De novo sterologenesis in the skin. II. Regulation by cutaneous barrier requirements

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Abstract Recent studies suggest: *1*) that the epidermis and pilosebaceous epithelium are important sites of de novo sterol synthesis, and *2*) that the rate of cutaneous cholesterol synthesis does not change with alterations in circulating sterol levels. Since cutaneous sterols may be important for permeability barrier function, we studied the effect of experimentally altered barrier function on de novo sterologenesis in the epidermal and dermal layers of the skin. Epidermal sterologenesis appeared to be modulated by the skin's barrier requirements because topical detergent and acetone treatment stimulated de novo synthesis of nonsaponifiable lipids in the epidermis, but not in the dermis. Synthetic activity paralleled both the return of barrier function toward normal and the extent of prior damage to the barrier. Moreover, plastic-wrap occlusion of solvent-treated sites simultaneously corrected both the barrier abnormality and normalized sterol synthesis, further linking increased epidermal sterologenesis to barrier requirements. Whereas topical applications of a variety of other topical lipids did not down-regulate synthesis, epicutaneously applied 25-hydroxycholesterol appeared to diminish synthesis. These results suggest that maintenance of barrier function is one purpose of epidermal de novo nonsaponifiable lipid synthesis, and demonstrate further that, despite a lack of low density lipoprotein receptors, epidermis can regulate its lipid-synthetic apparatus in response to certain specific requirements. —**Menon, G. K., K. R. Feingold, A. H. Moser, B. E. Brown, and P. M. Elias.** De novo sterologenesis in the skin. II. Regulation by cutaneous barrier requirements. *J. Lipid Res.* 1985. **26:** 418–427.

Supplementary key words skin lipids; • epidermis • cholesterol • nonsaponifiable lipids • skin barrier

Previous studies have shown that the skin is a major site of sterologenesis in both rodents (1–3) and primates (4), accounting for up to 30% of total body sterol synthesis. Of this synthetic activity, we reported recently that about 80% was localized to the dermal layer in rodents, presumably emanating from the pilosebaceous epithelium which remained with the dermis (5). The remaining 20% of synthetic activity derived from the epidermis, where it was localized almost exclusively to the basal and spinous layers (5), which on a weight basis can be considered among the most active sites of sterol synthesis in the body. Prior work also has shown that circulating sterol levels do not effect cutaneous sterologenesis (6), and furthermore, de novo sterol synthesis, both by cultured keratinocytes and by pilosebaceous follicular epithelium, is not influenced by exogenous cholesterol, presumably due to the lack of LDL receptors on the membranes of these cells (7, 8).

Despite the suggestion from this work that cutaneous sterol synthesis is not subject to regulation, factors other than circulating lipoproteins could modulate epidermal sterologenesis. For example, both sterologenesis and HMG-CoA reductase activity of cultured keratinocytes are modulated by the polar sterol metabolite, cholesterol sulfate (9). Sterols are important constituents of epidermal cell membranes, where they may subserve permeability barrier function (10) and/or vitamin D_3 synthesis. In the studies reported here we explore the regulation of the skin's lipid biosynthetic machinery. We have found that epidermal sterologenesis is not affected by the host's vitamin D requirements; instead the permeability barrier requirements of the skin appear to modulate the intensity of epidermal nonsaponifiable lipid synthesis (11).

METHODS

Materials and animal procedures

[26-^{14}C]Cholesterol (0.5 mCi/0.33 mg), and $^{3}H_2O$ (1 Ci/g), were purchased from New England Nuclear. Thin-layer polygram silica gel G plates were purchased from Brinkmann Instruments. Ultrafluor was purchased from National Diagnostics. Synthetic lipids were obtained from Supelco (Bellefonte, PA). 25-Hydroxycholesterol and cholesterol sulfate were purchased from Research Plus (Bayonne, NJ). Hairless mice (Hr/Hr) were purchased from Jackson Laboratories (Bar Harbor, ME); all were males, aged 1 to 3 months at the time of experiments, and

Abbreviations: LDL, low density lipoproteins; TEWL, transepidermal water loss; DMSO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate.

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all were maintained on a normal light cycle and fed Simonsen mouse diet and water ad libitum.

Experimental permeability barrier disruption and transepidermal water loss determinations

Disruption of the permeability barrier to water was achieved by treatment of flank skin with either 10% sodium dodecyl sulfate (SDS) in distilled water or absolute acetone. The skin was scrubbed gently to minimize friction, and then washed with distilled water. Contralateral, control sites were washed with normal saline in the same manner as the detergent- or solvent-treated flank. Transepidermal water loss (TEWL) was measured 15 min later (when the skin surface was sufficiently dry) with a Meeco electrolytic water analyzer (Meeco Corp., Warrington, PA) (12). Dry nitrogen gas, 99.9% pure, was passed through the sample cup at 100 cc/min. The sampling cup was separated from its Parafilm® cover (sampling cup previously nitrogen gas-dried to basal levels), and slid onto the site to be measured, thus minimizing contamination with atmospheric air. TEWL was measured at regular intervals (every 6–12 hr) over a time period of 24–48 hr after a single SDS or acetone treatment. In some experiments treatments were repeated daily with SDS for 7 consecutive days to assess the long-term effects of chronic barrier perturbation. TEWL was recorded as parts per million (H_2O to nitrogen), when the reading had stabilized, in animals anesthetized with intraperitoneally administered chloral hydrate.

