspartate (Fig. 3).

Construction of a molecular model of a hypothetical protein in the β-pleated sheet conformation showed that with such an arrangement the surface area occupied by glutamate residues matches the cross-sectional area of closely packed hydroxyceramide chains

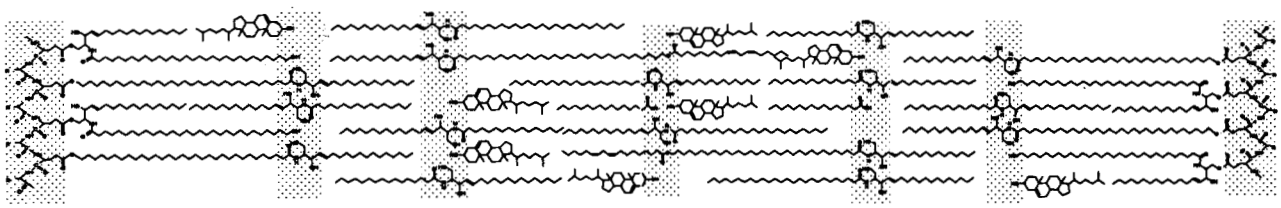


Fig. 6. A molecular interpretation of the appearance of stratum corneum intercellular lamellae. In this instance, lipid monolayers intervene on both sides between a bilayer pair and the adjacent lipid envelopes (10).

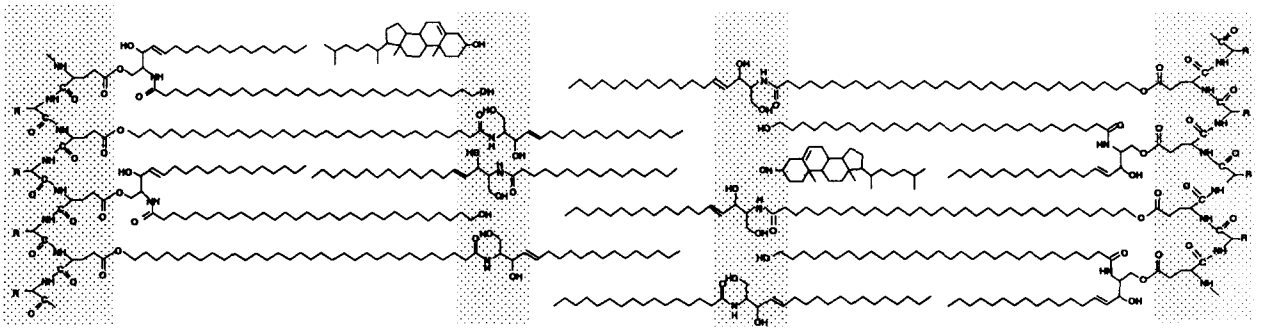


Fig. 7. A molecular interpretation of the interface between two corneocytes with no lipid bilayers intervening (10).

(33). As shown in Fig. 3, two amino acid side chains would bind the equivalent of three lipid chains. As measured on a scale model, two amino acid residues occupied 65 Å², while three lipid chains would occupy 56.4 Å² (34). Calculations showed that the hydroxyceramide chains would need to be tilted 21.5° in order that their projected cross-sectional area should exactly match the area occupied by the glutamate residues to which they are attached. Further experimentation with molecular models showed that a protein surface suitable for attachment of the hydroxyceramides could be provided by involucrin, one of the proteins known to be cross-linked into the corneocyte envelope.

HUMAN INVOLUCRIN

In human involucrin, glutamates constitute about 20% of the residues, which would allow 40% of the protein chain to contain the 50% abundance of glutamate that is required to provide the carboxylate surface for hydroxyceramide attachment (Fig. 3). This unusual protein appears to have evolved through multiple repeats of a 30-nucleotide sequence (15). The resulting protein has a central region containing 39 repeats of a 10-amino acid sequence, the consensus of which is highly preserved. The sequence may be folded as shown in **Fig. 8** on the grounds that glycine is favored as the second of the two amino acids involved in tight turns between β-pleated sequences (35).

