

Relationship of epidermal lipogenesis to cutaneous barrier function

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Abstract Although the lipids of mammalian stratum corneum are known to be important for the cutaneous permeability barrier, the factors that regulate epidermal lipid biosynthesis are poorly understood. Recent studies suggest that cutaneous sterol synthesis is regulated by cutaneous barrier requirements, while the levels of circulating sterols do not play a role. Whether cutaneous barrier requirements regulate epidermal lipogenesis in general and the nature of the signal that activates the lipid biosynthetic apparatus are unknown. We determined whether alterations of the cutaneous permeability barrier, induced by treatment with a solvent (acetone), a surfactant, sodium dodecyl sulfate (SDS), or essential fatty acid deficiency (EFAD), provoked a discrete versus global stimulation of epidermal and dermal lipid biosynthesis. Acetone treatment increased epidermal, but not dermal, sterol and fatty acid biosynthesis approximately threefold over controls at 1–4 hr, which returned to normal after 12 hr. SDS treatment likewise stimulated epidermal sterol and fatty acid biosynthesis, but the increase was less dramatic than in acetone-treated animals. Since plastic occlusion blocked the expected increase in de novo lipid biosynthesis in acetone-treated animals, it is possible that water flux provides the molecular signal for de novo synthesis. Finally, EFAD mice also demonstrated enhanced epidermal sterol and fatty acid biosynthesis in comparison to normals, an effect that also was abolished when transepidermal water loss was normalized by occlusion, despite the presence of ongoing EFAD. These results demonstrate that disruption of the cutaneous permeability barrier stimulates a parallel, global boost in both sterol and fatty acid biosynthesis that is limited to the epidermis. Since such stimulation is reversed by restoration of barrier function, transepidermal water gradients may regulate epidermal lipogenesis.—**Grubauer, G., K. R. Feingold, and P. M. Elias.** Relationship of epidermal lipogenesis to cutaneous barrier function. *J. Lipid Res.* 1987. 28: 746–752.

Supplementary key words epidermis • barrier function • sterologenesis • fatty acid biosynthesis • lipogenesis • essential fatty acid deficiency

The principal function of mammalian epidermis is to generate a water-tight outer layer, the stratum corneum, which protects the organism from desiccation due to excessive transcutaneous loss of water (1–4). Because of their unusually high content of hydrophobic species, including sphingolipids, free and esterified sterols, and free fatty acids, lipids are thought to play a key role in the

modulation of cutaneous permeability barrier function (5, 6). Yet, the factors that regulate epidermal lipid biosynthesis are poorly understood. Until recently, attempts to ascribe function to specific classes of epidermal lipids have utilized an analytic approach, comparing the lipid content of stratum corneum to living layers, and as a result particular importance has been ascribed to ceramides in barrier function (7, 8), because their content increases dramatically in the stratum corneum (9–11). Although such an analytic approach can provide suggestive associations, it does not provide direct evidence of a role for specific lipid classes in barrier function. To link specific lipids more clearly to barrier function, we have studied how epidermal lipid biosynthesis is regulated by barrier perturbation. To date, these studies have shown that the mammalian epidermis is a major site of sterol biosynthesis (12), and that epidermal sterol synthesis is modulated when the barrier is disrupted with lipid solvents, such as acetone (13). Epidermal sterologogenesis appears to be autonomous from circulating lipids, since plasma sterol levels do not influence cutaneous sterologogenesis (14), and since neither epidermal appendages (15) nor confluent cultured keratinocytes possess low density lipoprotein receptors (16).

Although these studies strongly suggest that the epidermis synthesizes sterols in response to cutaneous barrier requirements, sterols comprise only 15–25% of the stratum corneum lipid mixture (9–11, 17). Yet, both free and esterified fatty acids, the latter acylated to free sterols as well as both glycerol- and sphingolipids, are also present in abundance, and may be important for barrier function (op. cit.). In contrast to sterols, which appear to be generated within the epidermis, circulating fatty acids accumulate in the epidermis. Not only do dietary fatty acids become incorporated into cell membrane lipids under

Abbreviations: EFAD, essential fatty acid deficiency; NSL, total non-saponifiable lipids; SDS, sodium dodecylsulfate; TEWL, transepidermal water loss.