To directly assess the relationship of lipid synthesis to barrier disruption, several animals, previously treated bilaterally or unilaterally with acetone or saline, were covered with loose-fitting polyethylene wrap or tight-fitting flexible latex tubes for 3–6 hr, followed immediately by measurement of both TEWL and sterol synthesis.

Topical applications of lipid

Within 10 min of measuring TEWL (post-SDS treatment), solutions of synthetic lipids were applied to one flank of the animals (three or four animals per group). In one group, 20 μl of cholesterol (1 mg/ml in a 1:1 mixture of ethanol–dimethyl sulfoxide (DMSO)) was gently applied to the SDS-treated flank with a gloved hand. In another group of animals, 20 μl of cholesterol sulfate solution (1 mg/1 ml in absolute DMSO) was applied similarly. A third group of animals received 20 μl of a mixture of synthetic lipids (1 mg/ml in ethanol–DMSO 1:1, by vol), intended to approximate the lipid composition of extracts of human stratum corneum (13). The fourth group received 1.5 mg/ml of 25-hydroxycholesterol (dissolved in chloroform–DMSO, 1:1 by vol; a total of 20 μl). In each case control flanks received an equal volume of the respective vehicles alone. TEWL was measured over both blanks 45 min after each topical application, followed by injection of ^3H_2O (see below).

Radioisotope labeling and tissue separation

At appropriate intervals the animals were injected i.p. with ^3H_2O (20 mCi/mouse). Three hours after injection, the animals were killed, weighed, and a blood specimen was obtained. The epidermis and dermal layers were separated by exposure to dry heat for 60 sec at 60°C, and weighed. This procedure yields a homogenous sheet of epidermis with no basal cells left attached to the dermis (**Fig. 1**). The separated skin fractions were removed and saponified by refluxing overnight in a solution of 45% KOH–water–70% ethanol 2:1:5, by vol).

Transport of cholesterol to skin

To assess whether barrier disruption results in increased transport of cholesterol out of the circulation into the skin, two groups of four animals each were injected intraperitoneally with ~ 1 million dpm each of [^{14}C]cholesterol (as above), solubilized in monopalmitate (Tween 40, Sigma). Immediately prior to injection, one side of each animal was treated with SDS (group one) or acetone (group two) to disrupt the barrier (TEWL readings > 100 ppm/cm^2 per hr in all animals), while the other side was left untreated. After 24 hr skin layers were separated and the recovery of label in each layer was determined (**Table 1**).

Analysis of newly synthesized nonsaponifiable lipids

The saponification flasks were cooled and an internal standard of [^{14}C]cholesterol was added to the flasks before extracting the nonsaponifiable material three times with petroleum ether. The petroleum ether extract was dried, dissolved in chloroform, and applied to thin-layer chromatography plates. The plates were developed in ethyl acetate–benzene 1:5 for 50 min and the radioactive bands corresponding to cholesterol, lanosterol, and squalene were cut from the plates and placed in scintillation vials containing 10 ml of counting solution. The counting techniques, including calculations for background, spillover, and specific activity/animal, were performed as described previously (5). Results were calculated per gram net weight of skin per 3-hr pulse, because soluble protein data from epidermis were not reproducible (5). Statistical differences were determined using a two-tailed Student's *t*-test.

Microscopy procedures

Skin biopsies of treated sites were obtained from all animals at the time of killing, snap-frozen in liquid nitrogen, and stored at −70°C until sectioned. Frozen cryostat sections (8 μm) were cut at −20°C and stained with either hematoxylin and eosin to indicate the level of cleav-

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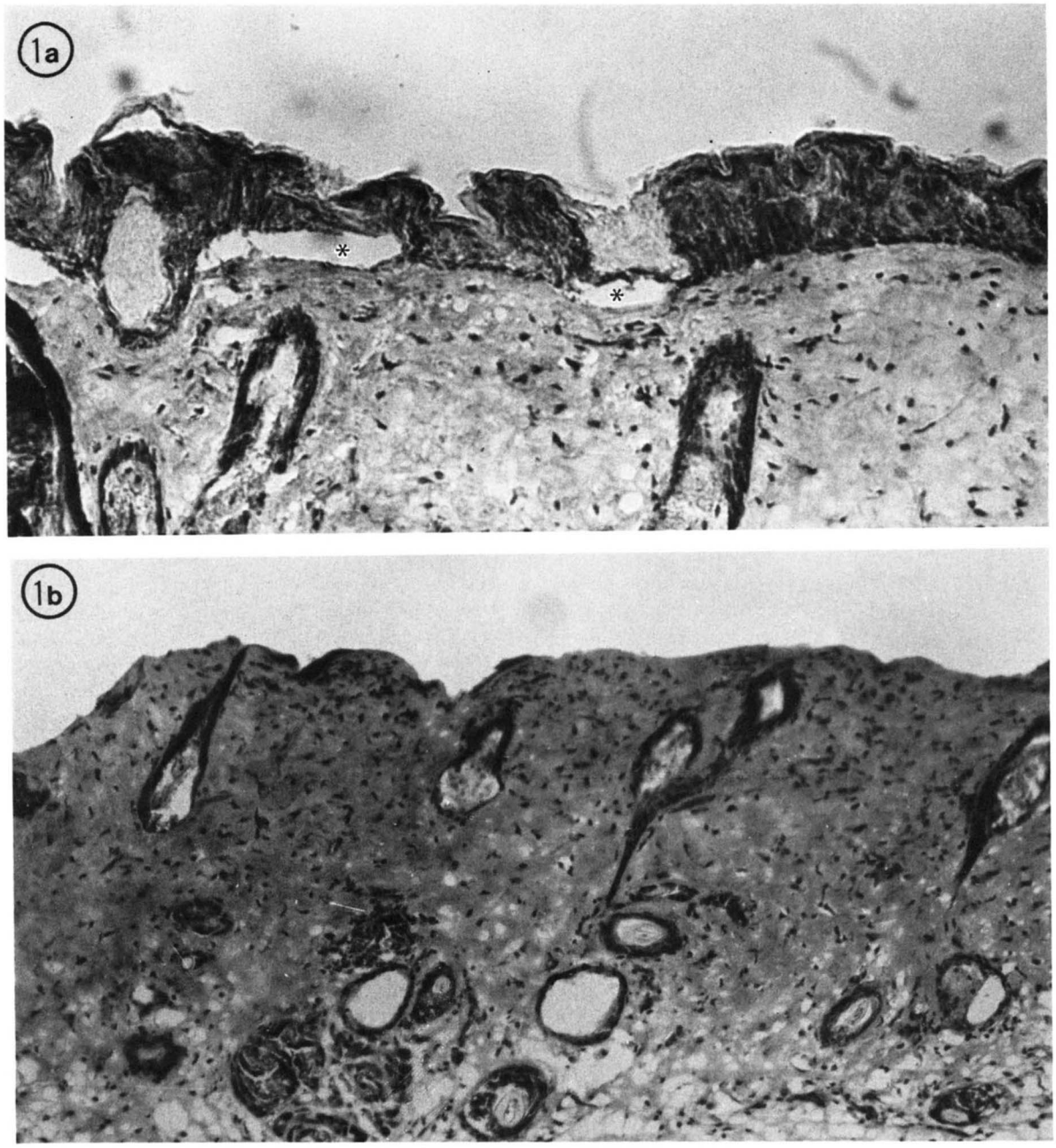