This arrangement of human involucrin indicated that most of the molecule can adopt a β-pleated sheet conformation with 10-residue courses. Arranged in this way, straight columns of glutamate residues became apparent (Fig. 8). However, the frequency of glutamate was not sufficient to support the glutamate-surface hypothesis. Nevertheless, molecular models constructed of portions of the involucrin sequence (33) showed that most of the glutamate residues would be directed towards one surface of the β-pleated sheet, while all of the nonpolar residues (notably leucine and valine) would be directed to the other surface.

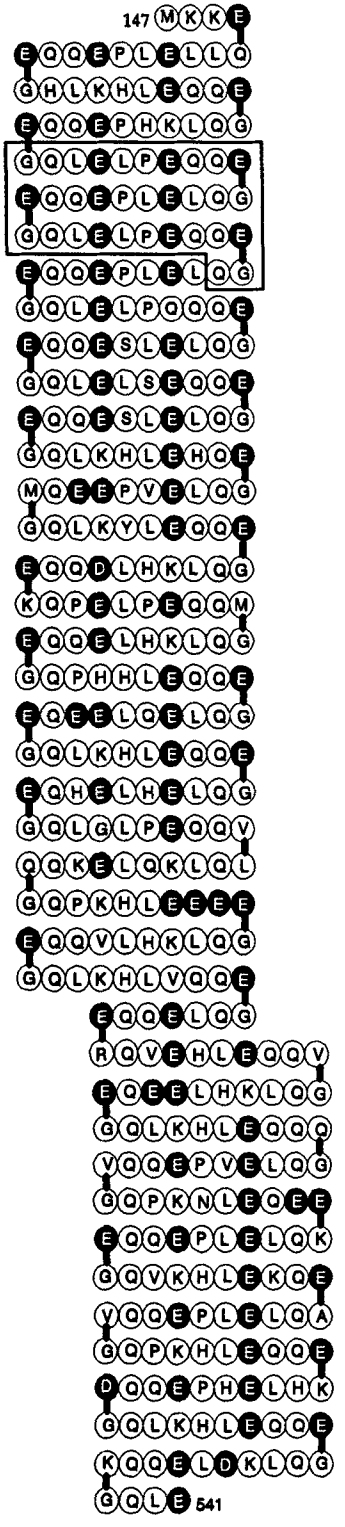
Inspection of this construction showed that if most of the glutamine residues were to be hydrolyzed to glutamate, this would produce a surface on which virtually all of the exposed residues were carboxylic, fulfilling the requirement of the hypothesis. It would be helpful to know whether epidermis contains an enzyme capable of the post-translational hydrolysis of glutamine to glutamate, as is the case with certain bacteria (36).

It may be that the arrangement and frequency of glutamate residues required for lipid envelope attachment could only be assembled in an indirect manner. If the primary sequence of the protein were to contain all of the glutamates preformed, it might be impossible for the β-pleated sheet to exist, because of repulsion between the like charges on the glutamate side chains. Likewise, simple hydrolysis of the glutamine residues in the β-pleated sheet could cause it to unfold. Therefore, it seems likely that hydrolysis of glutamines to glutamate would have to be preceded by cross-linking, and perhaps accompanied by esterification of the carboxylic residues to the hydroxyceramide molecules, to prevent unfolding. However, van der Waals attraction between the very long, predominantly saturated, hydrocarbon chains may outweigh the effects of amino acid sequence on folding.

Human-type involucrin has been found in six other anthropoids, although, not surprisingly, there is some variation in composition, mostly concerning the number of amino acids in the repeated sequences and in the number of repeats of the sequence, as well as in the mutation of individual codons before or after duplication of certain sequences. Such variation has been detected even within the human species (16). What is truly remarkable is that in prosimians, and in all other mammals that have been studied, the molecule that performs the same function as anthropoid involucrin is quite different in amino acid composition and sequence.

PRE-ANTHROPOID INVOLUCRIN

In involucrin of the pig, dog, owl monkey, and galago, the repeated sequence apparently originated in a separate section of the gene and has a different number, composition, and sequence of amino acids (17). The resulting protein is even more rich in glutamate and glutamine than anthropoid involucrin, containing as much as 70% of such residues in the repeat region. The species differ considerably in the number and identity of amino acids in the repeated sequences, as well as in the number of times the sequence is repeated.