certain pathological circumstances (18), but the epidermis also lacks the Δ^6 desaturase and, therefore, it presumably must obtain the arachidonic acid required for eicosanoid generation from the circulation (19). The fundamental unresolved issue remains to what extent the epidermis synthesizes its own fatty acids versus deriving all or most of these molecules from the circulation. Moreover, if these substances are synthesized by the epidermis, to what extent is the synthesis of these lipid constituents also modulated by alterations in the permeability barrier. In this study, we have examined the effect of a variety of manipulations on epidermal and dermal lipid biosynthesis that alter the cutaneous permeability barrier. Our results indicate: *a*) that the epidermis synthesizes large quantities of fatty acids; and *b*) that barrier requirements regulate not only cholesterol biosynthesis, but also epidermal fatty acid synthesis (20).

METHODS

Materials

Radioisotopes. Tritiated water ($^3\text{H}_2\text{O}$, 1 Ci/g) was purchased from ICN Biochemicals. $[4\text{-}^{14}\text{C}]$ cholesterol, $[1\text{-}^{14}\text{C}]$ oleic acid, and $[9, 10\text{-}^3\text{H}]$ oleic acid were purchased from New England Nuclear Corporation (Boston, MA), and $[1,2\text{-}^3\text{H}]$ cholesterol and $[1\text{-}^{14}\text{C}]$ stearic acid were obtained from Amersham (Arlington, IL).

Chemicals. Sodium dodecylsulfate (SDS) was purchased from Bio-Rad Laboratories (Richmond, CA). Trypsin and potassium hydroxide (KOH) were obtained from Sigma (St. Louis, MO) and Mallinckrodt, Inc. (Paris, KY), respectively.

Solvents. The following solvents, from Fisher Scientific (Fairlawn, New Jersey), were utilized: acetone, chloroform, methyl alcohol, and petroleum ether. Thin-layer polygram silica gel G plates were obtained from Brinkmann Instruments (Westbury, NY), and scintillation counting fluid (Ultrafluor[®]) was purchased from National Diagnostics (Somerville, NJ).

Animals. Male hairless mice (Hr/Hr) purchased from Jackson Laboratories (Bar Harbor, ME) were between 1–3 months of age at the time of study. They were fed Simonson mouse diet (Gilroy, CA) and water ad libitum. Male hairless, essential fatty acid-deficient (EFAD) animals were maintained on an EFAD diet (21) from 2–3 weeks of age until reaching 1–3 months of age. Cutaneous signs of EFAD appeared after 6–8 weeks and there was elevated transepidermal water loss by 7–8 weeks (see below).

Experimental design

Disruption of the permeability barrier was achieved by unilateral treatment of one flank with either absolute ace-

tone or 10% sodium dodecylsulfate (SDS) in distilled water. The skin surface was scrubbed very lightly for about 10 min to avoid friction and afterwards washed gently with 0.9% saline. The contralateral (control) side was treated with 0.9% saline for a similar period of time, with similar applied pressure. Neither acetone nor saline treatment affected the total wet weight of the skin (see below). Transepidermal water loss (TEWL) was quantitated with a Meeco[®] electrolytic water analyzer, as described previously (13), and recorded in parts per million (ppm/cm² per hr).

To assess directly the effect of occlusion, which instantly lowers the measurable rates of TEWL to zero, on lipid biosynthesis, groups of acetone-treated animals were covered with a tightly fitting plastic (or Latex[®]) wrap immediately after treatment (13), until the animals were killed. Similarly, to determine the effect of barrier restoration in EFAD mice, the same, tightly fitting, plastic occlusive wrap was placed around the EFAD mice for 72 hr prior to killing the animals. Non-solvent-treated mice, on a normal diet, were also wrapped, serving as a control cohort for both experiments.

Lipid metabolic studies

All animals were injected intraperitoneally with tritiated water ($^3\text{H}_2\text{O}$, 20 mCi/mouse) 1, 4, and 12 hr after barrier disruption. Three hours after $^3\text{H}_2\text{O}$ administration, the animals were killed, the treated skin samples were obtained and weighed (accuracy ± 1 mg), and the epidermal and dermal fractions were separated by heat treatment (60°C for 60 sec). This procedure has been shown to yield a homogenous sheet of epidermis and dermis with no basal cells left attached to the dermis (13). Each skin fraction was saponified individually by refluxing overnight in a solution of 45% KOH, water, and 70% ethanol (2:1:5, by volume). The saponification flasks then were cooled and an internal standard of $[^{14}\text{C}]$ cholesterol and $[^{14}\text{C}]$ oleic acid was added. The subsequent extraction, fractionation, and counting of the nonsaponifiable lipids were performed as described previously (12, 13).

