Transepidermal water loss: the signal for recovery of barrier structure and function

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Abstract Previous studies have demonstrated that perturbations in barrier function stimulate epidermal lipid synthesis and that this increase can be prevented by occlusive membranes. These observations suggest that epidermal lipid synthesis might be related to barrier function and raised the question whether transcutaneous water flux might regulate epidermal lipogenesis. In the present study we first abrogated the barrier with acetone, and then compared the rate of repletion of stainable lipids, barrier recovery, and epidermal lipogenesis in animals covered with occlusive membranes or vapor-permeable membranes versus uncovered animals. Acetone treatment of hairless mice removed stainable neutral lipids from the stratum corneum, with repletion evident both biochemically and histochemically within 48 hr in uncovered animals. In contrast, when the animals were covered with an occlusive membrane, the usual return of stratum corneum lipids was aborted. Since application of vapor-permeable membranes allowed normal lipid repletion, occlusion alone is not responsible for the inhibition of lipid repletion. Acetone treatment also perturbed epidermal barrier function, which returned to normal in uncovered animals in parallel with the reappearance of stratum corneum lipid. However, when animals were covered with an occlusive membrane, barrier function did not recover normally. In contrast, occlusion with vapor-permeable membranes allowed barrier function to recover normally. Finally, whereas occlusive membranes prevented the characteristic increase in epidermal lipid synthesis that follows barrier perturbation, epidermal lipid synthesis was increased in animals covered with a vapor-permeable membrane. These results point to transepidermal water flux itself as the signal that regulates epidermal lipid synthesis, which is associated first with the redeposition of stratum corneum lipids and then the normalization of stratum corneum barrier function.—**Grubauer, G., P. M. Elias, and K. R. Feingold.** Transepidermal water loss: the signal for recovery of barrier structure and function. *J. Lipid Res.* 1989. **30:** 323–333.

Supplementary key words epidermal lipogenesis • occlusive membrane • hairless mice • stratum corneum • water flux

Although lipids account for only a small percentage of total stratum corneum weight (~10%), they provide the permeability barrier that is required for terrestrial life (1–6). Their ability to regulate barrier function can be attributed to their extreme hydrophobicity, their intercellular location, and to their organization into broad, lipid-enriched bilayers (7). Recent studies have shown that the epidermis is a very active site of both sterol and fatty acid synthesis, with most of the lipids accounting for the intact cutaneous barrier synthesized in the epidermis itself rather than deriving from extracutaneous sources (8–10).

Epidermal lipid synthesis appears to be relatively autonomous from systemic influence. Neither dietary cholesterol nor circulating sterol levels affect cutaneous sterologenesis (11, 12); furthermore, de novo sterol synthesis both by confluent cultured keratinocytes and by follicular epithelium is not influenced by exogenous cholesterol, presumably due to the paucity of LDL receptors on the membranes of these cells (13, 14). Although systemic factors do not appear to influence epidermal sterol and fatty acid synthesis, local perturbation of the cutaneous permeability barrier by organic solvents markedly stimulates epidermal lipid synthesis, which returns to normal in parallel with the return of barrier function (8, 9). Furthermore, essential fatty acid-deficient mice, who exhibit a dietary perturbation in barrier function (15), also demonstrate increased epidermal sterol and fatty acid synthesis that normalizes following replenishment with linoleic acid (9, 16).

When the defect in barrier function, induced by either acetone or essential fatty acid deficiency, is corrected by application of a water-impermeable membrane, the expected increase in epidermal sterol and fatty acid synthesis does not occur (8, 9, 16). These studies suggest that barrier function regulates epidermal lipid synthesis and raise the possibility that water flux itself might serve as the regulatory signal. To examine the role of water flux as the potential signal, we have correlated barrier function, stratum corneum lipid content, and epidermal lipid biosynthesis in acetone-treated animals covered with either occlusive or vapor-permeable membranes. These studies clearly link epidermal lipid synthesis with both stratum

Abbreviations: TLC, thin-layer chromatography; TEWL, transepidermal water loss.

corneum lipid replenishment and normalization of barrier function, establishing a strong case for water flux as the critical regulatory influence.

