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REVIEW

Barrier function regulates epidermal lipid and DNA synthesis

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Summary The stratum corneum, the permeability barrier between the internal milieu and the environment, is composed of fibrous protein-enriched corneocytes and a lipid-enriched intercellular matrix. The lipids are a mixture of sphingolipids, cholesterol and free fatty acids, which form intercellular membrane bilayers. Lipid synthesis occurs in the keratinocytes in all nucleated layers of the epidermis, and the newly synthesized lipids are delivered by lamellar bodies to the interstices of the stratum corneum during epidermal differentiation. Disruption of barrier function by topical acetone treatment results in an increase in the synthesis of free fatty acids, sphingolipids and cholesterol in the living layers of the epidermis, leading to barrier repair. Cholesterol and sphingolipid synthesis are regulated by the rate-limiting enzymes HMG CoA reductase and serine palmitoyl transferase (SPT), respectively. Acute barrier disruption leads to an increase in both enzymes, but with a different time curve: increase in HMG CoA reductase activity begins at 1·5 h, whereas the increase in SPT activity occurs 6 h after barrier impairment. Topical application of HMG CoA reductase or SPT inhibitors after acetone treatment delays barrier repair, providing further evidence for a role of cholesterol and sphingolipids in epidermal barrier function. Repeated application of lovastatin to untreated skin results in disturbed barrier function accompanied by increased DNA synthesis and epidermal hyperplasia. Therefore, we have examined the specific relationship between barrier function and epidermal DNA synthesis. After acute and chronic disturbances not only lipid, but also DNA synthesis, is stimulated. Thus, stimulation of DNA synthesis leading to epidermal hyperplasia may be a second mechanism by which the epidermis repairs defects in barrier function. The link between barrier function and both lipid and DNA synthesis is supported further by occlusion studies. Artificial barrier repair by latex occlusion prevents an increase in both lipid and DNA synthesis. In addition, increased epidermal lipid and DNA synthesis in essential fatty-acid deficiency can be reversed by topical applications of the *n*-6 unsaturated fatty acids, linoleic or columbinic acid. These studies may be of relevance in understanding the pathogenesis of hyperproliferative skin diseases, such as ichthyosis, psoriasis, atopic dermatitis, and irritant contact dermatitis.

The major function of the epidermis is to form a permeability barrier which prevents excessive loss of the body fluids required for terrestrial life. This barrier is located mainly in the stratum corneum, which is organized into a heterogeneous two-compartment system of protein-enriched cells embedded in lipid-enriched intercellular membrane bilayers.[1–6] The lipid synthesis required for barrier function occurs in the keratinocytes in all nucleated layers of the epidermis. The lipids are stored and delivered by lamellar bodies (Odland bodies, keratinosomes). Lamellar body formation is first visible ultrastructurally at the level of the spinous layer. In the upper granular layer the contents of the lamellar bodies are secreted into the intercellular domains of the stratum corneum.[1–7] The lamellar bodies in the living epidermal layers contain cholesterol, phospholipids and glucosylceramides.[1–7] Hydrolytic enzymes, which are also located in the lamellar bodies, convert phospholipids and glucosylceramides to free fatty acids and ceramides after secretion.[1–7] The lipids in the stratum corneum contain approximately equal quantities of ceramides, cholesterol and free fatty acids, as well as lesser amounts of non-polar lipids and cholesterol sulphate.[1–7] This mixture forms the membrane bilayer system, which regulates barrier function.

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Barrier function and epidermal lipid synthesis

To determine the regulation of barrier function, hairless mice were treated with a solvent (acetone) and a surfactant (sodium dodecylsulphate [SDS]). These treatments remove stratum corneum lipids, leading to a marked disruption of barrier function (increase in transepidermal water loss). Measurement of the incorporation of tritiated water into epidermal lipids revealed an increase in biosynthesis of cholesterol, total non-saponifiable lipids (NSL) and fatty acids compared with normal, beginning 1 h after barrier disruption. Lipid biosynthesis in the dermis was unchanged. Chronic barrier disruption by administration of an essential fatty-acid-deficient diet (EFAD) also enhanced epidermal cholesterol, total NSL and fatty acid biosynthesis in comparison with normal.[8–10]

To determine if the increase in epidermal lipid synthesis is specifically linked to barrier function, skin sites in hairless mice with increased TEWL after acetone or SDS treatment were occluded with a water-vapour-impermeable latex wrap. This artificial barrier repair prevented the expected increase in epidermal lipid biosynthesis. Moreover, in EFAD mice occlusion for 1–3 days resulted in a reduction in lipid synthetic rates towards normal.[8–10]

Although various studies support a role for sphingolipids and fatty acids in barrier function, the evidence for the importance of cholesterol is particularly persuasive. Biophysical studies have shown that the cholesterol content is critical for the permeability of water through lipid layers in model systems,[11] and also that the skin is an active site of cholesterol biosynthesis in both rodents and primates.[12–14] Within the epidermis, cholesterol synthesis occurs in both the basal (proliferating) and suprabasal (differentiating) cell layers.[12] Epidermal cholesterol synthesis seems to be independent of circulating cholesterol levels, presumably because of the paucity of low-density lipoprotein (LDL) receptors.[4,5,12–14]

In contrast with cholesterol, which appears to be generated within the epidermis, circulating fatty acids accumulate in the epidermis. Not only dietary fatty acids become incorporated into cell membrane lipids under certain pathological circumstances,[15] but the epidermis also lacks Δ^5 and Δ^6 desaturase and, therefore, it must obtain essential fatty acids (linoleic acid), and the arachidonic acid required for eicosanoid, from the circulation.[16]

Cholesterol synthesis in mammalian systems is regulated by the key enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase),[17,18] and it has been confirmed that this process also occurs in the epidermis.[19] When disturbed barrier function was achieved by acetone, SDS solution or tape stripping, there was a 43–60% increase in HMG CoA reductase activity compared with control. The level of increase correlated directly with the extent of disturbance in barrier function measured as TEWL. The increase in enzyme activity began after 1·5 h, reached maximal levels after 2·5–4·5 h, and returned towards normal as barrier function also returned to normal (after 4·5–7·0 h). After SDS treatment, both barrier recovery and HMG CoA reductase activity declined more slowly than after acetone treatment.[19] This difference may be due to the fact that acetone mainly extracts lipids and quickly evaporates from the skin surface, whereas SDS has a prolonged effect by binding epidermal proteins, in addition to the extraction of lipids.[20]