Molecular models of β-pleated sheets based on the porcine involucrin sequence showed most of the glutamate and glutamine residues arrayed on one side and the nonpolar residues on the other. Assuming the hydrolysis of most glutamines to glutamate, there would be regions where close-packed arrays of hydroxyceramide molecules could be attached. However, there was a region where two columns of histidine residues, and an intervening column of aspartate residues, interrupted the potentially glutamate-rich surface. It was found that the required surface could be obtained if the protein chain were wound into a right-handed helix having 16 amino acids per turn (**Fig. 9**). A molecular model showed that by flattening the cylinder, to the extent allowed by the side chains within, a planar surface was obtained on two sides (33), on one of which all of the exposed side chains were from glutamate or glutamine.

As with human involucrin, the conformation of porcine involucrin might result from forces imposed by attachment to the hydroxyceramide monolayer rather than the inherent folding tendency of the primary sequence. Cross-linking of the corneocyte protein envelope would then stabilize the final conformation. Calculations based on the thickness of the proposed conformation of porcine involucrin (2 nm) and the thickness of the corneocyte protein envelope (11 nm) showed that the proportion of involucrin in the envelope would be 36%, assuming that all of the planar region of the involucrin were exposed on the cell surface (33).

Fig. 8. Proposed conformation of the central region of human involucrin arrayed on the surface of the corneocyte protein envelope. Positions of folding of the β-pleated sheet were based on the positions of the glycine residues. A molecular model of the boxed region showed that one surface of the sheet exposed only glutamine and glutamate residues, while all of the nonpolar side chains were located on the other side (33). The amino acids are represented by their standard one-letter convention: A, alanine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; V, valine; Y, tyrosine.

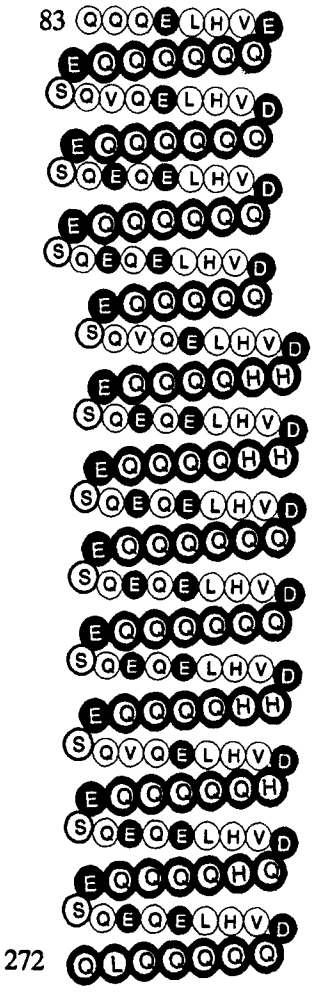


Fig. 9. Proposed conformation of the central region of porcine involucrin wound into a right-handed helix having 16 amino acids per turn. A molecular model showed that the coil could be flattened to provide one almost planar surface on which almost all of the amino acid side chains were glutamine or glutamate; all of the nonpolar side chains were on the other surface (33). The amino acids are represented by the standard one-letter convention given in the legend to Fig. 8.

EFFECT OF LIPID AND PROTEIN STRUCTURES ON EPIDERMAL PERMEABILITY

Effects of lipid composition

A number of experimental approaches satisfied earlier investigators that the epidermal barrier to water permeability resides in the stratum corneum and not in the lower layers of the skin. Furthermore, the consensus of opinion is that the barrier is provided by the lipids that are present as multiple lamellae between the cells of the stratum corneum. Attention has therefore concentrated on the biophysical properties of the stratum corneum lipids and on factors that may enhance or peturb barrier efficiency. It is intuitively obvious that the very-long-chain, highly saturated fatty acids that are present free, esterified, and in amide linkage in the stratum corneum are ideally suited for the construction of a barrier to water permeation. The role of cholesterol may be less obvious, but is widely recognized as providing plasticity in gel-phase bilayers (37), which may be a valuable property in lipid lamellae that are subject to frequent flexing and deformation. The free fatty acids may be essential for the formation and maintenance of bilayers composed of relatively nonpolar lipids such as ceramides and cholesterol. It has been shown, for instance, that liposomes cannot be formed from these lipids unless fatty acids or cholesteryl sulfate are present (38).

