Regulation of epidermal sphingolipid synthesis by permeability barrier function

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Abstract A mixture of sphingolipids, cholesterol, and free fatty acids forms the intercellular membrane bilayers of the stratum corneum which are presumed to regulate epidermal barrier function. Prior studies have shown that both cholesterol and fatty acid synthesis are rapidly regulated by epidermal barrier requirements. In contrast, the importance of sphingolipids in barrier function has not been directly demonstrated. Here, we have assessed both sphingolipid synthesis by $[^3H]H_2O$ incorporation and serine palmitoyl transferase (SPT) activity in relation to modulations in barrier function. Incorporation of $[^3H]H_2O$ into sphingolipids increased after barrier disruption with acetone, with maximal increase (170%) occurring 5–7 h after treatment ($P < 0.005$). As barrier function returned to normal over 24 h, incorporation of tritium into sphingolipids normalized. SPT activity also increased after barrier disruption, peaking at 6 h (150%) ($P < 0.05$), and returning towards normal by 24 h. Artificial restoration of the barrier with a water vapor-impermeable membrane prevented the increases in both $[^3H]H_2O$ incorporation into sphingolipids and enzyme activity. Finally, SPT activity was increased in two other models of barrier dysfunction, cellophane tape-stripping and essential fatty acid deficiency. Occlusion normalized SPT activity in both of these models as well. These studies: *a*) demonstrate a distinctive, delayed increase in epidermal sphingolipid synthesis in response to barrier requirements that contrasts with the immediate responses of cholesterol and fatty acid synthesis; and *b*) suggest that sphingolipids are important for the maintenance of the epidermal permeability barrier. —**Holleran, W. M., K. R. Feingold, M. Mao-Qiang, W. N. Gao, J. M. Lee, and P. M. Elias.** Regulation of epidermal sphingolipid synthesis by permeability barrier function. *J. Lipid Res.* 1991. **32:** 1151–1158.

Supplementary key words sphingolipids • serine-palmitoyl transferase • epidermal barrier function

Cornification of the epidermis of terrestrial mammals is accompanied both by sequestration of lipids to intercellular domains (1, 2), as well as profound alterations in lipid biochemical composition (3). Toward the outer cell layers of the epidermis, phospholipid content diminishes and a relatively nonpolar mixture of lipids emerges that is enriched in cholesterol, free fatty acids, and sphingolipids (4–7). These species are organized into a system of parallel membrane bilayers that is presumed to mediate the cutaneous permeability barrier (1, 2).

Recent metabolic studies have demonstrated the importance of both cholesterol and fatty acids for barrier homeostasis (8–12). However, the evidence to date for a role of sphingolipids in epidermal barrier function has been largely indirect and includes the following: first, a family of sphingolipids represents the predominant lipid species on a weight basis (35–40%) in the stratum corneum intercellular domains (5–7, 13). Second, these sphingolipids are also the principal repository for the highly saturated, very-long chain (C22:0–C26:0) fatty acids among esterified stratum corneum species (7). And, in marine cetaceans, the very long-chain, N-acyl fatty acids are replaced by shorter chain species (14), which may reflect the less stringent barrier requirements of the marine environment. Third, the majority of epidermal linoleic acid, an essential fatty acid known to be required for cutaneous barrier function (15–17), is esterfied to ceramide at the ω-hydroxy terminus of the N-acyl fatty acid (18–20). Decreased linoleic acid content in essential fatty acid deficiency leads to an abnormal permeability barrier (21, 22), which has been attributed to substitution of oleic for linoleic acid in the epidermal sphingolipids (23). Fourth, only polar organic solvents that remove sphingolipids as well as neutral lipids from the stratum corneum are capable of significant abrogation of the permeability barrier (24). Finally, topical applications of certain natural and synthetic ceramides correct the abnormal water-retaining properties of solvent- or detergent-extracted stratum corneum (25).

Abbreviations: SPT, serine-palmitoyl transferase; CH, cholesterol; FA, fatty acid; SPL, sphingolipid; TEWL, transepidermal water loss; EFAD, essential fatty acid deficiency; HPTLC, high performance thin-layer chromatography; 3-KDS, 3-ketodihydrosphinganine.