For extraction of fatty acids, after extracting the nonsaponifiable lipids, the saponified samples were acidified with 6 N HCl to pH 2. After acidification the material was extracted three times with petroleum ether and dried at 40°C. The saponified lipids were then resuspended in 1 ml of chloroform and an aliquot was removed for liquid scintillation counting. In addition, the specific activity of $^3\text{H}_2\text{O}$ in the plasma of each animal was determined, and the incorporation of $^3\text{H}_2\text{O}$ into epidermal and dermal lipids was calculated as has been described previously (12, 13).

Fatty acid transport to skin

To assess the influence of barrier disruption on transport of fatty acids to the skin, groups of hairless mice

were injected intraperitoneally with [^{14}C]stearic acid (10 $\mu\text{Ci}/\text{mouse}$), dissolved in a 10% solution of albumin in 0.9% saline, 1 hr after barrier disruption with acetone. Stearate was chosen both because it is the largest fatty acid that remains soluble in blood, and because very little C18:0 should be metabolized by β -oxidation in mitochondria, since very long chain fatty acids are metabolized in peroxisomes (22, 23), particularly under the short reaction conditions used in this experiment. The animals were killed 3 hr after injection, and the epidermal and dermal fractions were separated, as described above. In a second experiment, 20 mCi of $^3\text{H}_2\text{O}$ water were injected intraperitoneally and, after 3 h, the animals were killed, and incorporation of $^3\text{H}_2\text{O}$ into fatty acids was compared in the liver and epidermis. After saponification, an internal standard (^3H)cholesterol and (^3H)oleic acid) was added, and the lipids were assayed as described above.

Topical application of lipids

After acetone-induced barrier disruption, on one flank of the animal, TEWL was measured over different points. Immediately after TEWL measurements were performed, 1 mg of whole hairless mouse stratum corneum lipids, isolated as described previously (24) and dissolved in 0.3 ml of warm acetone-ethanol 3:1 (v/v), was applied with a pipette to a circumscribed area ($\sim 1\text{ cm}^2$) on the disrupted flank, while the rest of the treated flank did not receive lipids. The contralateral flank provided additional control sites, which were either treated with the same solvents or left untreated. After intraperitoneal injection of $^3\text{H}_2\text{O}$, the incorporation into cholesterol, total nonsaponifiable lipids, and fatty acids were determined, as described above.

Statistical significance was determined using either a paired t -test or a two-tail Student's t -test.

RESULTS

Impact of various treatments on permeability barrier function

After gentle treatment with acetone-soaked rayon balls, hairless mice displaced about a 50-fold increase (range: 30–100-fold) in TEWL above control sites, followed by a rapid decline in the first 5 hr after disruption (Fig. 1). This increase correlated with removal of all visible, oil red O-staining material from the stratum corneum, without evidence of damage to corneocytes or the underlying epidermis (13). TEWL levels had returned to almost normal levels by 33 hr after acetone treatment (Fig. 1). On the other hand, as shown in Table 1, treatment with 10% sodium dodecylsulfate (SDS) produced a less extensive defect in TEWL than occurred with acetone treatment, and TEWL returned to normal levels by 24 hr (13). Final-

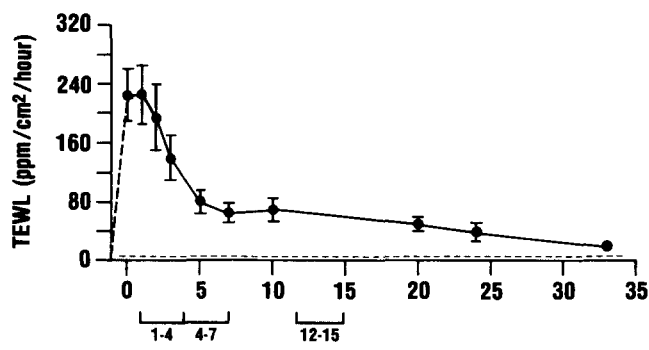


Fig. 1. Acetone treatment produces a dramatic increase in transepidermal water loss (TEWL) that returns to almost normal by 33 hr. The bars indicate the time periods in which the incorporation of $^3\text{H}_2\text{O}$ into lipids was measured. The dashed line represents normal TEWL levels in control mice ($n = 3$).

ly, as shown in Table 2, TEWL rates in EFAD rodents, due to dietary deprivation of linoleic acid, ranged from 80 to 170 ppm/cm^2 per hr (mean 106).