Control over the composition of the stratum corneum lipids appears to be maintained by the strong exclusion of exogenous lipids. Most of the lipids appear to be endogenous to the epidermis, although obviously the linoleate that is present in acylceramide and acylglucosylceramide must be obtained from the diet. Linoleate is strongly preferred for incorporation into these two lipids, but is excluded from all others (30). On the contrary, palmitate is strongly excluded from the positions for which linoleate is preferred, and oleate, although a major circulating fatty acid, replaces linoleate to a significant extent only under conditions of essential fatty acid deficiency (39, 40).

An addition to these selective processes, the composition of stratum corneum lipids is affected by the enzymatic processes that take place during and even after conversion of the epidermal cells to corneocytes. Most notable are the elimination of all phospholipids and glycolipids from the stratum corneum. This renders the lipid bilayers resistant to swelling in water, which would certainly be a biological advantage in avoiding maceration of the epidermis under moist conditions. Furthermore, the elimination of nutrient phosphorus and sugars from the skin surface must improve its resistance to bacterial colonization.

The composition of the stratum corneum lipid mixture, which has a chain-melting temperature in excess of 60°C, ensures that the bilayers will remain in the gel state under all conceivable ambient temperatures, and will therefore remain highly resistant to water permeation.

Effects of protein structures

The densely packed keratin fibers of the corneocyte interior, and the dense, cross-linked protein envelope, may not provide resistance to water permeation but might be expected to be resistant to the permeation of larger molecules, especially those that are lipid-soluble. This may have limited biological significance but is increasingly important pharmacologically with regard to transcutaneous delivery of drugs. However, the recent discovery that the corneocyte possesses a

lipid envelope bound to the exterior of the protein envelope suggests that the corneocyte may indeed have significant resistance to the entry of water and other small polar molecules. If so, this resistance is destroyed by chloroform–methanol extraction, even though the bound lipid is not removed. This could conceivably occur by denaturation of the protein sheet that supports the lipid envelope, rendering the lipid structure permeable. Certainly an intercellular route must be eliminated by the solvent extraction, after which the corneocytes are tightly apposed to each other, with their lipid envelopes in intimate contact (11, 19).

With the discovery of the corneocyte lipid envelope (18, 19), it became apparent that the intercellular lipid lamellae are in contact, not with the protein of the corneocyte envelope, but with a lipid layer resembling a bilayer in dimensions and surface properties. It was anticipated that this might have significant effects on the formation and properties of the intercellular lamellae. To investigate this possibility, isolated solvent-extracted corneocytes were added to a suspension of liposomes prepared from stratum corneum lipids (41). Electron microscopy revealed that during the subsequent few hours, liposomes tended to adsorb onto the corneocyte lipid envelope and then to become transformed into multiple bilayer sheets. This indicates that the corneocyte lipid envelope may act as a template in the slow formation of lipid sheets from the lamellar disks that are extruded into the intercellular space. It can also be deduced that this template action will tend to maintain the integrity of the intercellular lamellae in the face of perturbing effects. This resistance may extend to the prevention of penetration of less polar lipids from sebum, which does not appear to significantly disrupt the epidermal barrier.

In addition to the probable effects on permeability, it may also be deduced that the corneocyte lipid envelope may have an important influence on stratum corneum cohesion (11). The formation of an intervening lipid monolayer between corneocyte envelopes, in regions where unbound lamellae are absent, indicates the interdigitation of chains everted from both envelopes, which might be expected to contribute to cohesion. Similar interdigitation might provide cohesion between corneocyte envelopes and adjacent lipid bilayers. This might be particularly important over the large proportion of the corneocyte surface that is devoid of functional desmosomes (6). It may contribute to, but probably not exceed, the cohesion provided by desmosomes on the periphery of the corneocytes.