We have recently shown that both cultured human keratinocytes, as well as murine epidermis, are highly enriched in serine palmitoyl transferase (EC2.3.1.50; SPT) (26), the rate-limiting enzyme of sphingolipid base synthesis (27, 28). In order to assess the role of sphingolipids in the barrier, we measured both epidermal sphingolipid synthesis and SPT activity in animals undergoing various types of experimental barrier perturbation. We describe here elevations of both sphingolipid synthesis and SPT activity after acute perturbations of the permeability barrier. Moreover, both enzyme activity and synthesis were normalized when the barrier was artificially restored by occlusion, providing further evidence that sphingolipid synthesis is regulated by barrier requirements. Finally, these modulations in sphingolipid synthesis and SPT activity are different from those displayed by cholesterol (8, 12) and fatty acid (10) synthesis after barrier perturbation.

MATERIALS AND METHODS

Materials

Reagent grade organic solvents, pyridoxal phosphate, dithiothreitol, palmitoyl CoA, and sphingosine base were obtained from Sigma Chemical Co. (St. Louis, MO). HEPES buffer was purchased from Fisher Scientific (Santa Clara, CA); [^{3}H]H_2O (sp act 0.1 Ci/ml) and [3-^{3}H]L-serine (sp act 30 Ci/mmol) were obtained from Amersham (Arlington Heights, IL). High performance thin-layer chromatography (HPTLC) plates (silica gel 60) were obtained from Merck (Damstadt, FRG) (lot #5641). Protein reagent and bovine serum albumin standards were obtained from Bio-Rad (Richmond, CA).

Animals

Male hairless mice (Hr/Hr) between 4 and 12 weeks of age were purchased from Jackson or Simonsen Laboratories (Bar Harbour, ME and Gilroy, CA). They were fed Purina mouse diet and water ad libitum. For the essential fatty acid deficiency (EFAD) studies, mice were maintained on an EFAD diet (17) for 7–8 weeks until transepidermal water loss levels exceeded 1.0 mg/cm^2 per h.

Experimental design

Disruption of the permeability barrier was achieved by unilateral treatment of one flank of each animal with absolute acetone, as described previously (8, 10, 24, 29). Control animals were treated with 0.9% sodium chloride alone. Transepidermal water loss (TEWL) rates were measured with a Meeco^R electrolytic moisture analyzer (9, 10, 29), recorded in parts per million/0.5 cm^2 per h over background, and converted to mg H_2O/cm^2 per h according to the formula: $J = 6(18P/22.4A) \times 10^{-3}$; (P = increase over background, A = area of skin). To assess directly the effects of occlusion, which instantly lowers TEWL rates to zero, groups of acetone-treated tape-stripped, and EFAD animals were immediately covered with a tight-fitting, water-impermeable membrane (one finger of a Latex^R glove) (9, 10, 29). The wrap was removed just prior to excision of the whole skin samples for the biochemical studies described below.

Lipid incorporation studies

Approximately 8 cm^2 on one flank of each hairless mouse was gently swabbed with acetone-soaked cotton balls until TEWL readings exceeded 4.0 mg/cm^2 per h, measured over at least two separate sites. Animals were injected intraperitoneally with [^{3}H]H_2O (20 mCi/0.2 ml) at various time points after acetone treatment (1, 5, 10, and 22 h). Two hours after injection, blood samples were taken and the animals were killed. Whole skin was excised from each flank (acetone-treated vs. untreated sides), heated to 60°C for 60 sec, and the epidermis was separated from the dermis by gentle scraping (12). Samples were blotted dry, weighed, minced, immediately placed into screw-cap glass test tubes containing Bligh-Dyer solution (30), and total lipid extracts were obtained, as described previously (6). The lipid components then were separated by HPTLC using the following solvent sequence: *1*) chloroform–methanol–water 90:10:1 (by volume); *2*) petroleum ether–diethylether–acetic acid 70:50:1 (by volume); and *3*) chloroform–methanol–water–acetic acid 60:35:4.5:0.5 (by volume) to approximately 15 cm. Lipids were visualized by Woods light fluorescence after staining with 8-anilino-1-naphthalene sulfonic acid (ANS) (7), and identified by co-chromatography against known standards. The lipid spots, were scraped into scintillation vials and counted by liquid scintillation spectrometry. Total incorporation into sphingolipids was obtained by combining the ceramide, glycosphingolipid, sphingomyelin, and sphingosine base fractions. Using the specific activity of [^{3}H]H_2O in serum samples from each animal, results were expressed as μmoles incorporated per 2 h per mg of epidermal wet weight, as described previously (8–10, 31).

