Skin Lipids: An Update

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The stratum corneum lipids, responsible for the epidermal water barrier, consist principally of ceramides, cholesterol, cholesteryl sulfate, and free fatty acids. These lipids are arranged in multiple intercellular lamellae that provide an efficient water barrier because of the crystalline array of the straight and predominantly saturated lipid chains. Interlamellar linkages provided by lipids based on 30-carbon ω-hydroxyacids may be responsible for holding together the intercellular lamellae as well as for assembly of the lamellar granules of the granular cells. The normally ordered exfoliation of corneocytes as they arrive at the surface seems to require hydrolysis of the cholesteryl sulfate to free cholesterol.

The sebaceous glands secrete continuously, producing sebum that consists predominantly of triglycerides, wax esters, and squalene. High rates of sebum production per sebocyte result in low levels of linoleate in the sebaceous esters, subjecting the follicular epithelium to essential fatty acid deficiency and the characteristic hyperkeratosis that results in comedo formation. Suppression of sebum production by drugs elevates sebum linoleate concentration and relieves follicular hyperkeratosis. Thus, sebum continues to be a prime suspect in the crime of acne. Low levels of sebaceous gland activity are not correlated with the occurrence of dry skin. *J Invest Dermatol 88:2s–6s, 1987*

Index Medicus contains the information that A. M. Kligman has produced over 80 publications since 1978. Inspection of these reveals him to be as erudite and contentious as ever. From the breadth of his publications, a great many dermatologic investigators must feel that Dr. Kligman has a special interest in their field, and the present authors are no exception. Although this review of recent progress in the field of skin lipids principally covers the contributions from this laboratory over the past 2 years, we have no suspicion that Dr. Kligman requires informing of these developments. It is hoped therefore, that this summary will be perceived as homage rather than as edification.

EPIDERMAL LIPIDS

Knowledge of the composition and physiologic role of the epidermal lipids has undergone rapid development in the 10 years since they first were illuminated by the works of Gray and colleagues, and the field is now ripe for exploitation. In reviewing these developments, our most vivid image is of a practical demonstration by Kligman of the efficiency of the epidermal water barrier: a sheet of isolated stratum corneum tied over the orifice of an inverted vial effectively prevented evaporation of the water within [1]. Recent investigations have not changed the long-standing perception that the epidermal barrier is located in the stratum corneum, and that it consists of lipids.

Structure of the Water Barrier ". . . *all* the layers of the horny layer contribute to its barrier function." [1]

By the mid-twentieth century, it was well established that the epidermal barrier to water loss resided within the stratum corneum; however, there still existed controversy concerning the specific location of the barrier within this epidermal compartment. On the basis of carefully performed Scotch tape–stripping experiments, Kligman made the first suggestion that all of the layers of the stratum corneum contributed to its barrier function [1]. At the time at which this proposal was made, the detailed microanatomy of the epidermis was still largely unknown; however, it is now apparent that the spaces between the layers of horny cells are filled with multiple lipid bilayers that constitute the water barrier, and indeed, the entire stratum corneum does constitute the barrier.

The stratum corneum extracellular membranes are composed mainly of ceramides, free fatty acids, and cholesterol, with small proportions of triglycerides, steryl esters, and cholesteryl sulfate. Phospholipids and glycolipids are absent. The ceramides, which constitute about 40% of human stratum corneum lipids, are a structurally heterogenous and complex mixture, the detailed component structures of which have only recently been elucidated [2,3]. Epidermal ceramides contain sphingosine or phytosphingosine bases in amide linkage with nonhydroxy, α-hydroxy- or ω-hydroxyacids. In the ceramides that contain ω-hydroxyacids, the ω-hydroxyl groups are esterified with fatty acids, predominantly linoleic, to form unusual *O*-acylceramides. These may have a specialized function in epidermis, as discussed below.

Like other membrane-forming lipids, the epidermal ceramides each have both a polar head group and a nonpolar tail. The polar ends of these molecules are formed by the functional groups attached to the first 4 or 5 carbon atoms of the long-chain bases. These functional groups include the amide group, 2 or 3 free hydroxyl groups, and in some cases a *trans* olefinic bond between the fourth and fifth carbons of the long-chain base. The hydrophobic tails, which make up the interior of the bilayer, range from 14–30 carbon atoms in length, with the most abundant chain lengths being 24, 26, and 28 carbons.