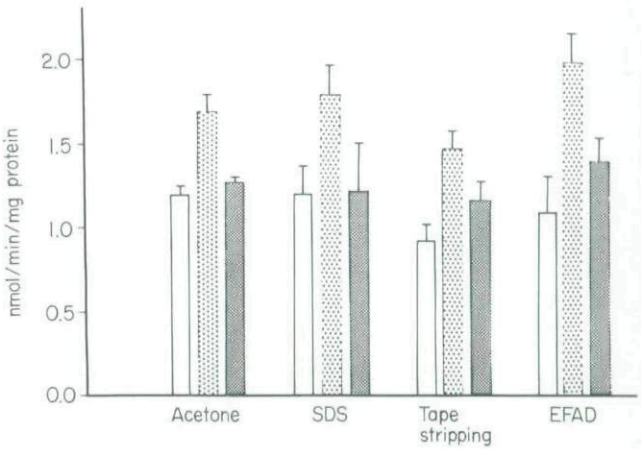

Figure 1. Effect of various treatments ± occlusion on HMG CoA reductase activity. Mice were treated with acetone or SDS (TEWL 8–10 mg/cm² h), and immediately occluded with a tightly fitting latex wrap. Control mice were treated with saline and left exposed to air. After 2·5 h the skin was excised, the epidermis was separated from the dermis, and HMG CoA total activity was measured. Tape-stripped animals were studied 4·5 h after treatment. EFAD mice were occluded for 3 days. Control mice for the EFAD experiment were re-fed a diet containing linoleic acid. Data are mean ± SEM (n = 4–6).[19] □, control; ▨, treated; ▩, treated + occlusion. (Adapted from Proksch *et al. J Clin Invest* 1990; **85**: 874–82.)

Artificial barrier repair by occlusion after treatment with acetone or SDS treatment, or tape stripping largely prevented the expected increase in HMG CoA reductase activity. In EFAD mice, occlusion for 3 days markedly reduced the increased (+83%) enzyme activity (Fig. 1).

HMG CoA reductase activity is dependent both on the total quantity of enzyme present and on its activation state. The activity can be modulated by a reversible phosphorylation–dephosphorylation process with the phosphorylated and dephosphorylated forms of the

enzyme in an inactive and active state, respectively.[17,18] In normal epidermis 46 ± 2% of the enzyme is in the active (dephosphorylated) form. Barrier disruption by treatment with acetone or SDS, tape stripping, or an EFAD diet not only produced an increase in total HMG CoA reductase activity, but also a shift in the proportion of enzyme in the activated state. The increase was directly proportional to the extent of barrier disruption. Whereas treatment of mice with acetone produced an increase in total enzyme activity 1·5 h after treatment, an increase in activation state occurred much sooner, i.e. by 15 min after barrier disruption. In addition, enzyme activation occurred at lower levels of barrier disruption; the threshold is different, and this may be clinically relevant. Furthermore, occlusion immediately after barrier disruption with acetone or SDS treatment or tape stripping largely prevented the increase in enzyme activation state. Finally, in EFAD mice, where the activation state was increased to 100%, occlusion markedly reduced the proportion of enzyme in the activated state.[19] These results demonstrate that the increase in cholesterol synthesis which occurs in the epidermis after barrier disruption can be attributed both to stimulation of HMG CoA reductase content and modulations in enzyme activation state. The specific relationship of this enhanced activity to barrier function is supported further by the blockade of both increased total enzyme activity and enzyme activation following application of an occlusive wrap.

Cholesterol synthesis and HMG CoA reductase activity in different epidermal layers were also determined. Prior studies have shown that all nucleated layers actively synthesize cholesterol, and that high rates of synthesis persist into the stratum granulosum, whereas most other forms of synthetic activity in this layer are either in decline or absent.[21] Whether such persistance of lipid biosynthetic activity reflects a special role for this layer in providing lipids for barrier function was examined. To obtain individual epidermal cell layer preparations two different methods were used.

1. Hairless mice were injected intradermally with staphylococcal exfoliative toxin. This treatment resulted in separation into upper epidermis, comprising stratum corneum and stratum granulosum, and lower epidermis, comprising stratum spinosum and stratum basale.[22]

2. Incubation of excised skin pieces in dithiothreitol solution (10 mM) resulted in separation into upper epidermis, comprising stratum corneum, stratum granulosum and stratum spinosum, and lower epidermis, comprising the stratum basale alone.

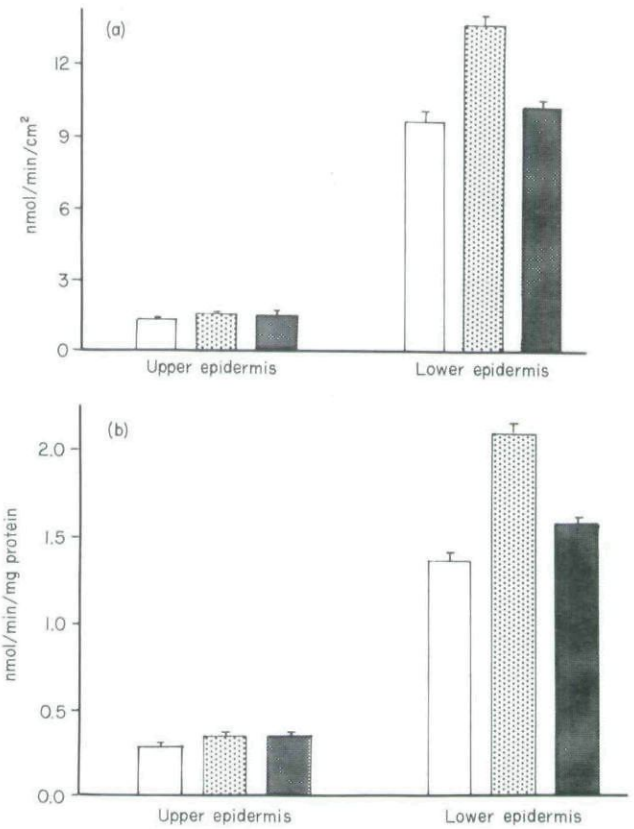

Figure 2. Effect of acetone treatment on the localization of epidermal HMG CoA reductase activity. (a) Total activity (per cm^2); (b) specific activity (per mg protein). Mice were treated with acetone on one flank and with saline on the contralateral flank. One group of mice was immediately occluded with tightly fitting latex wrap. After 2·5 h staphylococcal exfoliative toxin was injected intradermally in both flanks. After 4·5 h the upper and lower epidermis were isolated and HMG CoA reductase activity was determined. Data are mean ± SEM.[23] □, control; ▨, acetone; ▩, acetone + occlusion. (Adapted from Proksch *et al. Biochim Biophys Acta* 1991; **1083**: 71–9.)