Microsomal isolation

Prior to assessment of SPT activity, microsomes were prepared from murine epidermis at various time points after barrier disruption as described previously (12). Briefly, whole skin was excised, incubated at 37°C for 45 min in phosphate-buffered saline (calcium/magnesium-free) containing 10 mM EDTA. The epidermis was peeled off the dermis with a scalpel blade, weighed, minced into small pieces (< 1 mm^3), and stored in small plastic tubes overnight at −70°C. Samples then were thawed on ice, and five volumes of homogenization buffer were added (HEPES 50 mM, pH 7.4, containing 10 mM EDTA, 5

TABLE 1. Tritiated water incorporation into sphingolipids in acetone-treated versus untreated flanks

Time after Acetone Treatment	Synthesis Rate[a] Treated Side (Right)	Synthesis Rate[a] Untreated Side (Left)	Significance[b] (P)	Ratio[c] (R/L)
	μmol/mg/2 h			
Untreated	2.92 ± 0.44 (n = 7)	2.86 ± 0.42 (n = 7)	NS	1.0 ± 0.1
1–3 h	2.83 ± 0.40 (n = 6)	2.57 ± 0.29 (n = 6)	NS	1.2 ± 0.2
5–7 h	4.59 ± 0.38 (n = 7)	2.85 ± 0.25 (n = 7)	<0.005	1.7 ± 0.2
12–14 h	3.10 ± 0.35 (n = 5)	2.18 ± 0.21 (n = 5)	<0.005	1.4 ± 0.1
22–24 h	3.37 ± 0.28 (n = 5)	2.77 ± 0.19 (n = 5)	<0.05	1.2 ± 0.2

[a]Mean ± SEM (n).
[b]Determined by paired *t*-analysis, two-tailed.
[c]Mean (± SEM) of ratios for individual animals.

mM DTT, and 0.25 M sucrose). Cell homogenates were prepared using a Polytron PCU2 tissue homogenizer (Kinematica GmbH, Lucerne, Switzerland) followed by sonication using a Fisher Sonic Dismembranator (Model 300, Artec Systems Corporation, Farmingdale, NY), as described previously (12). Differential centrifugation (4°C) was performed, and the microsomal pellet (100,000 *g*) was resuspended in storage buffer containing 50 mM HEPES (pH 7.4), 5 mM EDTA, 5 mM DTT, and 20% glycerol (v/v) using a Dounce homogenizer, and stored at −70°C until use. No loss of enzyme activity was observed upon freezing or subsequent thawing; activity was stable for over 6 months at −70°C. In experiments with large numbers of tissue samples (n > 10), brief sonication was used to re-solubilize microsomal pellets. The enzyme activity for each experiment was related to a parallel normal control group. The protein content of various subcellular fractions was determined by the Bradford procedure (32) using bovine serum albumin as standard. DNA content was determined using the method of LaBarca and Paigen (33).

Serine palmitoyl transferase (SPT) assay

Our assay for SPT activity was modified from the method of Williams, Wang, and Merrill (28), as recently described (26). Briefly, the assay buffer contained 100 mM HEPES, pH 8.3, 5.0 mM DTT, and 2.5 mM EDTA, while the reaction mixture contained 50 μM pyridoxal phosphate, 150 mM palmitoyl-coenzyme A, 1.0 mM [^{3}H]L-serine (sp act 45 to 50,000 dpm/nmol), and 50 to 100 μg of microsomal protein in 0.1 ml total assay volume. The assay mixture (protein, buffer, and pyridoxal phosphate) was preincubated for 10 min (37°C), and the assay was initiated by simultaneous addition of palmitoyl CoA and [^{3}H]L-serine, incubated at 37°C for 10 min, and terminated by the addition of 0.2 ml of 0.5 N NH_4OH. The reaction product, 3-ketodihydrosphinganine (3KDS), was isolated as described previously (26) and counted by liquid scintillation spectrometry. Enzyme specific activity was expressed as pmoles of 3KDS formed per minute per mg of microsomal protein. Total SPT activity was obtained by multiplying the specific activity by the total protein (total pmol 3KDS formed/min).