Fig. 1. Heat-treatment (60°C for 60 sec) of hairless mouse skin causes detachment of epidermis from dermis (* denotes cleavage plane in Fig. 1a). Entire epidermis peels off, leaving pilosebaceous units with underlying dermis (Fig. 1b). × 150.

age, or with oil red O, with or without hematoxylin counterstaining, to demonstrate neutral lipids.

RESULTS

Relationship of sterologenesis to barrier function

SDS treatment. To abrogate the barrier in hairless mice, we initially employed SDS solutions. A single SDS treatment removed all oil red O-stainable material (**Fig. 2**) and produced a minor, early defect in cutaneous barrier function, which corrected rapidly over a subsequent 24-hr period (**Table 2**). When SDS treatments were continued daily for an additional 2–7 days, correction occurred more quickly during each successive 24-hr interval, so that little or no additional barrier destruction resulted from repeated applications (data not shown).

Despite abrogation of the barrier with SDS, 24 hr after

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TABLE 1. Influence of SDS-treatment and acetone-treatment on transport of [^{14}C]cholesterol to the skin (dpm/g tissue per 24 hr)[a]

Animal #	Epidermis		Dermis	
	Treated	Untreated	Treated	Untreated
Group 1 (SDS)				
1	2,800	3,987	11,966	16,787
2	6,452	4,550	18,980	21,370
3	9,987	4,913	24,875	28,397
4	5,112	5,578	16,256	16,816
Mean ± SEM[a]	6,058 ± 1,477	4,765 ± 332	18,019 ± 2,703	20,843 ± 2,738
Group 2 (Acetone)				
1	6,558	6,782	66,387	70,083
2	2,373	2,505	11,616	12,833
3	3,536	3,913	43,746	47,144
4	4,895	4,622	33,010	36,364
Mean ± SEM[a]	4,340 ± 901	4,456 ± 892	38,690 ± 11,394	41,606 ± 11,892

Transport of systemically administered cholesterol to SDS-treated and acetone-treated skin versus normal skin. Hairless mice received intraperitoneal injections of [^{14}C]cholesterol (approx. 1 × 10^6 dpm) immediately after one side was treated with SDS (group 1) or acetone (group 2), while the other side was left untreated. After 24 hr, the label in treated versus untreated sides was measured (see Methods).

[a]Differences between treated and untreated sites in both cell layers are not significant by the usual Student's *t* test. On paired *t* testing, significant differences were not observed in the epidermis, but in both the acetone and SDS experiments the transfer of cholesterol to the untreated dermis was slightly but significantly increased.

such treatment there was no evidence of increased transport of systematically administered cholesterol to the skin (Table 1). In contrast, over the same period of time and under the same conditions, incorporation of ^3H_2O into cholesterol and total nonsaponifiable lipids was greatly increased (see below). This suggests that transport of newly synthesized cholesterol from extracutaneous sites to the skin could not account for the increase in labeled epidermal cholesterol following SDS treatment.

SDS treatment provoked a statistically significant burst of epidermal sterol synthetic activity, which remained elevated even after 21–24 hr, despite return of barrier function to normal by that time (**Fig. 3**). In several preliminary experiments, the control, saline-treated site demonstrated levels of synthesis comparable to sites left completely untreated, and moreover, neither SDS nor saline treatment affected dermal sterologenesis.