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The presence of lipid bilayers in the stratum corneum is not in itself sufficient to account for the water impermeability of the stratum corneum, because typical biologic bilayers are known to permit the free passage of water. The key to the water barrier is in the array of hydrophobic chains that make up the interior of the extracellular bilayers. These hydrocarbon chains are straight and almost entirely saturated. This lack of structural perturbation among the hydrophobic chains provides the opportunity for the formation of bilayers with closely packed interiors, and such highly ordered bilayers are ideally suited for water impermeability. The high degree of chain length variation among the epidermal ceramides may also be significant in that this could result in interdigitation of the chains in the center of the bilayer. This interdigitation may add to the stability of the membranes. It is also noteworthy that the minimal unsaturation of the stratum corneum lipids limits the opportunity for oxidative damage on exposure to the atmosphere.

The *O*-acylceramides, though small in amount, are thought to serve a specialized function of major importance in maintaining the multi-bilayer organization of the barrier lipids [4]. This sphingolipid consists of a sphingosine base with an amide-linked long-chain ω-hydroxyacid and linoleic acid esterified to the ω-hydroxyl group. The major ω-hydroxyacids are the 30-carbon saturated and 32-carbon monenoic species. It has been proposed that this unusual acylceramide serves as a molecular rivet to hold together the multiple broad bilayers found within the stratum corneum, a suggestion based largely on the dimensions and geometry of the acylceramide molecule. Examination of molecular models leads to the conclusion that this molecule prefers an extended configuration in which the ω-hydroxyacyl chain extends all the way through a typical lipid bilayer and the linoleoyl chain is free to insert into a second closely apposed bilayer. This sort of interaction can account for the observed stacking of the extracellular bilayers of the stratum corneum.

Epidermal Differentiation ". . . the raison d'etre of the viable epidermis is to make the horny layer." [1]

The lipids of the barrier layer are synthesized throughout the viable epidermis, where they are packaged in lamellar granules. The contents of the lamellar granules appear to be stacks of flattened lipid vesicles, and their lipid components include an acylglucosylceramide, which has been shown to be structurally the same as the acylceramide described above except that it bears a β-D-glucosyl group on the primary hydroxyl of the sphingosine [4]. It has been suggested that this molecule may provide the driving force for assembly of the stacks of flattened disks that are seen within the lamellar granules. This proposal is essentially the same as the molecular rivet hypothesis described above for the acylceramide.

In the uppermost viable cells, the lamellar granules move to the apical surface of the cell. The bounding membranes of the granules then fuse with the plasma membrane and the lamellar contents are extruded into the extracellular space. After extrusion, the lipids undergo a rearrangement to form the broad multilamellar sheets that constitute the water barrier. At some point the acylglucosylceramide of the living layers loses glucose to become the acylceramide of the stratum corneum. It is not clear what function this removal of glucose may have, because both lipids probably perform similar structural roles in holding lipid bilayers together.

Support for the hypothesis that acylceramides and acylglucosylceramides can act as molecular rivets has come from several distinct observations. First, incorporation of acylglucosylceramides causes synthetic liposomes to flatten and aggregate to resemble stacks of coins [5]. These stacks of flattened liposomes bear a striking resemblance to the contents of lamellar granules. Second, it has been shown that the acylglucosylceramides and acylceramides are unique to keratinizing, barrier-forming epithelia. They are not found in water-permeable regions of oral epithelia [6] or in other organs [7]. Finally, the *O*-acylsphingolipids have thus far been found only in the epidermis of those vertebrates that produce epidermal lamellar granules. They are absent from frogs, fish, and invertebrates (Wertz and Downing, unpublished observations).

In essential fatty acid–deficient animals, oleic acid is substituted for linoleic acid in the *O*-acylsphingolipid structures [8]. Under these conditions, the contents of the lamellar granules appear to be amorphous rather than striated, the extracellular spaces of the thickened horny layer contain only sparse, fragmented membranous structures, and water barrier function is severely impaired. Thus, the essential function of linoleic acid in the epidermis would appear to be in the synthesis of the *O*-acylsphingolipids that function in formation and maintenance of the water barrier. However, it is not yet clear why substitution of oleic for linoleic acid seems to produce nonfunctional molecular rivets.