Epidermal cholesterol and fatty acid synthesis versus serum delivery

Prior studies have demonstrated the capacity of the mammalian epidermis to synthesize substantial quantities of sterols (12), and showed that the transport of sterols from extracutaneous sites does not contribute greatly to the total pool of labeled sterols in the epidermis (13). In these studies, there also was no increase in the delivery of cholesterol to perturbed skin sites (data not shown). To assess whether labeled fatty acids in the epidermis could be ascribed primarily to transport of fatty acids from extracutaneous sites of synthesis to the skin or local synthesis, we first calculated total cutaneous delivery and compared the transport rates of fatty acids to acetone-treated versus control skin sites. The quantity of albumin-bound [^{14}C]stearic acid (10 $\mu\text{Ci}/\text{animal}$) transported to the epidermis and dermis in four mice is shown in Table 3. Not only were the absolute quantities of fatty acids delivered to the skin relatively small ($\sim 0.02\%$),¹ but also the same amount of [^{14}C]stearic acid was transported to both the treated and the control epidermis. Thus, the transport of extracutaneously derived fatty acid to perturbed skin was not increased. Second, we compared fatty acid synthesis in the liver and epidermis. In the entire epidermis an average of 109,114 dpm/g was calculated based upon the individual specific activity in each animal, whereas the counts in the entire liver were only 208,213 dpm. Since

¹In a typical 20-g hairless mouse, total skin weight is about 1.0 g (data not shown). Of the administered dose of 2.2×10^7 dpm of ^{14}C stearate, about 4,400 dpm would enter the entire epidermis (see Table 3). Accordingly, $4.4 \times 10^3 + 2.2 \times 10^7 = 2 \times 10^{-4} = 0.02\%$

TABLE 1. The effect of SDS treatment on epidermal and dermal lipid biosynthesis^a

	TEWL (ppm)	Epidermis			Dermis		
		Cholesterol	NSL	FA	Cholesterol	NSL	FA
Controls (n = 5)	9 ± 3	0.89 ± 0.11	2.18 ± 0.21	3.69 ± 0.41	1.62 ± 0.09	3.42 ± 0.22	6.46 ± 0.46
SDS-treated (n = 5)	184 ± 56	1.31 ± 0.09	3.00 ± 0.20	5.06 ± 0.38	1.75 ± 0.13	3.66 ± 0.36	7.50 ± 0.66
Significance	<i>P</i> < 0.01	<i>P</i> < 0.01	<i>P</i> < 0.025	<i>P</i> < 0.025	NS	NS	NS

Epidermal and dermal de novo lipid synthesis was quantitated in mice in which one flank was disrupted with 10% SDS in distilled water; n = number of animals; NS, not significant; NSL, total nonsaponifiable lipids; TEWL, transepidermal water loss (ppm/cm² per hr).

^aMicromoles of ³H₂O incorporated per 3 hr per g (mean ± SEM).

only 0.02% of systemically administered dpm enter the epidermis, and considering that hepatic synthesis, as the main site of systemic fatty acid production was only slightly greater than epidermal synthesis, the contribution of extracutaneously synthesized fatty acids to the epidermal pool would be negligible when compared to the activity already present in the epidermis.

De novo lipid biosynthesis after barrier disruption

As described above, acetone treatment provoked a defect in skin barrier function, as indicated by a considerable increase in TEWL. Measurement of the incorporation of ³H₂O into newly synthesized epidermal cholesterol, total nonsaponifiable lipids (NSL), and fatty acids over a span of 3 hr, beginning 1 hr after breaking the barrier, revealed a 2.5–3-fold (*P* < 0.05) increase in incorporation into the respective lipid fractions in comparison to controls (Table 4). At later time points after barrier disruption, lipogenesis returned to normal despite persistence of slightly elevated TEWL rates. Whereas lipid biosynthesis was increased 1.4-fold (*P* < 0.05), 1.4-fold (*P* < 0.05), and 1.2 fold (*P* < 0.05) over controls in the 4–7 hr period after barrier abrogation, by the 12–15-hr time period, ³H₂O uptake into newly synthesized lipids was not significantly increased in comparison to untreated controls (Table 4). Acetone treatment affected the epidermis specifically, since the biosynthesis of dermal lipids at all time points was the same in both the control and acetone-treated sites (Table 4). The different quan-

ties of ³H₂O water incorporation in control animals at the various time points represents diverse variation in the baseline rates of lipid synthesis in control animals.