MATERIAL AND METHODS

Materials

Hairless male mice (HR/HR), 8–10 weeks old, were purchased from Jackson Laboratories (Bar Harbor, ME). They were fed Simonsen mouse diet (Gilroy, CA) and water ad libitum. The age ranged between 1 to 3 months at the time of study. Tritiated water (1 Ci/g) was purchased from ICN Biochemicals (Costa Mesa, CA). [4-^{14}C]Cholesterol and [1-^{14}C]oleic acid were obtained from New England Nuclear Corporation (Boston, MA). Acetone was purchased from Fisher Scientific (Fairlane, NJ). Thin-layer polygram silica gel G plates were obtained from Brinkmann Instruments (Westbury, NY). Ready-Solv scintillation counting fluid was purchased from Beckman (Fullerton, CA). Latex®, a vapor-impermeable barrier, was obtained from Fisher Scientific (Pittsburgh, PA). Two different vapor-permeable membranes were used in our studies. Op-Site®, a moisture vapor-permeable adhesive drape, was obtained from Acme United Corporation (Bridgeport, CT). Goretex® was produced by W. L. Gore and Associates and purchased at REI (Berkeley, CA). Goretex® is impermeable to water but is permeable to water vapor (17). Oil red O and nile red were purchased from Polysciences, Inc. (Warrington, PA).

EXPERIMENTAL PROCEDURES

Solvent treatment and water loss measurements

Hairless mice were treated on one flank with acetone-soaked cotton balls. The other flank, serving as a control, was treated with saline-soaked cotton balls. Each was rolled gently with approximately equal pressure to the skin surface and neither acetone nor saline treatment produced visible or microscopic (8) damage to the stratum corneum.

After treatment, transepidermal water loss was measured as described previously (8, 9). In the experiments in which animals were covered with membranes, transepidermal water loss was measured 30 to 60 min after the removal of the membrane.

Ultrastructural studies

Barrier function was also assessed morphologically by visualization of transport of the electron-dense, water-soluble tracer lanthanum nitrate through the stratum corneum (18). Briefly, untreated animals, animals treated with acetone and left exposed to air for 48 hr, and animals treated with acetone and occluded with an impermeable Latex® membrane for 48 hr were killed. Skin samples were immediately obtained, and placed in a petri dish containing the lanthanum nitrate solution, stratum corneum side downward to restrict lanthanum nitrate exposure to the outer epidermis. After 2 hr of treatment with 2% aqueous lanthanum nitrate, pH 7.4, samples were fixed in 2% cacodylate-buffered glutaraldehyde, and processed for electron microscopy (18).

Water loss inhibition

Immediately after solvent treatment the animals were either wrapped in a tightly fitting vapor-impermeable membrane (Latex®) or in vapor-permeable membranes (Goretex® or Op-Site®). Op-Site® was adhesive and therefore the animals could be wrapped in the membrane. Goretex® was wrapped around the animal's trunk and then covered with a Latex® membrane to hold the Goretex® in place. In the area to be studied, the Latex® membrane was removed, creating a window (2 cm × 2 cm) with Goretex® below.

Radioisotope incorporation studies

A detailed description of our methods has been published elsewhere (8, 9). Briefly, 1 hr after barrier disruption the animals were injected intraperitoneally with tritiated water (20 mCi/mouse) and killed 3 hr later. Solvent- and saline-treated skin was removed from the carcass, weighed (accuracy ± 1 mg), and separated by heat treatment (60°C for 60 sec) into epidermis and dermis. Each specimen was saponified separately overnight in a solution of 45% KOH, water, and 70% ethyl alcohol (2:1:5). After adding internal standards of [^{14}C]oleic acid and [^{14}C]cholesterol, the lipids were extracted with petroleum ether, dried, dissolved in chloroform, and then applied to TLC plates. Bands corresponding to standards of cholesterol, lanosterol, and squalene were scraped from the plate and counted by liquid scintillation spectrometry. The window settings of the scintillation counter were adjusted so that less than 0.2% of the tritium counts were counted in the ^{14}C window, and 10% of the ^{14}C counts in the tritium window. Calculations were corrected for their respective spillovers, background, and recovery of internal standards. Fatty acids were extracted with petroleum ether after acidification of the remaining saponification fluid. The material was dried, dissolved in chloroform, and an aliquot was counted as described above.

Histochemical staining

Oil red O: a working solution was prepared 1 hr before use by mixing three parts of the stock solution (saturated in 99% isopropranol) with two parts of 1% dextrin (to eliminate dye precipitation) (19). The solution was filtered just before use. Four-μm fresh-frozen cryostat sections were allowed to dry on the glass microscopic slide and

then transferred to the working dilution for 6 min at room temperature followed by rinsing in distilled water. The sections were counter-stained in hematoxylin for 30 sec at room temperature, rinsed in distilled water, and mounted for microscopic examination.