Desquamation ". . . near the surface, the hypothetical cement is no longer an effective glue, and the inevitable cracking leads to desquamation." [1]

In addition to their role in the water barrier, epidermal lipids have been implicated as determinants of the cohesion-dishesion properties of the stratum corneum. Specifically, cholesteryl sulfate has been implicated as an intercellular cement substance, the hydrolysis of which coincides with the desquamation of cells from the surface [9]. These results have been further substantiated by comparison of the lipids from intact and desquamated stratum corneum collected from mouse ear explants maintained in culture for several weeks [10]. Thus, cholesteryl sulfate hydrolysis accompanies desquamation and may be an essential part of the desquamation process. Also, it has been shown that cholesteryl sulfate constitutes 20% of the total lipid in horse hoof [11]. This supports the contention that cholesteryl sulfate is an intercellular cement, since the hoof is a keratinized tissue that does not undergo desquamation.

Evolutionary Significance of the Water Barrier "Without this delicate but tough outermost wrapping . . . . dry-land life would be impossible." [1]

Although comparative studies of the water barrier, which were initiated by Kligman, are still incomplete, it is appreciated that the appearance of a watertight skin was a major evolutionary step and a requirement for the development of terrestrial life. In this regard, it is significant that the *O*-acylsphingolipids, which are thought to be of major importance for formation of the water barrier, have been found in all terrestrial mammals thus far examined. Acylceramides and acylglucosylceramides have also been found in chicken epidermis [12,13], but are lacking from whales, and from frogs, fish, and the several invertebrates that have been studied (Wertz and Downing, unpublished observation). Those species in which *O*-acylsphingolipids are present also contain relatively high proportions of other ceramides.

The cast skins from snakes appeared to contain acylceramides, as judged by thin-layer chromatographic comparisons, but this identification must be considered tentative because the material has not been isolated for detailed study. Acylglucosylceramide is absent from cast skins of snakes and from full-thickness skins excised from freshly killed snakes. However, snake skin contains lamellar granules only during a brief interval of the growth and shedding cycle. The lipids present during this period have not yet been studied.

In addition to the *O*-acylsphingolipids, several unusual lipids that may be relevant to the evolution of the water barrier are found in chicken epidermis [13]. These include glucosylsterols and acylglucosylsterols in which the sterols consist of cholesterol and cholestanol. The principal esterified fatty acid in the latter lipid is palmitic acid. These lipids have polarities similar to glucosylceramides and ceramides, respectively. The acylglucosylsterols would seem to have the structural requirements necessary to serve as an alternative to the *O*-acylsphingolipids as molecular rivets.

Much further work is necessary to evaluate the potential of these lipids for participation in the epidermal membrane system and to elucidate the molecular and physical structures of importance to the function of the stratum corneum. "We shall be greatly rewarded if we learn the secrets of its construction." [1]

SEBUM

It has long been taught that sebum has no biologic function in humans [14], and developments up to the present time have not altered this concept. Nevertheless, it has frequently been demonstrated that sebum secretion rate is under hormonal control, and its composition is species specific and under genetic regulation. These factors indicate that in other species, at least, there could be functions other than simply as a waterproofing material, but as yet it has not been established what these might be. Current studies of sebum, as in the past, are predominantly concerned with the role of sebum as a pathogenetic factor in acne.

Mechanism of Sebum Secretion "The gland secretes continually." [14]

Before the studies of Kligman it seems to have been universally accepted that sebaceous glands secrete sebum only until the "necessary" thickness of surface lipid film is produced, and then the glands shut down. This deduction was based on the repeated demonstration that when the skin is defatted, the lipid film is restored in a matter of hours, and then no further lipid accumulates. Kligman confirmed this observation, but then came to a quite revolutionary conclusion: "After a few hours the skin surface becomes saturated and the excess flows away or is otherwise lost." [15] This simple idea made possible the now well-established dogma that "the sebaceous gland functions continuously, without regard to what is on the surface." [15]