Acetone treatment. Oil red O-stainable material was completely removed from the stratum corneum by a single acetone wash (**Fig. 4**). Although staining did not reappear by 24 hr, some staining returned in the outer layers of the stratum corneum by 48 hr, and the intensity of staining

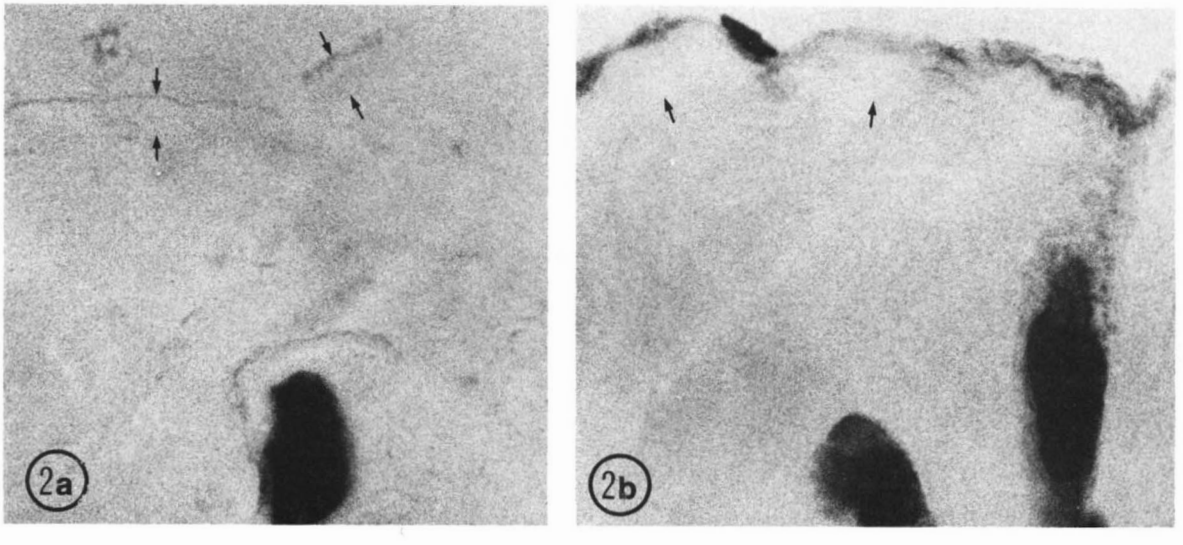

Fig. 2. SDS-treatment removes all oil red O-stainable material (Fig. 2a). Staining has not returned at 24 hr despite return of barrier formation to normal (cf. Table 2). Arrows denote upper and lower margins of stratum corneum. × 400.

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TABLE 2. Epidermal water loss and lipid metabolic activity after SDS treatment[a]

Time	TEWL	^3H_2O into Epidermal Lipid[b]	
		Cholesterol	TNS
hr	*ppm/cm² per hr*		
0–3	141 ± 21.1	+0.09 ± 0.01	+0.3 ± 0.02
6–8	47 ± 13.6	+0.16 ± 0.02	+0.6 ± 0.07
21–24	13 ± 2.2	+0.18 ± 0.11	+0.27 ± 0.14

Epidermal and dermal de novo sterologenesis are compared on sides of hairless mice treated with sodium dodecyl sulfate (SDS) versus saline-treated controls (three or four animals at each time point). Data shown are differences (± SEM) of SDS-treated versus control side. Epidermal cholesterol and nonsaponifiable lipid synthesis increased significantly ($P < 0.05$) at all time points in the epidermis, but showed no significant trend in the dermis. Although TEWL returned to normal by 24 hr, epidermal synthetic activity remained elevated.

[a]TEWL (transepidermal water loss) of untreated control side ranged from 4 to 12 ppm/cm² per hr. Values shown represent mean ± SEM.

[b]Values represent differences between SDS-treated and control sites ± SEM (μmol/g tissue per 3 hr); TNS, total nonsaponifiable lipids.

was indistinguishable from control by 96 hr (Fig. 4).

Although acetone treatment produced a more profound and reproducible defect in barrier function than did SDS treatment, TEWL levels returned rapidly to normal by 24 hr (**Fig. 5**). As with SDS treatment, acetone treatment did not result in increased transport of cholesterol from the circulation to the skin (Table 1). Epidermal cholesterol and nonsaponifiable lipid synthesis remained significantly elevated for 1–5 hr after acetone treatment, and again increased synthesis was limited to the epidermis; regardless of the severity of damage to the barrier, dermal synthesis remained unaffected (**Table 3**).

Yet, in contrast to SDS treatment, where epidermal sterologenesis remained high at 24 hr and beyond, sterologenesis following acetone treatment declined over 24 hr in parallel with the return of barrier function toward normal (Fig. 5). As can be seen in **Fig. 6**, the extent of synthetic activity following acetone treatment generally paralleled the severity of the barrier abnormality: higher rates of TEWL were accompanied by proportionately higher rates of de novo sterologenesis (data shown are from 9–12-hr time period).

As noted above, despite the return of metabolic activity to normal levels by 24 hr, oil red O staining required 4 days to reacquire normal intensity (Fig. 4). Since oil red O staining reappeared initially in the outermost layers of the stratum corneum (Fig. 4), the possibility was raised that the barrier might be corrected through the deposition of pilosebaceous lipid over the surface of the skin, rather than by reappearance of lipids within the depths of the stratum corneum. However, the barrier remained intact in animals 24–28 hr after acetone treatment, even when the outermost, surface lipid-containing, oil red O-positive layers were removed by tape-stripping (data not shown).