Application of a surfactant (10% SDS) resulted in less dramatic elevations in TEWL than was provoked by acetone treatment (Table 1). As in the acetone experiments, from 1 hr after barrier disruption through 4 hr, ³H₂O incorporation into sterols, NSL, and fatty acids was significantly elevated in the epidermis of treated sites in comparison to control sites (Table 1). Finally, as with acetone treatment, SDS did not significantly alter the rates of dermal biosynthesis; i.e., ³H₂O incorporation was comparable in both treated and untreated dermis (Table 1).

EFAD animals, which demonstrate a leaky barrier associated with lipid-depleted stratum corneum intercellular spaces (24–26), also demonstrated enhanced epidermal sterol and fatty acid biosynthesis in comparison to normals (Table 2). However, in contrast to acetone- and SDS-treated animals, EFAD animals also displayed elevated levels of dermal sterol and fatty acid synthesis (Table 2).

Occlusion experiments

When skin sites demonstrating increased TEWL after acetone treatment were occluded with a plastic wrap to block all TEWL, the characteristic increase in epidermal lipid biosynthesis was blocked (Fig. 2). In contrast, acetone treatment without occlusion again demonstrated the expected increase in ³H₂O incorporation (Fig. 2). In con-

TABLE 2. Epidermal and dermal lipid synthesis in EFA-deficient animals

	TEWL (ppm)	Epidermis			Dermis		
		Cholesterol	NSL	FA	Cholesterol	NSL	FA
Controls (n = 5)	9 ± 5	1.37 ± 0.20	2.85 ± 0.55	7.24 ± 0.99	1.49 ± 0.13	4.22 ± 0.66	9.28 ± 0.92
	<i>P</i> < 0.0005	<i>P</i> < 0.01	<i>P</i> < 0.025	<i>P</i> < 0.01	<i>P</i> < 0.005	NS	<i>P</i> < 0.025
EFAD (n = 5)	106 ± 18	2.51 ± 0.28	4.92 ± 0.66	12.35 ± 1.44	2.93 ± 0.45	5.52 ± 0.45	13.80 ± 1.58
		<i>P</i> < 0.005	<i>P</i> < 0.01	<i>P</i> < 0.005	NS	NS	NS
EFAD + occlusion (n = 5)	0	1.27 ± 0.22	2.48 ± 0.39	5.58 ± 0.91	2.16 ± 0.30	4.56 ± 0.45	11.80 ± 1.57

Epidermal and dermal de novo cholesterol, total nonsaponifiable lipids (NSL), and fatty acid (FA) synthesis are compared in control mice, essential fatty acid-deficient (EFAD) mice, and EFAD mice wrapped in Latex film for 72 hr; n = number of animals; NS, not significant. Significance data are presented between data of each group. TEWL, transepidermal water loss (ppm/cm² per hr).

^aMicromoles of ³H₂O incorporated per 3 hr per g (mean ± SEM).

TABLE 3. Influence of acetone treatment on transport of fatty acids to the skin^a

	Epidermis		Dermis	
	<i>dpm/g</i>			
Controls (n = 4)	4,020 ± 370		27,330 ± 3,430	
Acetone treatment (n = 4)	4,770 ± 940		25,870 ± 1,810	
Significance	NS		NS	

Transport of systemically administered [¹⁴C]stearic acid to acetone-treated skin. Hairless mice received intraperitoneal injections of [¹⁴C]stearic acid (10μCi/mouse), dissolved in a 10% solution of albumin in 0.9% saline, 1 hr after barrier disruption with acetone, while the other side served as a control. After 3 hr, the label in treated versus untreated sides was measured (see Methods).

^aDifferences between treated and untreated sites in both epidermis and dermis were not significant.

control animals, wrapping per se did not influence lipid biosynthesis in either the epidermis or dermis (data not shown).