A related, time-honored observation is that when sebum is collected at short intervals from a given anatomic site, more sebum is collected at the outset than at subsequent times, and the amount obtained gradually declines to a constant quantity, which is regarded as the true rate of sebum synthesis. In this instance, Kligman accepted an earlier view that this phenomenon results from a large reservoir of sebum available near the surface of the skin. "Huge amounts of preformed sebum are stored in the capacious ducts of sebaceous follicles." [15] Kligman also recognized that the reservoir was not readily depleted. "Thoroughly defatting the surface by keeping 5 ml of ether in a glass cup on the forehead for 5 minutes does not dissolve out the stored sebum." [15] The reservoir phenomenon may be partially responsible for the repeated observation that several collections performed over a given period at the same site will yield more sebum than a single collection after sebum has been allowed to accumulate for the same total time after defatting. It was proposed that this phenomenon results from avoidance of some of the loss of sebum that occurs once the surface has become saturated, but it can be argued that repeated rapid refatting might draw down the reservoir more than a single such occurrence. Probably both mechanisms are in operation in this instance.

Our own studies have shown that a dry absorbent in intimate contact with the skin surface will eventually equilibrate the sebum reservoir [16]. This process requires at least 12 h, after which successive 3-h collections produce virtually constant amounts of sebum. The collection device consists of a gel of bentonite clay in water, which is applied to the skin with a disk of nonwoven fabric embedded in the gel. The gel dries to an adherent, absorbent film within 15 min, after which it readily absorbs sebum. After a given time, usually 3 h, the disk and adherent clay are peeled off, leaving a clean, lipid-free surface. The sebum is then extracted from the disk with ether and quantified by thin-layer chromatography. When successive 3-h collections are made on the forehead after first cleaning the area with soap and water, the quantity of sebum recovered decreases rapidly, but usually does not reach a constant level until the end of the fourth collection (12 h). The constant quantity of sebum then collected in succeeding periods is taken to represent the rate at which sebum is being produced by the glands.

In practice, the reservoir equilibration is accomplished by having the subjects wear a patch of the bentonite clay on their foreheads overnight. The depletion patch is then removed and immediately replaced by a fresh application of the clay, into which a 2-cm collection disk is embedded. Using this procedure, measurements of sebum secretion rate have been made over 3-h periods in a wide variety of subjects, including acne patients and controls [17], children [18], and adults over the entire life span, as discussed later. These studies have not appreciably altered the view of sebum secretion dynamics as expressed by Kligman: "*It should be emphasized that the production of sebum is inseparably linked with the process of growth or reproduction of the glandular cells.* It is only by the division and multiplication of such cells that sebum can be produced." [15] However, certain quantitative data have now been produced that help to flesh out the basic framework of sebaceous gland physiology.

To determine the time between synthesis of sebum and its appearance on the skin surface, radioactive acetate was injected intradermally in the foreheads of 3 volunteers and surface lipid was collected and assayed for radioactivity daily for 2 weeks. A sharp maximum of radioactivity appeared on the surface 8 days after the injections, indicating that this is the secretion time of sebum in humans [19]. Each of the principal constituents of human sebum (wax esters, triglycerides, and squalene) showed the same secretion times. Similar studies with animals [19–21] showed secretion times of 5 days for guinea pigs, 6 days for sheep, and an astonishing 21 days for horses.

In a related series of experiments [22], subjects who were to undergo hair transplants were given a single intradermal injection of radioacetate at a prospective transplant site each day for 2 weeks. On the final day, all of the sites were harvested at one time, the lipids were recovered from the punch biopsies, and the amount and distribution of radioactivity was assessed. As expected, the quantity of radioactivity recovered began to decline rapidly about 5 days after injection as labeled lipid began to be lost to the skin surface. However, a curious transfer of label was noted between phospholipids and wax esters. This was interpreted as initial incorporation of label into the fatty acids of phospholipids, which are synthesized by the glands to provide for the enormous increase in cell membranes required to accommodate cell expansion. Then, in the senescent cells, phospholipids are broken down and the fatty acids released are free to be incorporated into the wax esters. Phospholipid degradation is implicit in the knowledge that sebum does not contain such lipids. The transformation of sebaceous phospholipids into nonpolar sebaceous lipids has been demonstrated also in the horse, where the end products are giant 34-carbon ω-lactones that are the principal sebaceous lipid in this species. The occurrence of the appropriate branched chain fatty acids in the sebaceous phospholipids of both human and horse has been confirmed [23].