Occlusion experiments. In order to assess directly the relationship between sterologenesis and barrier function, animals were first treated with acetone to disrupt the barrier, followed by the application to treated sites of an impermeable polyethylene or Latex® film. In a third group of animals, similarly treated sites were coated with an occlusive layer of petrolatum. As a result of occlusion, TEWL declined immediately from an average of over 500 ppm/cm² per hr to normal levels in the case of petrolatum, and subnormal levels in plastic-wrapped animals (**Table 4**). Coincident with correction of the barrier, sterologenesis also returned to normal or subnormal levels in animals occluded with both types of plastic films, but there was a paradoxical increase in sterologenesis in animals treated with petrolatum (Table 4). The reduced levels of sterologenesis in plastic-wrapped animals could not be ascribed to altered blood flow because similar results were obtained with both the loose-fitting polyethylene wrap and the more tight-fitting Latex tube. That occlusion per se was not responsible for the decline in synthesis was shown by the similarity of synthetic activity in saline-scrubbed occluded versus unoccluded skin (Table 4; cf. Table 1). The blockade in sterol synthetic activity produced by plastic occlusion was paralleled by a delay in the return of oil red O staining in frozen sections: at 48 hr no oil red O staining was seen in occluded stratum corneum (Fig. 4).

Topical lipid applications. To determine whether the metabolic response to acetone and/or SDS treatment resulted from barrier disruption per se or from loss of lipid from the stratum corneum, groups of animals were treated with

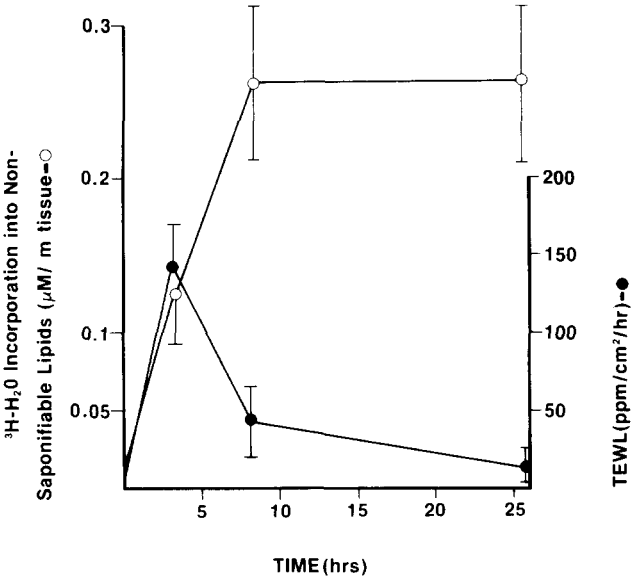

Fig. 3. SDS-treatment of hairless mouse skin causes a moderate increase in transepidermal water loss (TEWL, ●), which returns rapidly towards normal. Epidermal de novo sterol synthetic rates (○), however, remain high even after correction of the barrier (cf. Table 2).

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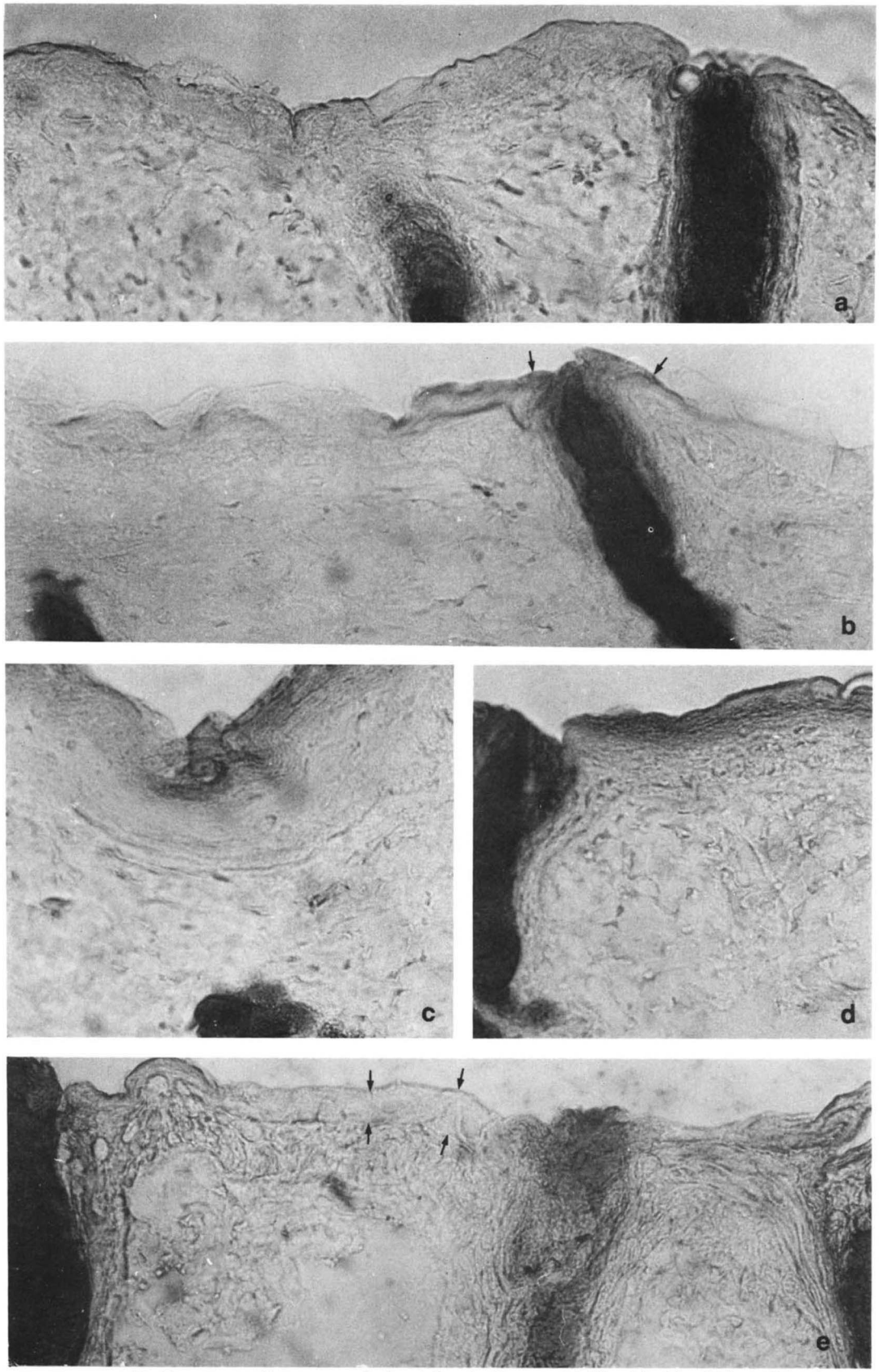