In untreated skin, after toxin separation, 13% of both cholesterol synthesis and HMG CoA reductase activity was localized in the upper and 87% in the lower epidermis. After dithiothreitol separation 29% of cholesterol synthesis and enzyme activity was located in the upper and 71% in the lower epidermis. Acute barrier disruption with either acetone or SDS provoked a significant increase in HMG CoA reductase activity in the lower epidermal layers, but only a small change in the upper layers (but in the upper epidermis the enzyme activation state was markedly increased). Correction of barrier function by occlusion with an impermeable latex wrap prevented the increase in enzyme activity in the lower layer (Fig. 2). In contrast, after chronic barrier disruption with an EFAD diet, HMG CoA reductase specific activity was increased only in the upper epidermis. Moreover, occlusion of EFAD mice prevented the

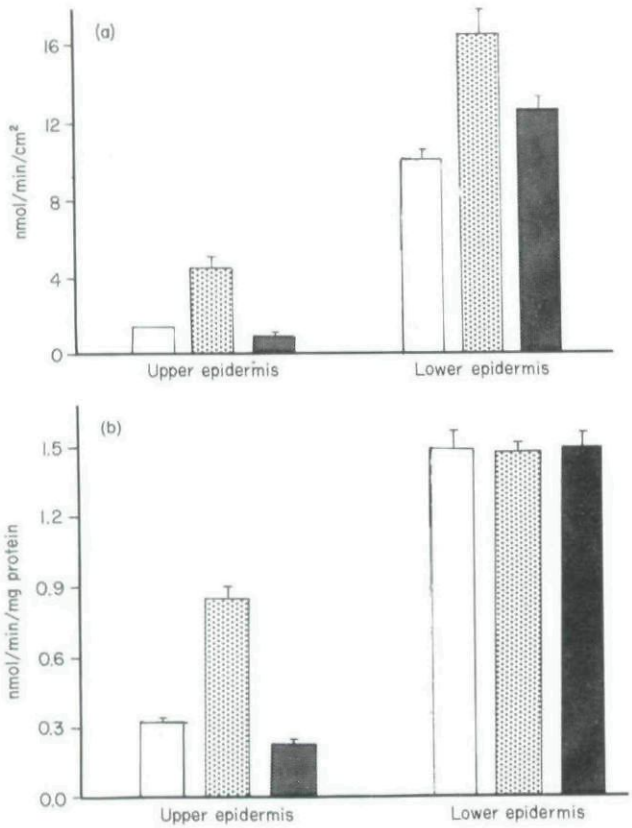

Figure 3. Effect of EFAD-diet on the localization of epidermal HMG CoA reductase activity. (a) total activity (per cm^2); (b) specific activity (per mg protein). Groups of EFAD mice occluded with a tightly fitting latex wrap for 3 days, were injected intradermally with staphylococcal exfoliative toxin. After two hours the upper and lower epidermal layers were isolated, and HMG CoA reductase activity was determined. Data are mean ± SEM.[23] □, control; ▨, EFAD; ▩, EFAD + occlusion. (Adapted from Proksch *et al. Biochim Biophys Acta* 1991; **1083**: 71–9.)

increase in activity in the upper layers (Fig. 3). Finally, application of an occlusive wrap to normal (untreated) mice for 3 days produced a 20–30% decrease in HMG CoA reductase activity in the upper epidermis.[23] In summary, these results show: (i) that in untreated mice about two-thirds of cholesterol synthesis and HMG CoA reductase is localized in the lower epidermis; (ii) that acute barrier disruption stimulates enzyme activity in the lower epidermis; (iii) that chronic insults provoke an increase in enzyme specific activity in the upper layers. The upper epidermis may be most important for the chronic regulation of the cholesterol content of stratum corneum lipid bilayers.

As a further test of the importance of cholesterol synthesis for barrier function, the effect of topical lovastatin on barrier function in the skin was assessed. Lovastatin (mevinolin, Mevacor®) is a potent competitive inhibitor of HMG CoA reductase,[24] and is used for the

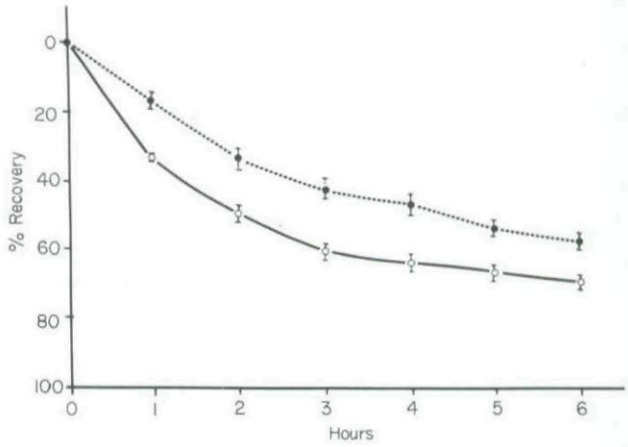

Figure 4. Effect of lovastatin on barrier recovery. Hairless mice were treated topically with acetone to disrupt the barrier, followed by either lovastatin or vehicle alone. TEWL was measured hourly. The data are presented as the per cent recovery of TEWL, and are expressed as mean ± SEM. $n = 45$ for control, $n = 50$ for lovastatin; $P < 0{\cdot}001$ for all time points.[25] –●–, lovastatin; –○–, control.