A beneficial consequence of having determined the secretion time of sebum has been an independent method for measurement of sebum secretion rate. If the average secretion time of sebum is 8 days, then at all times the skin contains 8 days worth of sebum synthesis. It requires only a punch biopsy of known cross-section and measurement of its sebum content to be able to calculate an absolute measure of the rate of sebum synthesis. This has been done with biopsies obtained from hair transplant patients [24], but the method is not otherwise practical for human subjects. Nevertheless, the method might prove useful and relatively convenient for animal studies.

Sebum Composition "The diversity of fatty acids is staggering, ranging in chain length from about C_6 to C_{22}, including odd and even numbered, branched and unbranched, saturated and unsaturated amounting to literally hundreds of discrete substances." [25]

Since this passage was written, the known complexity of human sebum fatty acid composition has increased considerably, due largely to the efforts of Nicolaides and coworkers. Furthermore, it is now known that the fatty acid composition can differ greatly between individuals [26]. Studies with monozygotic and dizygotic twins have indicated that sebum fatty acid composition is genetically determined [27], although sebum secretion rates also have some influence on composition [28].

The major classes of human skin surface lipids (triglycerides, wax esters, and squalene) are still regarded as sebaceous in origin, whereas free cholesterol is suspected to originate mainly from the epidermis. Cholesteryl esters may arise from both sources [29]. These questions become important when considering the composition of sebum in relation to acne.

Sebum and Acne "Sebum plays a central, though not fully defined role in the pathogenesis of acne."[14]

Numerous studies have indicated a connection between sebum secretion rates and the occurrence of acne. The relationship can also be inferred from the knowledge that acne usually first begins when the sebaceous glands blossom at puberty. Also, the occasional cases of acne seen in infants occur at a time when sebum secretion rates are known to be elevated as the result of the hormonal status of the newborn. Furthermore, "Acne regresses promptly when sebum secretion is suppressed by any means." [14] Inhibitors of sebum secretion, including estrogens, antiandrogens, eicosatetraynoic acid, 13-*cis*-retinoic acid, and x-rays, all alleviate acne to a varying degree; the greater the inhibition the more profound is the clinical response. In spite of this evidence, there is still no firm conclusion as to how sebum is involved in the pathogenesis of acne. Nevertheless, there are indications that sebum is involved in comedo formation. Kligman demonstrated, for instance, that sebum is comedogenic in the rabbit ear test [30], and he was of the opinion that it was the free fatty acids that were the most active. Our recent studies support this suspicion and have suggested a mechanism by which the effect might be produced [31]. One of the effects of an increase in sebum secretion is to decrease the concentration of linoleic acid in the sebaceous lipids [32]. As a result, the follicular epithelium is bathed in essential fatty acid–deficient lipid, and responds with the hyperkeratosis that is characteristic of essential fatty acid deficiency. Reduction in sebum secretion rate by treatment with an antiandrogen [33] or 13-*cis*-retinoic acid (our unpublished observations) restores the levels of linoleate, and follicular keratinization returns to normal. It has been inferred that the linoleate concentration in sebum reflects the amount of sebum synthesized per sebocyte rather than per gland. Although these two parameters normally are related, it seems that in the immediately prepubertal period the lipogenic activity of individual sebocytes increases before there is much increase in the surface secretion rate. Linoleate concentration declines during this period, coinciding with the initiation of comedo formation. We speculate that this constitutes the mechanism by which sebum is involved in the pathogenesis of acne [31].

Sebum and Dry Skin ". . . skin can be healthy and have charming cosmetic properties in the virtual absence of sebum." [14]

Kligman drew attention to prepubertal children, who produce almost no sebum, to support his thesis that skin does not depend upon sebum for maintaining its barrier to water loss: ". . . there can be no doubt of the insignificance of sebum as a waterproofing material." [14] Our recent studies at the other end of the human age spectrum have supported this conviction. In a survey of sebum secretion rates and the incidence of dry skin among subjects aged 65 to 97, no correlation was found between sebaceous gland activity and the presence or severity of dry skin [34]. Kligman recognized that sebum could mask the scaliness of dry skin without producing any actual change in the condition: "Sebum, like any oil, has some emollient or smoothing effect when a sufficient quantity is rubbed into dry, scaling skin." [14] In spite of the clear inference to be drawn from the cutaneous characteristics of children and the experimental data obtained from the elderly, it remains difficult to dispel the myth that low sebum secretion rates *cause* dry skin. It is a rare individual who realizes that "dry" is not the obverse of "oily."

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