Fig. 4. Acetone-treatment of hairless mouse skin removes all oil red O-stainable material (Fig. 4a). After 24 hr, staining has not returned, but some lipid can be seen adjacent to hair follicles (Fig. 4b, arrows). After 48 hr, more lipid has coated surface (Fig. 4c), but only at 96 hr has staining returned to normal (Fig. 4d). Note that the epidermis of acetone-treated skin is thicker (more hyperplastic) at 24 and 48 hr than either control epidermis, or epidermis occluded after acetone treatment. Plastic-wrap occlusion for 48 hr retards all oil red O-staining (Fig. 4e). × 400.

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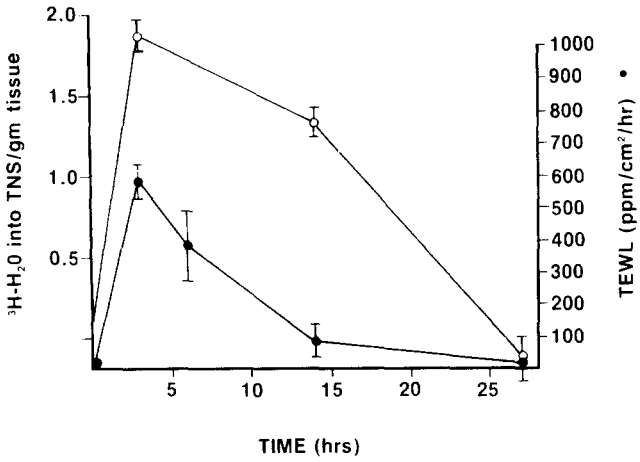

Fig. 5. Acetone-treatment produces a dramatic increase in transepidermal water loss (TEWL, ●) that returns to normal by 24 hr. In contrast to SDS, however, de novo sterol synthesis (○) returns to normal over the corresponding time period. TNS, total nonsaponifiable lipid (cf. Table 3).

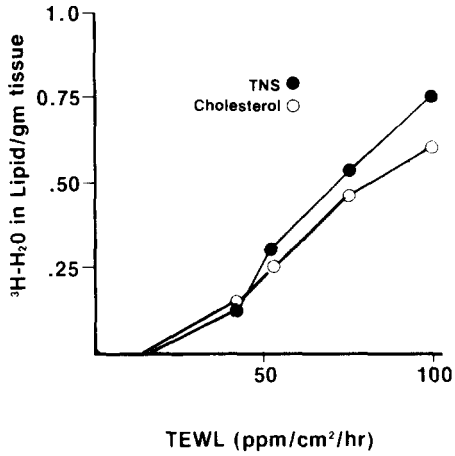

Fig. 6. When the rate of de novo synthesis against transepidermal water loss (TEWL) 6-9 hr after acetone treatment is compared to synthetic activity, the severity of the barrier defect is proportional to the extent of synthesis (closed circles, total nonsaponifiable lipids; open circles, cholesterol).

solutions of cholesterol, cholesterol sulfate, 25-hydroxycholesterol, a mixture of synthetic neutral and sphingolipids approximating stratum corneum lipid composition (14), or vehicle alone (see Methods). Whereas topical cholesterol, cholesterol sulfate, and the synthetic lipid mixture, at these concentrations and in these vehicles, neither corrected the barrier abnormality nor appeared to down-regulate de novo synthesis (**Table 5**), 25-hydroxycholesterol appeared to decrease sterologenesis significantly, but not to levels encountered in untreated skin (**Table 6**). Moreover, while 25-hydroxycholesterol appeared to reduce sterologenesis, cholesterol and cholesterol sulfate produced a statistically significant increase in de novo synthesis over the vehicle alone (Table 5), an effect that was also observed in animals treated with topical n-alkanes in the form of petrolatum (cf. Table 4). Moreover, the failure of cholesterol, cholesterol sulfate, or the whole stratum corneum lipid mixture to suppress synthetic activity could not be ascribed to failure of the applied lipids to reach the viable epidermis, because similar stimulation of synthesis occurred even when the lipids were applied directly to the viable epidermis after removal of the stratum corneum by tape-stripping (data not shown).