treatment of systemic hypercholesterolaemia. After a single topical application of lovastatin to acetone-disrupted skin, the normal rapid return of cholesterol to the stratum corneum and recovery of barrier function was impaired. Measurement of the TEWL revealed a significant inhibition of barrier function during the first 6 h (Fig. 4). Cholesterol synthesis was reduced by lovastatin, 1–4 h after treatment, by 70–88% in the epidermis and 45–70% in the dermis. As expected, lovastatin did not affect either epidermal or dermal fatty-acid synthesis. When lovastatin-treated animals were simultaneously treated topically with either mevalonate, the immediate product of HMG CoA reductase, or cholesterol, the final end-product of the pathway, the recovery of the barrier was normalized.[25] Histochemical staining with filipin (a specific fluorescence probe for free 3-β-hydroxysterols) in skin treated with acetone followed by lovastatin application, showed a delay in the reaccumulation of histochemically stainable cholesterol in the stratum corneum.[25]

Repeated topical applications of lovastatin to normal untreated hairless mice for 7 days led to a disruption of barrier function, and a progressive increase in transepidermal water loss. The histology of lovastatin-treated skin revealed epidermal hyperplasia accompanied both by accelerated DNA synthesis and increased DNA content. Initially, there was a reduction in epidermal cholesterol synthesis, whereas fatty-acid synthesis was unchanged. With further treatment, however, cholesterol synthesis normalized, and fatty-acid synthesis accelerated greatly, leading to an altered ratio of fatty

acid/free sterol in parallel with the disruption of barrier function. HMG CoA reductase activity was decreased after 1 day. After 5–10 days there was a compensatory increase in enzyme activity, mainly in the upper epidermis (+1600%), but also in the lower epidermis (+30%) (E.Proksch, P.M.Elias, K.R.Feingold, unpublished data). Electron microscopy revealed abnormalities of both lamellar body structure and their deposited, intercellular contents. The deposition of abnormal lamellar body contents resulted in the compartmentalization of lamellar and non-lamellar materials at the stratum granulosum/stratum corneum interface, with increased permeability shown by lanthanum perfusion.[26] Finally, concomitant application of cholesterol reversed the permeability abnormality and the abnormalities in the lamellar body secretory system which occur with the inhibitor alone.

In summary: (i) topical application of lovastatin, a competitive inhibitor of HMG CoA reductase, inhibits epidermal cholesterol synthesis; (ii) after acetone disruption of the barrier, lovastatin impaired the normal return of cholesterol to the stratum corneum and the recovery of barrier function; (iii) repeated topical applications of lovastatin to intact skin produced a progressive defect in the cutaneous permeability barrier—the barrier abnormality may result from an imbalance in epidermal lipid synthesis and stratum corneum lipid composition; (iv) in both the acute and chronic models, lovastatin treatment resulted in defects in lamellar body structure and deposition, associated with abnormalities in intercellular membrane domains in the lower stratum corneum.

In previous studies the evidence for a role of sphingolipids in epidermal barrier function has been largely indirect, and includes the following: (i) sphingolipids represent the predominant lipid species on a weight basis (35–40%) in the stratum corneum intercellular domains;[7,27–29] (ii) these sphingolipids are the principal repository for the highly saturated, very-long-chain (C22:0–C26:0) fatty acids among esterified stratum corneum species;[7] (iii) the majority of epidermal linoleic acid, an essential fatty acid known to be required for cutaneous barrier function,[30–32] is esterified to ceramide at the omega-hydroxy terminus of the N-acyl fatty acid[33–35] and decreased linoleic acid content in essential fatty acid deficiency leads to an abnormal permeability barrier,[36,37] which has been attributed to substitution of oleic for linoleic acid in the epidermal sphingolipids;[38] (iv) topical applications of certain natural and synthetic ceramides correct the abnormal water-retaining properties of solvent- or detergent-extracted stratum corneum.[39]

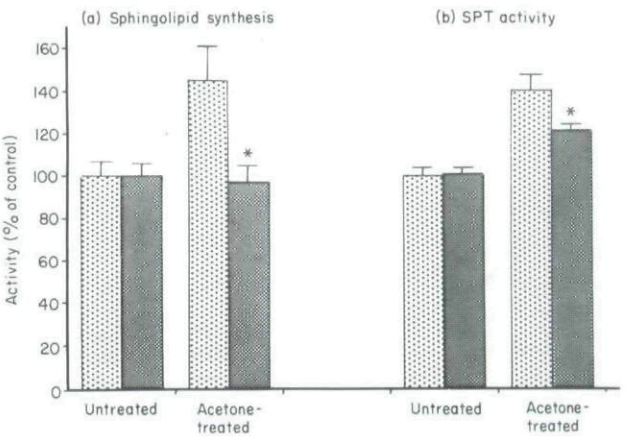

Figure 5. Effect of occlusion on total sphingolipid synthesis and SPT activity after acetone treatment. In each experiment, two groups of animals were treated with acetone to disrupt the barrier. One group was covered with latex wrap, and the second remained uncovered. Activity plotted as a percentage of normal, untreated controls for the same experiment ($n=>4$ animals in each group); mean ± SEM.[43] (a) Sphingolipid synthesis in untreated controls, and 5–7 h after acetone treatment. Results from 2 h [^{3}H]-H_2O incorporation are presented for occluded as well as unoccluded animal groups (*$P<0{\cdot}05$); (b) SPT activity in untreated, normal controls and 6 h after acetone treatment. The occlusive wrap was applied over the entire 6 h in both the untreated and acetone-treated groups. (*$P<0{\cdot}05$). ▨, unoccluded; ▩, occluded. (Adapted from Holleran *et al. J Lipid Res* 1991; **32**: 1151–8.)