When EFAD animals, demonstrating increased TEWL due to their defective barrier, were occluded for 72 hr, epidermal sterol, NSL, and fatty acid lipid biosynthesis returned to normal levels. In the EFAD animals, occlusion caused an approximately 50% reduction in epidermal lipid biosynthesis (Table 2). Biosynthetic rates in the dermis were not significantly affected by occlusion in EFAD animals (Table 2).

Topical replenishment experiments

To determine whether short-term, topical administration of extracted, hairless mouse stratum corneum lipids might influence de novo lipid biosynthesis after solvent-induced barrier disruption, the barrier function of one flank of each animal was disrupted with acetone. One hour after application of either 1 mg of mouse stratum corneum lipids or the acetone-ethanol vehicle, barrier

function had not changed significantly. Lipid applications to acetone-treated sites induced a very slight, but not statistically significant, suppression of epidermal lipid biosynthesis (data not shown).

DISCUSSION

It is now generally accepted that intercellular lipid domains of the stratum corneum are responsible for maintaining the mammalian cutaneous permeability barrier (reviewed in 27, 28). These lipids, by maintaining a low level of transcutaneous water loss, allow mammals to survive in a terrestrial environment. The studies reported here and prior studies from this laboratory indicate that there is a relationship between the barrier to transcutaneous water loss and epidermal sterol synthesis (13, 29). When the barrier was damaged with either a surfactant (SDS) or a lipid solvent (acetone) there was an increase in epidermal sterogenesis that was proportional to the degree of barrier disruption (13). Moreover, prior studies in EFAD animals, with disturbed barrier function, also demonstrated increased epidermal sterol synthesis (29, Table 2). Finally, when the defect in the permeability barrier was corrected with an impermeable occlusive film, sterogenesis remained normal in the detergent- and lipid solvent-treated animals (13), and returned to normal in the EFAD animals (29). These earlier observations, confirmed here in the same three experimental models, suggest that cutaneous water barrier status regulates epidermal sterogenesis.

In addition to studying the regulation of sterol synthesis by barrier function, here we have evaluated the capacity of the epidermis to synthesize fatty acids, and determined whether the regulation of synthesis of lipids other than sterols is also regulated by perturbations in the

TABLE 4. The effect of acetone treatment on epidermal and dermal lipid biosynthesis^a

Time after Treatment	Animals	Epidermis			Dermis		
		Cholesterol	NSL	FA	Cholesterol	NSL	FA
<i>hr</i>							
1-4	Controls (n = 5)	1.30 ± 0.40	2.20 ± 0.61	5.85 ± 1.8	4.16 ± 0.39	5.77 ± 0.54	19.70 ± 2.49
1-4	Acetone-treated (n = 5)	3.74 ± 0.89	6.43 ± 1.3	14.43 ± 2.75	4.41 ± 0.70	6.13 ± 1.08	17.56 ± 1.70
	Significance	<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> < 0.05	NS	NS	NS
4-7	Controls (n = 9)	0.94 ± 0.08	1.95 ± 0.22	4.98 ± 0.51	1.65 ± 0.16	3.15 ± 0.38	8.54 ± 0.62
4-7	Acetone-treated (n = 9)	1.31 ± 0.09	2.49 ± 0.24	6.07 ± 0.36	1.63 ± 0.12	2.96 ± 0.28	7.68 ± 0.42
	Significance	<i>P</i> < 0.005	<i>P</i> < 0.05	<i>P</i> < 0.05	NS	NS	NS
12-15	Controls (n = 5)	1.21 ± 0.17	2.22 ± 0.38	5.71 ± 0.94	2.56 ± 0.47	4.57 ± 0.82	9.20 ± 1.50
12-15	Acetone-treated (n = 5)	1.34 ± 0.12	2.30 ± 0.21	4.96 ± 0.52	2.32 ± 0.34	4.23 ± 0.52	7.36 ± 0.50
	Significance	NS	NS	NS	NS	NS	NS

The effect of acetone treatment on de novo epidermal and dermal cholesterol, total nonsaponifiable lipids (NSL), and fatty acid synthesis 1-4, 4-7, and 12-15 hr after barrier disruption with acetone on one flank; n = number of animals; NS, not significant. Significance data is presented under data for each group.

^aMicromoles of ³H₂O incorporated per 3 hr per g (mean ± SEM).