Nile red: nile red, a recently described fluorescent probe for lipids (20), was used to depict the distribution of lipids in the stratum corneum. Nile red displays a yellow-gold fluorescence in nonpolar lipid-enriched domains and a reddish brown fluorescence in polar lipid-enriched domains (20). A stock solution of nile red (500 μg/ml) in acetone was prepared, stored at −20°C, and protected from light. A fresh staining solution of nile red was prepared by the addition of 15–20 μl of the stock solution per 1 ml 75% glycerol followed by brisk vortexing. To stain fresh-frozen cryostat sections, a drop of the glycerol staining solution was added to each section and the preparation was covered with a glass coverslip. After 10 min at room temperature in darkness, the sections were examined by fluorescence microscopy on a Leitz Ortholux II fluorescent microscope equipped for epifluorescence, utilizing 450- and 500-nm excitation and emission frequencies, respectively. Depending upon the amount of tissue lipid present and section thickness, 1–5 μg dye/ml of 75% glycerol was required for maximum fluorescent staining (20).

Lipid analysis of stratum corneum

Stratum corneum sheets were obtained from full thickness skin of hairless mice by incubation, granular layer downward, in 0.5% trypsin in phosphate-buffered saline (pH 7.4) for 2 hr at 37°C which yields an intact sheet of stratum corneum plus granular cells. An additional incubation in 0.5% trypsin for 20 min followed by vortexing removes granular cells; the remaining stratum corneum sheet has been shown repeatedly to be devoid of nucleated cells (21, 22). The stratum corneum sheets were dried overnight in a Speed-vac concentrator (Savant) and weighed. The lipids were extracted by the method of Bligh and Dyer (23), dried, and weighed. The lipid weights were calculated as μg per cm^2.

Because of the small quantities of lipid available, we utilized microchromatography (Iatroscan®) to fractionate the lipids (24). The lipids were suspended in chloroform–methanol 2:1 to a final lipid concentration of approximately 20 μg/μl and spotted (1 μl) on each Chromarod which was first developed in hexane–diethylether–formic acid 80:20:1 and then in hexane alone. After development the rods were heated for 30 sec at 100°C and then charred and lipids were quantitated by flame ionization in the Thio-Mark III-TCC analyzer (Ancal Inc.) using an air flow of 2000 ml/min, a hydrogen flow of 160 ml/min (high purity hydrogen), and a scanning speed of 3.0 sec/cm (25). Detector response data was collected and integrated with a computing integrator Model #SP4100 (Spectra Physics).

Statistical significance was determined using a paired Student's t test.

RESULTS

Barrier function

Acetone-treated controls. Hairless male mice (n = 4) were treated on one flank with acetone-soaked cotton balls. After 4–5 min of gentle rolling, elevated rates of transepidermal water loss occurred. Barrier disruption required removal of substantial quantities of lipid (0.7–1.4 mg/7 cm^2) (26). In these studies water loss ranged from 235 to 655 ppm/hr per cm^2. **Fig. 1** demonstrates the recovery of barrier function following disruption with acetone. During the first hour after acetone treatment, barrier function did not change significantly, but between 1–6 hr barrier function recovered by 60%. After 6 hr transepidermal water loss continued to improve but more gradually, so that by 48 hr transepidermal water loss returned to the normal range (5–15 ppm/hr per cm^2). This demonstrates that following acetone-induced barrier perturbation, an initial rapid recovery occurs which is followed by a slow recovery phase that results in the complete normalization of barrier function by 48 hr.

Acetone treatment followed by occlusion with an impermeable membrane. When acetone-treated animals were covered with an impermeable membrane (Latex®), thereby preventing water flux, barrier function did not recover normally (Fig. 1). In fact, transepidermal water loss was actually somewhat higher in acetone-treated mice previously covered with an occlusive impermeable film for 48 hr (Fig. 1). In contrast, transepidermal water loss over the control, saline-treated side of the same animals was not affected by occlusion with an impermeable membrane for 48 hr (transepidermal water loss remains between 5–15 ppm/hr per cm^2). These results demonstrate that artificial

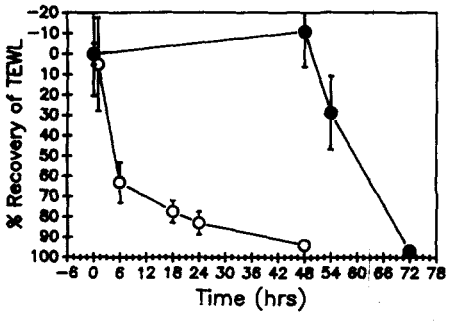

Fig. 1. Recovery of barrier function after acetone treatment. Acetone treatment causes an immediate marked increase in TEWL, which returns to normal within 48 hr (○). In animals covered with a vapor-impermeable membrane (Latex®) following acetone treatment, TEWL does not recover (●). After removal of the vapor-impermeable membrane at 48 hr, barrier function recovers. Data are expressed as the mean ± SEM.

restoration of the barrier with an impermeable membrane prevents the expected recovery of barrier function.