Recently, it was shown that both cultured human keratinocytes, and murine epidermis, are rich in serine palmitoyl transferase (SPT),[40] the rate-limiting enzyme of sphingolipid base synthesis,[41,42] and our group have investigated the regulation of sphingolipid synthesis in response to barrier function. Both epidermal sphingolipid synthesis and SPT activity in hairless mice undergoing various types of experimental barrier disruption were assessed.[43] First, the incorporation of [^{3}H]-H_2O into sphingolipids was examined at various time points following acetone treatment. One to three hours after barrier disruption, lipid synthesis was unchanged. However, by 5–7 h after acetone treatment, a significant increase in sphingolipid synthesis was observed. Synthesis remained elevated 12–14 h after treatment, and returned towards normal by 22–24 h. Enzymatic regulation of sphingolipid synthesis by SPT showed a similar time curve. SPT activity was increased after 6 h, remained elevated after 9 and after 12 h, and returned to normal by 24 h, following acetone treatment.[43] The increase in sphingolipid synthesis and SPT activity could be prevented by artificial barrier repair with a latex wrap (Fig. 5). These results show that the increase in sphingolipid synthesis in response to barrier disruption is regulated by SPT activity.

To determine whether the observed changes in

sphingolipid metabolism are a general phenomenon associated with barrier repair, SPT activity in two other models of barrier dysfunction was subsequently determined. Cellophane tape stripping of hairless mouse epidermis, which resulted in a rapid and pronounced break in the barrier (TEWL $>50{\cdot}0$ g/m^2 h), and an EFA-deficient diet, which resulted in a chronic abnormality in barrier function (TEWL $>10{\cdot}0$ g/m^2 h), produced an increase in SPT activity compared with normal controls ($+75\%$ and $+50\%$, respectively).[43]

In order to determine whether the changes in sphingolipid synthesis and SPT activity relate directly to barrier dysfunction, groups of animals were treated with acetone, tape stripping or an EFA-deficient diet, and then covered with a water-vapour-impermeable latex wrap. wrap. Artificial restoration of barrier function resulted in a return to normal of sphingolipid synthesis and SPT activity in our models.[43] These results confirmed that sphingolipid synthesis and SPT activity are regulated by barrier function.

A comparison of the time course of sphingolipid synthesis and SPT activity with the recovery of epidermal barrier function in acetone-treated animals showed that significant barrier repair (up to 60%) preceded the acceleration in epidermal sphingolipid synthesis. Thus, sphingolipid synthesis may not be required during the first few hours of barrier recovery. This is in contrast with cholesterol and fatty-acid synthesis, both of which are accelerated during the early phases of barrier recovery, returning towards normal levels shortly after 6 h.[8,14] Moreover, the activity of HMG CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, increased 2–3 h following barrier disruption with acetone, and returned to normal by 7 h. The activation state of this enzyme (i.e. the dephosphorylated state) increases within the first 30 min after acetone treatment.[19] In contrast, neither sphingolipid synthesis nor SPT activity increased significantly during the first 6 h after barrier disruption with acetone. In addition, SPT activity does not appear to be regulated by a similar dephosphorylation mechanism.[43] These studies suggest a difference in the role of cholesterol synthesis vs. sphingolipid synthesis in the early phase of barrier recovery. A pre-stored pool of sphingolipids in the stratum granulosum may suffice for the early repair of the barrier, and the newly synthesized sphingolipids may not be required either for the later phase of barrier recovery (complete recovery requires 30–35 h),[8] or to provide sphingolipids for replenishment of the storage pool. In contrast, the preformed cholesterol and fatty-acid pool in the stratum granulosum cells may not suffice for early repair, and thus synthesis of these two species is stimulated immediately following barrier disruption.

Our group has also assessed the effect of inhibition of sphingolipid synthesis on epidermal barrier function. β-chloro-L-alanine, an irreversible inhibitor of serine-palmitoyl transferase (SPT) was applied to acetone-treated skin of hairless mice. A single topical application of β-chloro-L-alanine resulted in $>75\%$ inhibition of SPT activity after 30 min. After 6 h SPT activity showed a 53% inhibition; after 18 h SPT activity returned to normal. Sphingolipid synthesis was decreased by 45% after 1–3 h. This degree of inhibition was comparable with the extent of SPT inhibition over the same period. In addition to a global decrease in sphingolipid synthesis, each major sphingolipid class was also decreased in response to β-chloro-L-alanine. Sphingolipid content in the epidermis remained low up to 18 h following treatments. Barrier recovery was determined 2–24 h following acetone treatment and β-chloro-L-alanine application, by measurements of TEWL. Barrier recovery was normal over the first 6 h. However, at later time points (15, 18 and 22 h), β-chloro-L-alanine produced a significant delay in the rate of barrier recovery.[44]

Light-microscopy studies with the fluorescent dye nile red also revealed a delay in the reappearance of sphingolipids in animals treated with acetone followed by β-chloro-L-alanine application. Ultramicroscopy studies showed that acetone treatment produced an immediate secretion of lamellar bodies from the outermost cells of the stratum granulosum, which was accompanied by cytosolic depletion of these organelles. β-chloro-L-alanine application produced a significant reduction in both the number of lamellar bodies and their contents 3–18 h after treatment. The reformation and secretion of lamellar bodies in the outer epidermis, and the internal structure of these organelles were impaired.[44]

These studies demonstrate a requirement for sphingolipid synthesis, in addition to cholesterol and fatty acids, for the maintenance of epidermal barrier homeostasis. Together, these species form the membrane bilayers which mediate epidermal barrier function. It is likely that a deficiency of any of these species will result in defective membrane structures, incapable of impeding transepidermal water flux. This view is supported not only by the inhibitory studies with lovastatin[25,26] and β-chloro-L-alanine,[44] but also by data from selected metabolic disorders where altered proportions of one or more of these species are accompanied by abnormal barrier function. In neutral lipid storage disease,[45] Refsum's disease, certain other inherent disorders of

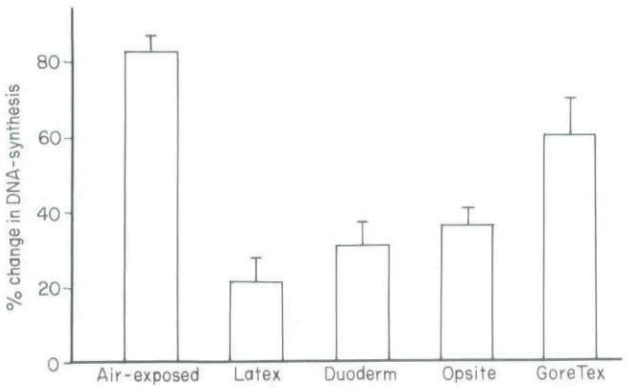

Figure 6. Prevention of expected burst in DNA synthesis in acetone-treated mice by occlusion with vapour-impermeable latex, partially vapour-permeable (Opsite®, Duoderm®), and vapour-permeable (GoreTex®) membranes. Mice were treated with acetone on one flank and immediately occluded with various membranes. After 18 h the skin was excised, the epidermis was separated from the dermis, and DNA synthesis was measured.[49] (Adapted from Proksch *et al. J Clin Invest* 1991; **87**: 1668–73.)