Statistical analysis

Statistical evaluation of data was performed using either a two-tailed Student's *t*-test or paired *t*-test.

RESULTS

Sphingolipid synthesis

To determine whether epidermal barrier requirements regulate sphingolipid synthesis, we first examined the incorporation of [^{3}H]H_2O into these lipids after acetone treatment versus untreated controls. Since considerable variation occurred within experimental groups, the values for treated versus untreated side from each group are shown (**Table 1**). In addition, the ratios of the data from the treated versus untreated flanks for each group are shown in Table 1. The untreated control animals showed no difference in mean incorporation into total sphingolipids (μmol/2 h per mg epidermis) between left and right flanks. As expected, the ratio for the untreated control animals (left vs. right sides) was near unity (1.02 ± 0.04). At the first time point after barrier disruption (1–3 h), no significant change in incorporation into sphingolipids was observed between the treated and untreated flanks. Although the synthesis ratio was 1.2 ± 0.2, this difference did not achieve statistical significance. However, by 5–7 h after acetone treatment, a significant increase in [^{3}H]H_2O incorporation into sphingolipids was observed (170%, $P < 0.005$). The incorporation rate remained elevated at the 12–14 h timepoint, and returned toward normal by 22–24 h. These results strongly suggest that disruption of the barrier by acetone produces a burst in total sphingolipid synthesis, which first appears after 5 h and is sustained to at least 24 h.

A comparison of the time course of [^{3}H]H_2O incorporation into sphingolipids (Table 1) with the recovery of epidermal barrier function (**Fig. 1A**) shows that significant barrier repair (35–40%) preceded the acceleration in epidermal sphingolipid synthesis. These results indicate that sphingolipid synthesis increases in response to barrier disruption, but that the response lags behind the early repair of barrier function.

We then determined the relative incorporation of [^{3}H]H_2O into individual sphingolipid species after acetone treatment. Although the incorporation of tritium into total sphingolipids was increased from 5 h onward, the distribution of radioactivity within individual sphingolip-

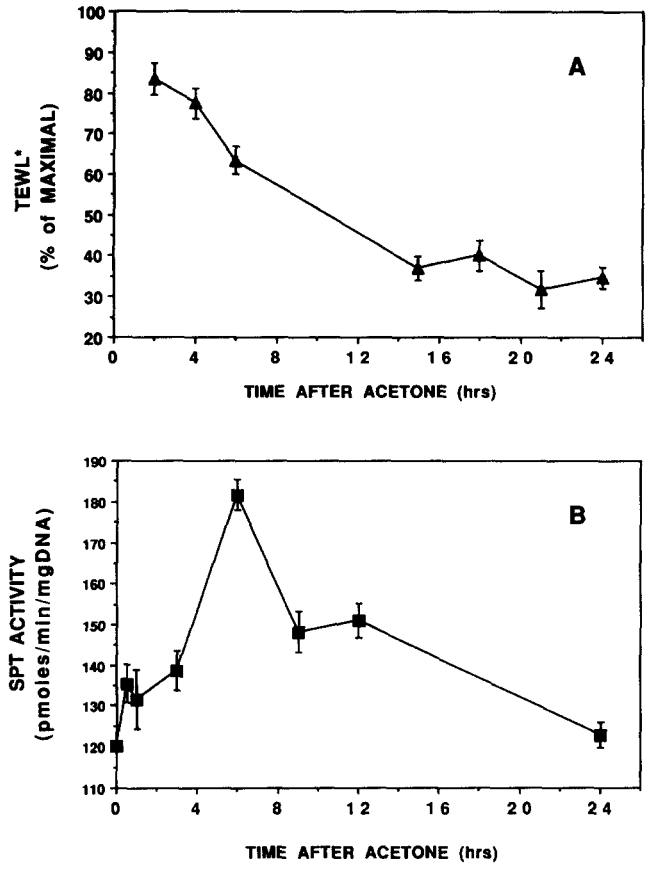

Fig. 1. A: Recovery of epidermal barrier to water loss (TEWL) with time after acetone treatment. Five measurements of TEWL were taken on each of five animals at the indicated timepoints. *Results are presented as the mean percent of the maximal TEWL reading (time 0). B: The total SPT activity versus time. Each point represents the mean SPT activity (± SEM) of triplicate assays determined on at least three separate animals.