TABLE 3. Time course of barrier disruption and epidermal lipid metabolic activity after acetone treatment

		^3H_2O into Epidermal Lipid[a]	
Time	TEWL	Cholesterol	TNS
hr	*ppm/cm² per hr*		
1-4	396 ± 62	+0.21 ± 0.12	+0.28 ± 0.14
6-9	256 ± 136	+0.06 ± 0.03	+0.16 ± 0.09
12-15	67 ± 15	+0.42 ± 0.11	+0.43 ± 0.16
24-27	20 ± 5.7	−0.05 ± 0.05	−0.08 ± 0.05

Epidermal and dermal de novo sterologenesis are compared on sides of hairless mice treated with acetone- versus saline-treated controls (three or four animals at each time point). Data shown are differences (± SEM) of acetone-treated versus control sides. Epidermal cholesterol and nonsaponifiable synthesis was significantly higher than control ($P < 0.05$) at 1-4 and 12-15 hr, but returned to normal or subnormal levels by 24 hr, in parallel with transepidermal water loss (TEWL, cf. Fig. 5). In contrast, the dermis showed no significant differences in synthesis at any of the time points.

[a]Values represent mean ± SEM of differences between acetone-treated versus saline-treated sites of each group (μmol/g tissue per 3 hr); TNS, total nonsaponifiable lipids.

DISCUSSION

Although prior studies have demonstrated that the skin is a major site of sterol synthesis in both rodents and primates (1-4), no light was cast on the regulation of this synthetic activity. Moreover, since epithelial cells in the skin appear to lack LDL receptors (7, 8), it is possible that cutaneous sterologenesis may not be influenced by circulating sterols, a conclusion supported by the failure of cutaneous sterologenesis to change with different blood levels of cholesterol (6). Although it could be argued from the above that cutaneous sterologenesis continues unabated, free from all regulatory influences, the present study demonstrates that cutaneous sterologenesis responds to certain specific regulatory influences. The recent finding that the polar sterol metabolite, cholesterol sulfate, also regulates sterologenesis in cultured human keratinocytes further demonstrates that epidermal sterologenesis is not entirely autonomous (9).

In contrast to all other epithelia, the lipids in keratinizing squamous epithelia are unique because they are

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TABLE 4. Effect of occlusion on de novo lipid synthesis following barrier disruption

Treatment	TEWL[a]	Epidermis				Dermis			
		Acetone		Control		Acetone		Control	
		Chol	TNS	Chol	TNS	Chol	TNS	Chol	TNS
Expt. #1 Latex glove	0	0.20 ± 0.02	0.43 ± 0.05	0.34 ± 0.03	0.62 ± 0.07	0.63 ± 0.09	0.98 ± 0.14	0.68 ± 0.11	1.04 ± 0.17
Expt. #2 Plastic wrap	0	0.19 ± 0.03	0.31 ± 0.05	0.26 ± 0.03	0.45 ± 0.06	1.59 ± 0.22	2.22 ± 0.28	1.48 ± 0.30	2.01 ± 0.35
Expt. #3 Petrolatum	28 ± 8.5	1.11 ± 0.25	1.54 ± 0.31	0.50 ± 0.08	0.74 ± 0.13	2.47 ± 0.35	3.69 ± 0.44	2.66 ± 0.27	4.19 ± 0.31

The effect of occlusion, either by a synthetic plastic film or by petrolatum (a mixture of long chain n-alkanes) on epidermal and dermal de novo synthesis is compared after disruption of the barrier with acetone (n = three or four animals in each group). All three occludents reduced transepidermal water loss (TEWL) to normal (or subnormal) levels. Whereas the plastic films, during a subsequent 3-hr period following ^3H_2O incorporation, demonstrated decreased epidermal synthesis, petrolatum produced a paradoxical increase in synthesis above control rates. Dermal synthetic rates did not differ significantly in any of the three groups of animals. $P \leq 0.05$ for all acetone-treated versus control in experiments #1, 2, and 3.

[a]TEWL in experiments #1 and 2 ranged from 400 to 700 ppm/cm^2 per hr for all animals after treatment and prior to occlusion; in experiment #3, TEWL prior to occlusion was 490 ± 35 ppm/cm^2 per hr versus untreated sites that ranged from 4 to 12 ppm/cm^2 per hr.

[b]Values given as μmol of ^3H_2O incorporated/g tissue per 3 hr.

secreted and sequestered in intercellular domains during terminal differentiation (14). Because of their intercellular location, they are in an appropriate location to modulate cutaneous barrier function (10), or to deliver sterols to the intercellular spaces for subsequent conversion and absorption into the circulation (15). Although cutaneous lipids have long been known to play a critical role in waterproofing (reviewed in ref. 16), direct evidence that epidermal sterologenesis is regulated by this function is still missing. The results reported here demonstrate a dynamic relationship between the host's water-barrier requirements and epidermal sterol metabolism. When the barrier was damaged with either detergents (SDS) or solvents (acetone, DMSO—latter data not shown), a burst of nonsaponifiable lipid synthetic activity followed which was limited to the epidermis. In parallel experiments, we also found that saponifiable lipid synthesis was similarly enhanced (K. R. Feingold, unpublished observations), suggesting that not only sterologenesis, but lipogenesis in general is regulated by barrier requirements. The following observations suggest that enhanced nonsaponifiable lipid synthesis does not represent a nonspecific response to skin injury, but rather, a specific response to barrier dysfunction. First, increased synthesis was limited to the epidermis with no amplification of dermal metabolic activity. Second, the time course of synthetic activity, at least after acetone treatment, closely paralleled barrier repair, and after a single treatment with acetone, both barrier function and de novo sterologenesis had returned to normal by 24 hr. This is particularly noteworthy inasmuch as epidermal alterations were still evident histologically at 24 hr (Fig. 4). Third, there was a strong correlation between the degree of barrier abrogation and the intensity of synthetic activity. Fourth, lipid synthesis abruptly normalized when acetone-damaged skin was occluded with a polyethylene or latex film. The fact that