cornification,[46,47] and in rodent essential fatty acid deficiency,[30–33] free sterol and sphingolipid content are reportedly normal, but fatty acid content is defective, and in rodent EFA deficiency elevated TEWL rates are a prominent feature. Thus, it is likely that a mixture of sphingolipids, cholesterol, and free fatty acids form the membrane bilayer system which is required to maintain epidermal barrier function. Further studies will be required to elucidate how cholesterol, free fatty acid and sphingolipid synthesis are co-ordinated by barrier requirements.

Barrier function and epidermal DNA synthesis

Chronic barrier disruption by repeated topical applications of lovastatin is accompanied by an increase in DNA synthesis and epidermal hyperproliferation. In addition, in several model systems, such as tape stripping[48] or in EFAD,[31] abnormal barrier function is known to be accompanied by epidermal hyperplasia. Therefore it was questioned whether this increase in DNA synthesis represented either a non-specific response to injury, or a specific response to disruption of barrier function.

Acute barrier disruption by tape stripping produced a 127% increase in DNA synthesis 18 h after treatment, and this response was greatly reduced by artificial restoration of barrier function by occlusion.[49] Acetone treatment also stimulated DNA synthesis, reaching an 80–100% increase compared with control at 20 h. Artificial restoration of barrier function by latex occlusion again largely blocked the expected increase in DNA synthesis (Fig. 6).

As a check for non-specific effects of occlusion, we have compared DNA synthesis in acetone-treated mice covered with four different, tightly fitting wraps of varying permeability. Occlusion with the water vapour-impermeable latex membrane completely restored barrier function, and largely prevented the increase in DNA synthesis. Occlusion with the partially vapour-permeable wraps, Opsite® and Duoderm® only partially restored barrier function, and therefore showed a smaller effect on DNA synthesis. An even smaller effect on DNA synthesis was measured after the application of the water vapour-permeable GoreTex® wrap (Fig. 6).[49] These results demonstrate the relationship of barrier function and epidermal DNA synthesis in several acute models of barrier dysfunction.

The histology of acetone-treated epidermis revealed an intact stratum corneum, with no evidence of epidermal injury.[49] At 24 h, a slight increase was evident in the number of nucleated cell layers. Autoradiography showed an increased incorporation of ^{3}H-thymidine limited to the basal cell layer.[49]

As a further test for a direct link between barrier function we applied three different unsaturated fatty acids to EFAD-skin.

1. Prostaglandin E_2, which has been shown to correct epidermal hyperplasia without effects on barrier function.[50]
2. Linoleic acid, which repairs barrier function and epidermal hyperplasia.[51]
3. Columbinic acid ((C 18:3(n-6,9,13-*trans*) = linoleic acid + a third double bond), which corrects barrier function, but which should not influence hyperplasia, because it cannot be metabolized to prostaglandin E_2.[51]

In EFAD animals, the barrier abnormality was accompanied by an increase in DNA synthesis.[31,49] Artificial barrier repair by occlusion for 3 days with a latex wrap largely reduced the increased levels of DNA synthesis and epidermal hyperplasia (as well as lipid synthesis). Reduction of increased levels of biosynthetic activity in EFAD, which are an attempt to repair barrier function despite linoleic acid deficiency, resulted in a further deterioration in barrier function. Topical application of linoleic acid and columbinic acid in EFAD hairless mice reversed increased levels in epidermal lipid and DNA synthesis, reversed epidermal hyperplasia, and restored barrier function. In contrast, neither epidermal lipid synthesis, nor DNA synthesis, nor epidermal hyperplasia in EFAD hairless mice were significantly influenced by topical application of prostaglandin E_2 (Fig. 7).[49,52]

These results demonstrate that barrier repair alone is sufficient to reduce epidermal DNA synthesis and hyper-

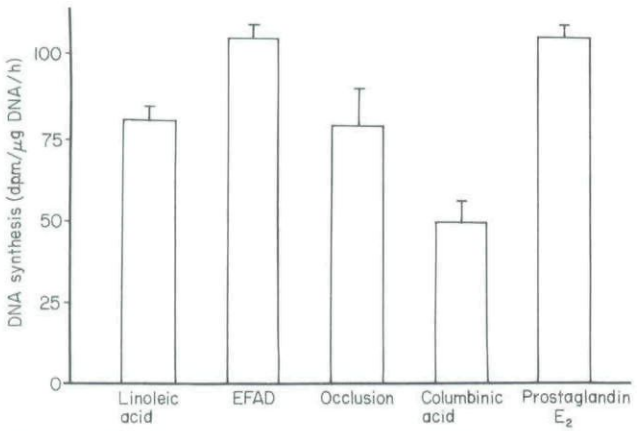

Figure 7. Effect of various treatments on DNA synthesis in EFAD mice. EFAD mice were occluded with a latex wrap for 3 days, re-fed with linoleic acid, or treated topically with columbinic acid or prostagladin E_2. The skin was excised, the epidermis separated from the dermis, and DNA synthesis was measured.[49] (Adapted from Proksch *et al. J Clin Invest* 1991; **87:** 1668–73.)

plasia in EFAD animals. Epidermal hyperplasia in EFAD seems to be independent of prostaglandin E_2.

Although it has been shown that occlusion in both the acute and chronic models greatly reduces the expected burst in DNA synthesis, it should be noted that in none of the models does DNA synthesis completely revert to normal levels. Thus, it is likely that barrier function is not the only factor which drives epidermal DNA synthesis. Instead, cellular replacement or repair may themselves exert regulatory influences.