id species did not change (**Table 2**). In both treated and normal epidermis, the majority of incorporated tritium appeared in the ceramide and glucosylceramide fractions (≈85%). Sphingomyelin and sphingosine base accounted for far less of the total sphingolipid incorporation (in both treated and untreated epidermis), and this distribution did not change at later time points.

SPT activity after acetone treatment

Since SPT is the rate-limiting enzyme for sphingolipid synthesis (27, 28), we next assessed the alterations in SPT activity induced by disruption of the barrier with acetone (Fig. 1B). The enzymatic activity present in untreated epidermal samples served as the control, which in these experiments was 120 ± 5 pmol/min per mg DNA. Total SPT activity was not significantly increased for the first 5 h after barrier disruption. However, a marked increase occurred at 6 h (≈↑50%, $P < 0.05$), and enzyme activity remained elevated at 9 and 12 h ($P < 0.05$), with normalization by 24 h after acetone treatment. Moreover, the increase in SPT activity correlated directly with the increased $[^3H]H_2O$ incorporation into sphingolipids at each corresponding timepoint (**Fig. 2**; $r = 0.96$; $P < 0.01$). These studies show that barrier disruption induces an increase in SPT activity after 5 h, which parallels the modulations in $[^3H]H_2O$ incorporation, a measure of in vivo total sphingolipid synthesis. They further suggest that SPT activity accurately reflects total sphingolipid synthesis, and finally that SPT represents a key regulatory step of epidermal sphingolipid synthesis.

Occlusion studies

In order to determine whether the changes in sphingolipid synthesis and SPT activity relate directly to barrier dysfunction, groups of animals first were treated with acetone, and then immediately covered with a water vapor-impermeable Latex^R wrap. As described above, acetone treatment again produced a significant increase in both $[^3H]H_2O$ incorporation into sphingolipids and SPT activity (**Fig. 3**; cf, Table 1 and Fig. 1B). Occlusion of the acetone-treated sites completely inhibited the expected increase in $[^3H]H_2O$ incorporation into sphingolipids 5–7 h after acetone treatment (**Table 3**; Fig. 3A). In contrast, occlusion of normal untreated sites produced no changes in sphingolipid synthesis, suggesting that toxicity from occlusion did not account for the decreased synthesis rates in occluded acetone-treated animals (Fig. 3A). Likewise, the increase in SPT activity that is observed at 6 h after acetone treatment (cf, Fig. 1B) was significantly inhibited

TABLE 2. Distribution of $[^3H]H_2O$ incorporation into sphingolipids

Timepoint	n	Sphingolipid Distribution[a]		
		Sphingomyelin	Sphingosine Base	Glyco + Cer[b]
		lipid weight percent		
Baseline control	16	6.6 ± 0.38	7.2 ± 0.55	85.9 ± 0.75
Acetone-treated (5–7 h)	7	6.5 ± 0.68	6.8 ± 0.23	86.6 ± 0.79
Significance		NS	NS	NS

[a]Defined as the rate of incorporation into individual sphingolipid class/total sphingolipid incorporation × 100; values are mean ± SEM

[b]Represents combined glucosylceramide and ceramide components isolated by HPTLC (see Methods).