To insure that this failure of barrier function to recover was not due to an artifact or to excessive accumulation of water beneath the occlusive membrane, we also assessed barrier function by an entirely different, morphological technique. The water-soluble tracer lanthanum nitrate normally does not penetrate into intact stratum corneum. As shown in **Fig. 2C** (closed arrows) acetone treatment followed by 48 hr of exposure to air results in complete recovery of barrier function, as evidenced by the complete absence of lanthanum penetration into the stratum corneum. In fact, the pattern of lanthanum penetration in acetone-treated animals left exposed to air was identical to that seen in untreated control skin; lanthanum did not gain access to intercellular domains in either group (18). In contrast, the lanthanum tracer can be seen deep within the intercellular spaces of the stratum corneum and stratum granulosum in acetone-treated animals, both immediately after treatment and after occlusion with an impermeable membrane for 48 hr (Figs. 2A&B). These observations validate the transepidermal water loss (TEWL) results, and demonstrate that occlusion with an impermeable membrane prevents the expected recovery of barrier function.

To ascertain whether the failure of barrier function to normalize while under occlusion might be due to damage from prolonged occlusion with an impermeable membrane, we assessed the subsequent rates of recovery of barrier function after removal of the occlusive membrane. As shown in Fig. 1, immediately after treatment with acetone, transepidermal water loss was increased (547 ± 29 ppm/hr per cm^2) and prior occlusion resulted, as described above, in a further slight increase in transepidermal water loss (602 ± 103 ppm/hr per cm^2). However, following the removal of the impermeable mem-