Conclusion and pathophysiological implications

Barrier disruption is followed by two distinct metabolic responses. The first type of response is a burst in epidermal lipid biosynthesis. This begins immediately after barrier disruption, and is regulated both by the enzyme content and the activation state of the rate-limiting enzymes. The second response is a delayed burst in DNA synthesis, which peaks several hours after barrier disruption in the acute models, leading to epidermal hyperplasia. DNA synthesis and epidermal hyperplasia are specifically linked to barrier function.

The link between disturbed permeability barrier and increase in DNA synthesis may have relevance in the pathogenesis of hyperproliferative skin diseases, such as ichthyosis, psoriasis, atopic dermatitis and irritant contact dermatitis.[53–55] Occlusion of psoriasis lesions reduces lesion size and epidermal hyperplasia.[56,57] Similarly, the benefical effect of bland ointment bases on all hyperproliferative skin diseases could be explained as an effect of artificial restoration of a previously disturbed barrier. Therefore, these studies may lead to the development of more rational strategies for the treatment of hyperproliferative skin diseases. Finally, these studies are of importance for cleansing (removal of stratum corneum lipids and potential disruption of the permeability barrier) and skin care (replacement of lipids and restoration of the permeability barrier), as well as for transdermal drug delivery systems.

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References

1 Elias PM, Friend DS. The permeability barrier in mammalian epidermis. *J Cell Biol* 1975; **65:** 180–91.
2 Elias PM. Lipids and the epidermal permeability barrier. *Arch Dermatol Res* 1981; **270:** 95–117.
3 Elias PM. Epidermal lipids, barrier function and desquamation. *J Invest Dermatol* 1983; **80** (Suppl.): 44–9s.
4 Elias PM. The special role of stratum corneum: stratum corneum structure. In: *Dermatology in General Medicine* (Fitzpatrick TB, Eisen ZA, Wolff K, Friedberg IM, Austen KF eds), 3rd edn. New York: McGraw-Hill Book Company, 1983; 342–6.
5 Elias PM, Feingold KR. Lipid related barriers and gradients in the epidermis. Endocrine, metabolic and immunologic functions of keratinocytes. *Ann NY Acad Sci* 1988; **548:** 4–13.
6 Downing DT, Stewart ME, Wertz PW *et al.* Skin lipids: an update. *J Invest Dermatol* 1987; **88** (Suppl.): 2–6s.
7 Lampe MA, Burlingame AL, Whitney J *et al.* Human stratum corneum lipids: characterization and regional variations. *J Lipid Res* 1983; **24:** 120–30.
8 Grubauer G, Feingold KR, Elias PM. Relationship of epidermal lipogenesis to cutaneous barrier function. *J Lipid Res* 1987; **28:** 746–52.
9 Grubauer G, Feingold KR, Harris RM, Elias PM. Lipid content and lipid type as determinants of the epidermal permeability barrier. *J Lipid Res* 1989; **30:** 89–96.
10 Grubauer G, Elias PM, Feingold KR. Transepidermal water loss: the signal for recovery of barrier structure and function. *J Lipid Res* 1989; **30:** 323–33.
11 Finkelstein A, Cass A. Effect of cholesterol on the water permeability of thin lipid membranes. *Nature* 1967; **216:** 717–18.
12 Feingold KR, Brown BE, Lear SR *et al.* Localization of de novo sterologenesis in mammalian skin. *J Invest Dermatol* 1983; **81:** 365–9.