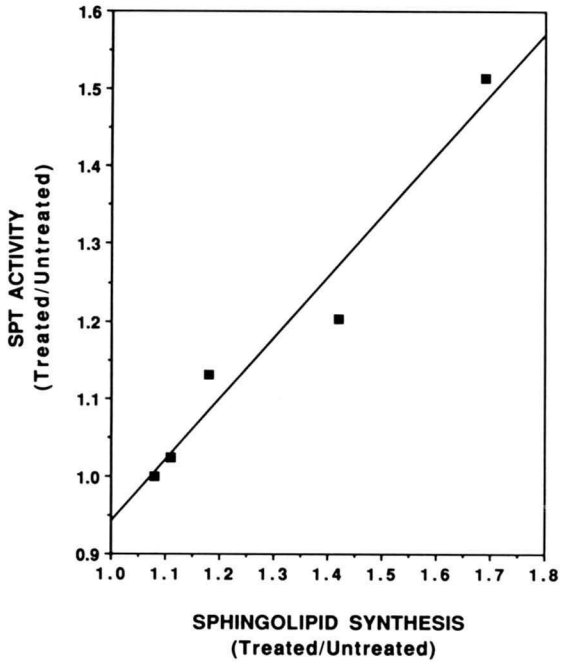

Fig. 2. Correlation between total sphingolipid synthesis and SPT activity after acetone disruption of barrier. The midpoint for each interval of sphingolipid synthesis was compared with the corresponding value for enzyme activity (e.g., 6 h for 5–7 h incorporation interval); $P < 0.01$; $r = 0.96$.

($P < 0.05$) although not completely reversed by occlusion (Fig. 3B). Again, occlusion of normal untreated skin produced no alteration in SPT activity. Finally, occlusion did not change the relative incorporation of $[^3H]H_2O$ into sphingolipid classes (data not shown). These results demonstrate that artificial restoration of barrier function blocks the expected increases in both $[^3H]H_2O$ incorporation into sphingolipids and SPT activity that occur 6 h after barrier disruption.

Other models

To determine whether the observed changes in sphingolipid metabolism are a general phenomenon associated with barrier repair, we next measured SPT activity in two other models of barrier dysfunction. Cellophane tape-stripping of hairless mouse epidermis, which resulted in a rapid and pronounced break in the barrier (TEWL >5.0 mg/cm^2 per h), also produced an increase in total SPT activity over normal controls (↑75%, $P < 0.01$ (**Fig. 4**). Moreover, EFAD animals, which exhibit a chronic abnormality in barrier function showed a nearly 50% increase in SPT activity over normal controls ($P < 0.05$) (Fig. 4). Furthermore, as in the acetone model, occlusion of both tape-stripped and EFAD animals with a vapor-impermeable wrap normalized enzymatic activity in both models (Fig. 4). These studies further confirm that increased sphingolipid production is a general response to barrier requirements in both acute and chronic models of barrier dysfunction.

DISCUSSION

The epidermis contains large quantities of sphingolipids that are concentrated in the stratum granulosum and stratum corneum layers (4–7). Based on a variety of indirect evidence, which is extensively reviewed in the Introduction, it has been hypothesized that the sphingolipids play an important role in the cutaneous permeability barrier. The stratum corneum is also enriched in cholesterol and free fatty acid, and prior studies have shown that cutaneous barrier requirements specifically regulate epidermal cholesterol and fatty acid synthesis (8–10). Recent studies by this laboratory have demonstrated that the activity of SPT is higher in the epi-