TABLE 5. Effect of topical lipids on epidermal barrier function and lipogenesis[a]

Exp. #	Lipid Fractions	TEWL[b] (Post-Rx)	^3H_2O in Epidermal Lipid[c]	
			Chol	TNS
1	Cholesterol	35 ± 6.2	0.93 ± 0.13	1.70 ± 0.22
	Vehicle	62 ± 21	0.45 ± 0.05	0.87 ± 0.12
2	Cholesterol sulfate	51 ± 4.5	1.14 ± 0.11	1.79 ± 0.10
	Vehicle	27 ± 2.3	0.49 ± 0.04	0.80 ± 0.06
3	Synthetic SC lipids	93 ± 56	0.13 ± 0.05	0.26 ± 0.10
	Vehicle	103 ± 35	0.15 ± 0.09	0.28 ± 0.16

Comparison of the effect of topical lipid applications versus vehicle on epidermal de novo sterologenesis after disruption of the barrier with SDS. None of the deposited lipids significantly affected TEWL. Moreover, none of the lipids reduced de novo synthesis and, in fact, each paradoxically increased synthetic activity significantly over vehicle ($P < 0.001$). Dermal synthetic activity was not affected significantly by any of the treatments (data not shown).

[a]TEWL, transepidermal water loss (ppm/cm^2 per hr); TNS, total nonsaponifiable lipids; SC, stratum corneum.

[b]TEWL prior to SDS treatment and topical applications ranged from 3 to 7 ppm/cm^2 per hr. Values shown are TEWL both after SDS treatment and 45 min after applications of topical lipids.

[c]Values are given as μmol/g tissue per 3 hr.

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TABLE 6. Influence of topical 25-hydroxycholesterol on barrier function and sterologenesis[a]

Treatment	Lipid Fraction	TEWL (ppm/cm² per hr)		Cholesterol Incorporation	
		Pre-Rx	Post-Rx	Epidermis	Dermis
SDS	25-Hydroxycholesterol[b]	107 ± 12.8	356 ± 6	1.63 ± 0.09	2.64 ± 0.13
	Vehicle alone[b]	155 ± 51.2	336 ± 54	2.26 ± 0.04	2.20 ± 0.10
Acetone	25-Hydroxycholesterol[c]	650	903	1.26	2.40
	Vehicle alone[c]	730	895	1.78	2.85
Untreated		35 ± 9		0.85 ± 0.16	4.15 ± 0.65

Effect of topical 25-hydroxycholesterol on barrier function and sterologenesis in hairless mice previously treated with SDS or acetone. TEWL levels were read immediately after barrier disruption, and both prior to and 45 min after applications of topical lipid or vehicle. Three to six animals are included in each group, except acetone-treated ($n = 2$, one animal died). Incorporation into cholesterol is shown; similar results (not shown) were found in total nonsaponifible lipids.

[a]Values given as μmol/g tissue per 3 hr.

[b]25-Hydroxycholesterol versus vehicle, $P < 0.01$.

[c]One site from each animal had value > 1,000.

occlusion alone normalized synthesis proves that enhanced synthesis after solvent or SDS treatment is not merely a response to removal of regulatory lipid molecules (see below). Fifth, synthesis was regulated, in part, by application of topical 25-hydroxycholesterol, which argues against increased synthesis as a nonspecific response to injury. Together, these observations strongly suggest that the host's water-barrier requirement represents one factor that can influence epidermal sterol metabolism.

It also should be noted that modulation in response to one functional requirement does not exclude other potential roles for epidermal sterol metabolism. For example, epidermal lipids are also considered to be regulators of stratum corneum disquamation (16, 17). In addition, epidermal sterols serve as important precursors for vitamin D_3 synthesis (18). Studies are underway to determine whether either of these factors might regulate sterologenesis.

Two other observations here deserve comment. First, the return of barrier function to normal in both SDS and acetone-treated skin was not accompanied by a comparable return of normal oil red O staining. The most likely explanation for this is that the barrier to water loss is quickly repaired by the secretion of lipid at the granular-cornified layer interface (15, 17). Either the amount of lipid required to repair the barrier is below the detection of oil red O staining, or the tinctorial properties of the initially deposited lipid may be different from the lipid residing throughout the remainder of the stratum corneum interstices. Yet, oil red O staining does increase with time, despite absence of increased sterologenesis. This discrepancy can be explained either by conversion of previously synthesized, polar lipid to neutral lipid (no de novo synthesis would be required), or a decreased rate of degradation (increased $T_{1/2}$) of intercellular neutral lipids. Further studies are needed to distinguish among these possibilities.

The second noteworthy observation was the difference in sterol synthetic response that followed SDS versus acetone treatment. Despite the much more profound defect in barrier function produced with acetone, epidermal sterologenesis returned to normal in 24 hr, while in contrast, synthetic activity continued unabated 24 hr after SDS treatment despite a much smaller defect in barrier function. We presume that this difference reflects the highly selective nature of the defect produced with acetone versus more sweeping damage that results from detergent treatment.

The nature of the chemical signal for enhanced synthesis in response to barrier abrogation is not known. Whereas the occlusion experiments imply that it is water itself which is the trigger, we have not excluded the possibility that specific regulatory molecules are removed during barrier disruption. Yet, we found that most mixtures of epicutaneously applied synthetic lipids did not down-regulate synthesis, but instead appeared paradoxically to stimulate synthetic activity. On the other hand, some degree of regulation appeared to be achieved with 25-hydroxycholesterol. Further studies are underway to determine whether other regulatory signals exist in the skin.

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