13 Feingold KR, Wiley MH, MacRae G *et al.* *De novo* sterologenesis in the intact rat. *Metabolism* 1983; **32:** 75–81.
14 Menon GK, Feingold KR, Moser AH *et al.* *De novo* sterologenesis in the skin. II. Regulation by cutaneous barrier requirements. *J Lipid Res* 1985; **26:** 418–27.
15 Dykes PJ, Mark R, Davies MG, Reynolds DJ. Epidermal metabolism in heredopathia atactica polyneuritiformis (Refsum's disease). *J Invest Dermatol* 1978; **70:** 126–9.
16 Chapkin RS, Ziboh VA. Inability of skin enzyme preparations to biosynthesize arachidonic acid from linoleic acid. *Biochem Biophys Res Commun* 1984; **124:** 784–92.
17 Beg ZH, Stonik JA, Brewer HB Jr. 3-hydroxy-3-methylglutaryl coenzyme A reductase regulation of enzymatic activity by phosphorylation and dephosphorylation. *Proc Natl Acad Sci USA* 1978; **75:** 3678–82.
18 Kennelly PJ, Rodwell VW. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by reversible phosphorylation-dephosphorylation. *J Lipid Res* 1985; **26:** 903–14.
19 Proksch E, Elias PM, Feingold KR. Regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in murine epidermis. Modulation of enzyme content and activation state by barrier requirements. *J Clin Invest* 1990; **85:** 874–82.
20 Imokawa G, Mishima Y. Cumulative effect of surfactants on cutaneous horny layers: adsorption onto human keratin layers *in vivo*. *Contact Dermatitis* 1979; **5:** 357–66.
21 Monger DJ, Williams ML, Feingold KR *et al.* Localization of sites of lipid biosynthesis in mammalian epidermis. *J Lipid Res* 1988; **29:** 603–12.
22 Elias PM, Fritsch P, Dahl MV, Wolff K. Staphylococcal toxic epidermal necrolysis: pathogenesis and studies of the subcellular site of action of exfoliatin. *J Invest Dermatol* 1975; **65:** 501–12.
23 Proksch E, Elias PM, Feingold KR. Localization and regulation of epidermal 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity by barrier requirements. *Biochim Biophys Acta* 1991; **1083:** 71–9.
24 Alberts AW, Chen J, Kuron G *et al.* Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc Natl Acad Sci USA* 1980; **77:** 3957–61.
25 Feingold KR, Mao-Qiang M, Menon GK *et al.* Cholesterol synthesis is required for cutaneous barrier function in mice. *J Clin Invest* 1990; **86:** 1738–45.
26 Feingold KR, Mao-Qiang M, Proksch E *et al.* The lovastatin-treated rodent: a new model of barrier disruption and epidermal hyperplasia. *J Invest Dermatol* 1991; **96:** 201–9.
27 Gray GM, Yardley HJ. Lipid compositions of cells isolated from pig, human, and rat epidermis. *J Lipid Res* 1975; **16:** 434–40.
28 Elias PM, Brown BE, Fritsch P *et al.* Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum. *J Invest Dermatol* 1979; **73:** 339–48.
29 Wertz PW, Downing DT, Freinkel RK, Traczyk TN. Sphingolipids of the stratum corneum and lamellar granules of fetal rat epidermis. *J Invest Dermatol* 1984; **83:** 193–5.
30 Prottey C. Essential fatty acids and the skin. *Br J Dermatol* 1976; **94:** 579–87.
31 Lowe NJ, Stoughton RB. Essential fatty acid deficient hairless mouse: a model of chronic epidermal hyperproliferation. *Br J Dermatol* 1977; **96:** 155–62.
32 Elias PM, Brown BE. The mammalian cutaneous permeability barrier: defective barrier function in essential fatty acid deficiency correlates with abnormal intercellular lipid deposition. *Lab Invest* 1978; **39:** 574–83.
33 Bowser PA, Nugteren DH, White RJ *et al.* Identification, isolation and characterization of epidermal lipids containing linoleic acid. *Biochim Biophys Acta* 1985; **834:** 419–28.
34 Abraham W, Wertz PW, Downing DT. Linoleate-rich acylglucosylceramides of pig epidermis: structure determination by proton magnetic resonance. *J Lipid Res* 1985; **26:** 761–6.
35 Hamanaka S, Asagami C, Suzuki M *et al.* Structure determination of glucosyl β 1-N-(ω-O-linoleoyl)-acylsphingosines of human epidermis. *J Biochem* 1989; **105:** 684–90.
36 Elias PM, Brown BE, Ziboh VA. The permeability barrier in essential fatty acid deficiency: evidence for a direct role for linoleic acid in barrier function. *J Invest Dermatol* 1980; **74:** 230–3.
37 Houtsmuller UMT, van der Beek A. Effects of topical application of fatty acids in essential fatty acid deficiency. *Progr Lipid Res* 1981; **20:** 219–24.
38 Wertz PW, Cho ES, Downing DT. Effect of essential fatty acid deficiency on the epidermal sphingolipids of the rat. *Biochim Biophys Acta* 1983; **753:** 350–5.
39 Imokawa G, Akasaki S, Hattori M, Yoshizuka N. Selective recovery of deranged water-holding properties by stratum corneum lipids. *J Invest Dermatol* 1986; **87:** 758–61.
40 Holleran WM, Williams ML, Gao WN, Elias PM. Serine-palmitoyl transferase activity in cultured human keratinocytes. *J Lipid Res* 1990; **31:** 1655–61.
41 Braun PE, Morell P, Radin NS. Synthesis of C18- and C20-dihydrosphingosines, ketodihydrosphingosines, and ceramides by microsomal preparations from mouse brain. *J Biol Chem* 1970; **245:** 335–41.
42 Williams RD, Wang E, Merrill AH Jr. Enzymology of long-chain base synthesis by liver: characterization of serine palmitoyltransferase in rat liver microsomes. *Arch Biochem Biophys* 1984; **228:** 282–91.
43 Holleran WM, Feingold KR, Mao-Qiang M *et al.* Regulation of epidermal sphingolipid synthesis by permeability barrier function. *J Lipid Res* 1991; **32:** 1151–8.
44 Holleran WM, Menon GK, Mao-Qiang M *et al.* Sphingolipids are required for mammalian barrier function: inhibition of sphingolipid synthesis delays barrier recovery after acute perturbation. *J Clin Invest* 1991; **88:** 1338–45.
45 Elias PM, Williams ML. Neutral lipid storage disease with ichthyosis. Defective lamellar body contents and intracellular dispersion. *Arch Dermatol* 1985; **121:** 1000–8.
46 Koone MD, Rizzo WB, Elias PM *et al.* Ichthyosis, mental retardation, and asymptomatic spasticity. A new neurocutaneous syndrome with normal fatty alcohol: NAD^+ oxidoreductase activity. *Arch Dermatol* 1990; **126:** 1485–90.
47 Ghadially R, Williams ML, Hou EYL *et al.* Membrane structured abnormalities in the stratum corneum of autosomal recessive ichthyoses. *J Invest Dermatol* 1992; **99:** 755–63.
48 Pinkus H. Examination of the epidermis by the strip method of removing horny layers. I. Observations on thickness of the horny layer, and on mitotic activity after stripping. *J Invest Dermatol* 1951; **16:** 383–6.
49 Proksch E, Feingold KR, Mao-Qiang M, Elias PM. Barrier function regulates epidermal DNA synthesis. *J Clin Invest* 1991; **87:** 1668–73.
50 Ziboh VA, Hsia SL. Effects of prostaglandin E_2 on rat skin: inhibition of sterol ester biosynthesis and clearing of scaly lesions in essential fatty acid deficiency. *J Lipid Res* 1972; **13:** 458–67.
51 Houtsmuller UMT, van der Beek A. Columbinic acid, a new type of essential fatty acid. *Prog Lipid Res* 1982; **20:** 889–96.
52 Proksch E, Feingold KR, Elias PM. Epidermal HMG CoA reductase

activity in essential fatty acid deficiency: barrier requirements rather than eicosanoid generation regulate cholesterol synthesis. *J Invest Dermatol* 1992; **99:** 216–20.

53 Pitz EP, Imerbeck RV, Rieger MM. An animal test procedure for the simultaneous assessment of irritancy and efficiency of skin care products. In: *Models in Dermatology* (Maibach HI, Lowe NJ, eds). Basel: Karger, 1985; 209–24.

54 Melnik B, Hollmann J, Plewig G. Decreased stratum corneum ceramides in atopic individuals—a pathobiochemical factor in xerosis? *Br J Dermatol* 1988; **119:** 547–9.

55 Schäfer L, Kragballe K. Abnormalities in epidermal lipid metabolism in patients with atopic dermatitis. *J Invest Dermatol* 1991; **96:** 10–15.

56 Petzold DG, Braun-Falco O, Wenig KH. Effect of plastic-foil-occlusion on psoriatic lesions. *Arch Klin Exp Dermatol* 1970; **238:** 160–8.

57 Friedman SJ. Management of psoriasis vulgaris with a hydrocolloid occlusive dressing. *Arch Dermatol* 1987; **123:** 1046–52.