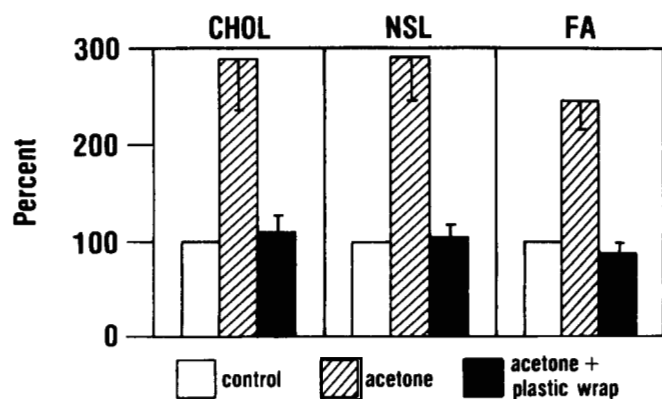


Fig. 2. The incorporation of $^3\text{H}_2\text{O}$ into cholesterol (chol), non-saponifiable lipids (NSL), and fatty acids (FA) in the epidermis 1-4 hr after 0.9% saline ($n = 5$), acetone treatment ($n = 5$), or acetone treatment immediately followed by occlusion with a Latex film ($n = 5$). Results are presented on a percentage basis with controls equal to 100%.

cutaneous permeability barrier. These studies demonstrate that the epidermis synthesizes fatty acids and, moreover, increases their synthesis in response to barrier requirements. We excluded the possibility that the large amounts of labeled fatty acids present in the epidermis were due to the transport of fatty acids from extracutaneous sites since the overall transport of [^{14}C]stearic acid to the skin was minimal. Moreover, since transport was comparable in perturbed versus control epidermal sites, this provides further evidence of endogenous synthesis. However, we cannot rule out the possibility that some transport of extracutaneously derived, very long-chain fatty acids may be taking place, since the plant-derived fatty acid, phytanic acid, accumulates in patients with Refsum's disease (18, 30). Moreover, since the epidermis lacks Δ^6 and Δ^5 desaturase activity (19), the epidermis presumably must obtain arachidonic acid from elsewhere. Furthermore, a role for dermal fibroblasts cannot be excluded, since these cells also generate very long-chain fatty acids (31). Nevertheless, these studies suggest that the epidermis possesses a substantial apparatus for de novo synthesis of fatty acids. To what extent fatty acids are synthesized locally versus some contribution from the dermis or extracutaneous sources under basal versus perturbed conditions still needs to be resolved.

We also have observed that epidermal fatty acid synthesis is stimulated when the permeability barrier is altered by either solvent or surfactant treatment, or by feeding a diet deficient in essential fatty acids. Moreover, the return of epidermal fatty acid synthesis to normal following acetone treatment parallels the restoration of normal barrier function. Furthermore, the relationship of fatty acid synthesis to permeability barrier function is supported by the specific localization of elevated synthesis to the epidermis; no augmentation of dermal fatty acid synthesis was noted.

These observations demonstrate that perturbations in barrier function not only regulate epidermal sterol synthesis, but also provoke a parallel stimulation of fatty acid synthesis.

A potential criticism of these studies might be that the burst in sterol and fatty acid synthesis represents a nonspecific response to skin injury rather than a specific response to barrier dysfunction. Against this argument are the following observations (13, 39, and present studies). First, increased synthesis is localized to the epidermis with no consistent effect in the dermis. Second, the time course of increased synthesis parallels the restoration of the barrier. Third, there is similarity of synthetic activity in three unrelated experimental models. Fourth, and perhaps most convincingly, the characteristic increase in epidermal lipid synthesis following acetone treatment is prevented by normalization of the barrier with a Latex film. Finally, in EFAD animals, restoration of the barrier with a Latex film restored epidermal lipid synthesis to normal, despite the presence of on-going, hyperplastic EFAD state (29, present studies). Taken together, these observations indicate that the synthesis of stratum corneum lipids is regulated in response to local disturbances in barrier function, and further, our data provide strong evidence that epidermal lipid biosynthesis is regulated by the rates of transcutaneous water loss. ■

We gratefully acknowledge the technical assistance of Barbara E. Brown and Steven Lear. Bil Chapman prepared the manuscript. Drs. Stephen Grayson and Daniel Monger provided helpful advice and criticism. This work was supported by NIH grant AM-19098 and the Veterans Administration Medical Research Service. Dr. Kenneth R. Feingold is a recipient of a Veterans Administration Clinical Investigatorship.

Manuscript received 12 November 1986 and in revised form 26 January 1986.

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