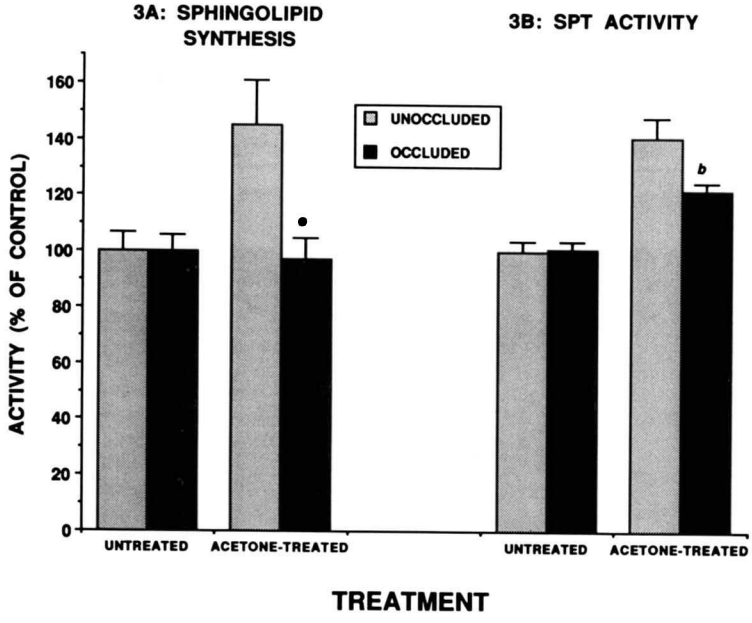

Fig. 3. Effect of occlusion on total sphingolipid synthesis and SPT activity after acetone treatment. In each experiment, two groups of animals were treated with acetone to break the barrier; one group was covered with Latex^R wrap, while the second group remained uncovered. Activity was plotted as the percent of normal untreated controls for the same experiment; ($n \geq$ four animals in each group; mean ± SEM). A: Sphingolipid synthesis in untreated controls and 5–7 h after acetone treatment. Results from 2 h $[^3H]H_2O$ incorporation are presented for occluded as well as unoccluded animal groups; $^aP < 0.05$. B: SPT activity in untreated normal controls and 6 h after acetone treatment. The occlusiv : wrap was applied over the entire 6 h in both the untreated and acetone-treated groups; $^bP < 0.05$.

TABLE 3. Effect of occlusion on sphingolipid synthesis 5–7 h after acetone treatment

Description	Synthesis Rate[a]		Significance (P)[b]
	Treated Side	Untreated Side	
	μmol/mg/2 h		
Acetone-treated, unoccluded	4.09 ± 0.44 (n = 4)	2.82 ± 0.20 (n = 4)	<0.05
Acetone-treated, occluded	2.72 ± 0.26 (n = 5)	2.81 ± 0.18 (n = 5)	NS

[a]Mean ± SEM (n).
[b]Determined by paired *t*-analysis, two-tailed.

dermis than in most other tissues (26), suggesting that sphingolipid synthesis is very active in this site.

In the present study we now demonstrate that sphingolipid synthesis, measured by the incorporation of $[^3H]H_2O$ in vivo, is increased in the epidermis after barrier disruption. This increase in incorporation into sphingolipids returned toward normal with the recovery of barrier function. Moreover, the activity of SPT increased in parallel with the changes in incorporation of tritium into sphingolipids after barrier disruption. We have thus demonstrated a direct correlation between incorporation of tritium into sphingolipids and SPT activity in the epidermis. This provides strong support for the view that SPT is the rate-limiting enzyme in sphingolipid synthesis (26, 27). Of particular note is that the increase in sphingolipid synthesis and SPT activity was seen in two acute models of barrier disruption (acetone and tape-stripping), as well as in one chronic model (EFAD). Additionally, artificial restoration of the barrier with the water-impermeable membrane inhibited the increase in both sphingolipid synthesis and SPT activity. Thus, the observed alterations in sphingolipid metabolism can be considered a specific response by the epidermis to the barrier defect, rather than being attributable to nonspecific toxicity or unrelated effects in each model. Since occlusion normalized SPT activity in all three models, the secondary effects which could occur in each (e.g., acetone: cytotoxicity; tape-stripping: cellular replacement; and EFAD: a general nutritional deficiency) are not likely to be the cause of the observed changes in sphingolipid synthesis.