Whether proteins contribute to corneocyte cohesion other than in the form of desmosomes or as a substrate for the lipid envelope is still an open question. Evidence against such a role for proteins or proteolipids includes the observation that treatment of stratum corneum with detergents, or even with hot water, results in the separation of corneocytes over their entire flat surfaces, with contact being maintained only at the peripheries where functional desmosomes are present (11). However, solvent extraction of stratum corneum does not result in separation and expansion of the intercellular spaces, but instead produces irreversible cohesion between the lipid envelopes of the cells. It has been proposed that this tight union might result from a new form of lipid interdigitation (11), in which sphingosine chains are exchanged between apposed lipid envelopes, as shown in **Fig. 10**.

Electron micrographs reveal that the close apposition (and presumed interdigition between lipid envelopes) is also present in stratum corneum that has been heated above 80°C. At this temperature, which is above the bulk melting temperature of the stratum

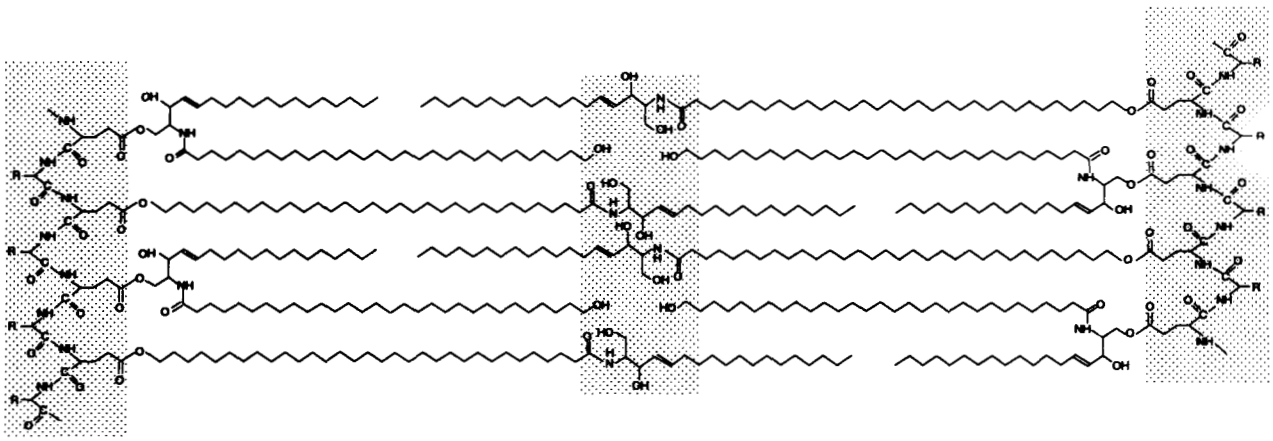


Fig. 10. Proposed interdigitation of lipid chains from apposing corneocyte lipid envelopes after extraction of all nonbound lipids. The resulting intercorneocyte cohesion prevents their separation by solvents, water or proteases (11).

corneum lipids, the unbound lipids appear to coalesce into large pools between the cells, and the lipid envelopes adopt the close apposition that is seen as two broad lucent bands, instead of the broad/narrow/broad pattern that is always seen in native stratum corneum where intercellular lamellae are absent (11). This observation would seem to have little biological significance, but does underscore the profound effects that the compound lipid/protein envelope of the corneocyte may have on the properties of the epidermis.

BOUND LIPIDS OF MAMMALIAN HAIR

Like the epidermal corneocyte, the cuticular cells of mammalian hair also possess a lipid envelope. However, the bound lipids of hair consist of branched-chain fatty acids, predominantly 18-methyleicosanoic acid, and cholesteryl sulfate (42, 43). It is tempting to speculate that the branched-chain acids, and the branched side chain of cholesterol, might provide cohesion between the cuticular cells in a manner similar to the protein cohesion known as the leucine zipper, which depends upon interdigitation of leucine side chains between α-helices (44).

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