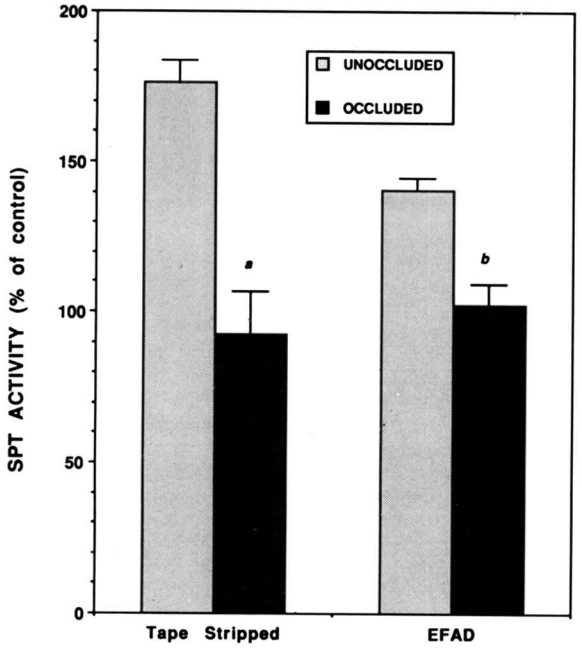

Fig. 4. SPT activity in tape-stripped and EFAD models with and without occlusion. SPT activity is reported as the percent of control SPT levels for each experiment. ($^{a,b}P < 0.01, 0.05$, respectively vs. untreated control). Tape-stripped animals were occluded for 24 h, while EFAD animals were occluded for 72 h.

The results presented here also suggest that de novo sphingolipid synthesis may not be required during the first few hours of barrier recovery, a time period during which up to 60% of barrier recovery has been reported to occur (8, 10, 24, 29). Earlier studies with $[^3H]H_2O$ showed that cholesterol and fatty acid synthesis are both accelerated during the early phases of barrier recovery (0–4 h), returning toward normal levels shortly after 6 h (8, 29). Moreover, the activity of hydroxymethylglutaryl CoA (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis, also increases by 2–3 h after barrier disruption with acetone, returning to normal by 7 h, while the activation state of this enzyme (i.e., dephosphorylated state) increases within the first 30 min after acetone treatment (12). In contrast, the present study demonstrates that neither incorporation of $[^3H]H_2O$ into sphingolipids nor SPT activity increases significantly during the first 5 h after barrier disruption with acetone (Table 1, Fig. 1B). The peak incorporation time for sphingolipids (5–7 h) is quite distinct from that for cholesterol and fatty acids (0–4 h) (8, 29). A lag in precursor pool labeling is not likely to be responsible for this difference in peak incorporation times for a number of reasons. First, the fatty acid pool is the most likely to affect sphingolipid incorporation rates, since ceramides represent the combination of two fatty acid moieties with

a serine molecule. However, the peak in fatty acid synthesis after acetone treatment occurs 3 h prior to peak incorporation into sphingolipids (29). Secondly, the delayed increase in incorporation into sphingolipids correlated well with the activity time course for SPT (Fig. 2). Peak SPT activity (6 h) also corresponded to the peak incorporation time point (5–7 h). From these data we conclude that a delayed increase in sphingolipid synthesis occurs after barrier disruption with acetone. The time course of sphingolipid synthesis after acetone treatment appears quite distinct from cholesterol synthesis in that peak SPT activity and sphingolipid synthesis are observed only after 6 h, while the peak for HMG-CoA reductase activity occurs 3 h earlier.

It has been suggested that the barrier to water loss is more dependent on the structural organization of lipids within the stratum corneum than on the individual lipid species present (34, 35). The data presented here and previously (8–10, 24, 29), as well as recent studies using inhibitors of lipid synthesis, argue that particular lipid species are important for barrier function. Inhibition of epidermal HMG-CoA reductase and cholesterol synthesis with topical lovastatin impaired the immediate recovery of barrier function after acetone treatment (11), confirming that new cholesterol synthesis is required for early barrier repair. Yet, specific inhibition of SPT and sphingolipid synthesis did not influence the same early stages of barrier repair after acetone treatment (36). Together, these studies again suggest a difference in the role of cholesterol synthesis versus sphingolipid synthesis in the early phases of barrier recovery. In addition, these results suggest that a pre-stored pool of sphingolipids in the stratum granulosum may suffice for the early repair of the barrier, and that newly synthesized sphingolipids may not be required for this stage. Rather, the late increase in sphingolipid synthesis may be required either for a later phase of barrier recovery (complete recovery requires 30–36 h) (10), 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 after barrier disruption.

In summary, we have demonstrated for the first time a strong correlation between epidermal sphingolipid synthesis and cutaneous permeability barrier homeostasis. Perturbations of the barrier provoked increased sphingolipid synthesis and SPT activity. However, in contrast to prior descriptions of cholesterol and fatty acid synthesis in the same models, the newly synthesized sphingolipids may not be reqired for the initial phase of barrier recovery, but rather they may be critical to replace depleted storage pools and/or for the replenishment of lipid required for the later stages of barrier recovery.

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