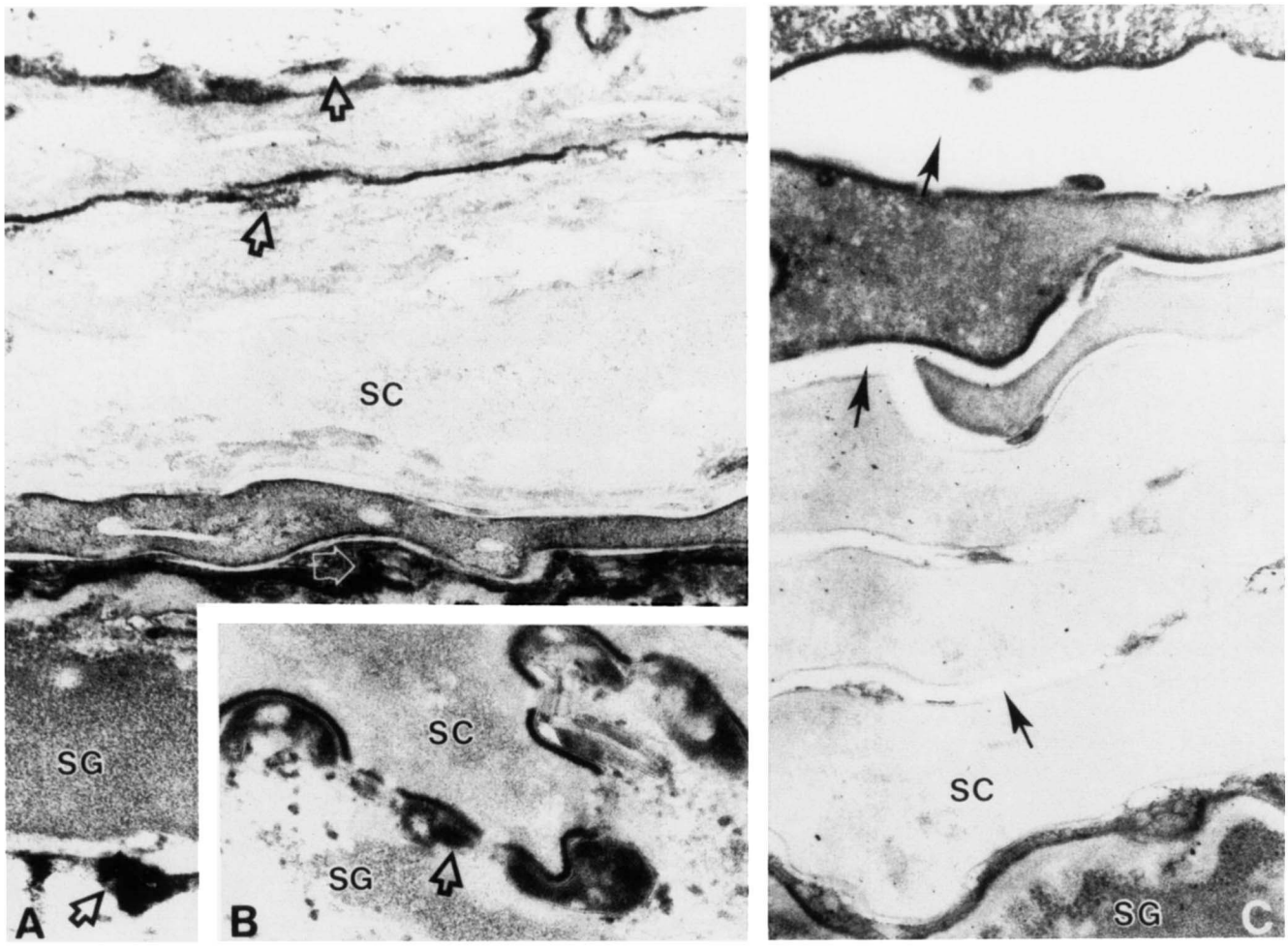

Fig. 2. Ultrastructure of lanthanum-treated epidermis. In acetone-treated epidermis allowed to remain exposed to air for 48 hr, no lanthanum tracer is found in the intercellular spaces of the stratum corneum (Fig. 2C, closed arrows). In contrast, when acetone-treated animals are kept occluded for 48 hr and then exposed to epicutaneously applied lanthanum, abundant tracer is present at all levels in the stratum corneum (Fig. 2A, open arrows), even reaching the stratum granulosum–stratum corneum interface (Fig. 2B, open arrows); SC, stratum corneum; SG, stratum granulosum. Fig. 2A, approx. 18,000 ×; Fig. 2B, approx. 23,000 ×; Fig. 2C, approx. 11,000 ×.

brane, transepidermal water loss recovered at the same rate as in acetone-treated uncovered animals; i.e., returning to 19 ± 14 ppm/hr per cm^2. These results indicate that prior application of an impermeable membrane does not impede the expected rate of recovery of barrier function, indicating that the impermeable membrane itself does not damage the epidermis.

Acetone treatment followed by occlusion with a vapor-permeable membrane. In order to determine whether water flux is the stimulus for the repair of barrier function, and to eliminate the possibility that the above results could be due to nonspecific effects of occlusion, we next carried out a series of experiments in which two different vapor-permeable membranes (Op-Site® and Goretex®) were applied for 48 hr to acetone-treated skin. Both of these membranes allow water vapor to pass through. As shown in **Fig. 3,** there was almost complete recovery of barrier function in animals occluded with the vapor-permeable membranes. Yet these vapor-permeable membranes did impede water transit: 48 hr after placement of the vapor-permeable membrane, transepidermal water loss was 17 ± 5 ppm/hr per cm^2 with the Op-Site® membrane in place, while following removal of the membrane transepidermal water loss increased to 62 ± 10 ppm/hr per cm^2. This observation suggests that the absence of total recovery of barrier function (or TEWL rates) is due to the fact that the vapor-permeable membrane itself provides a partial barrier to transepidermal water loss. These results demonstrate that membranes that allow water transit do not impede the recovery of barrier function following acetone treatment.

Histochemistry

Acetone-treated controls. **Fig. 4** shows representative pictures of mouse skin stained with nile red and oil red O before and after acetone-induced barrier disruption. In untreated uncovered specimens, as illustrated in Fig. 4A, nile red staining revealed a bright yellow fluorescence in the stratum corneum, a pattern typical for neutral lipids, which are the predominant lipid species in mammalian stratum corneum (21, 22). This pattern indicates that intact stratum corneum contains abundant hydrophobic lipids.

In contrast, as seen in Fig. 4B, 1 hr after acetone treatment, nile red staining was virtually absent in the stratum corneum. Since the stratum corneum itself remained intact, and since epidermal morphology appeared normal, acetone treatment did not appear to cause injury. Six hours after acetone treatment, as shown in Fig. 4C, portions of the cornified layers again displayed faint yellow fluorescence. Moreover, very slight reddish brown fluorescence was seen in the granular layer of the epidermis, which may be due to the more polar lipids found in this layer (polar lipids appear reddish brown with nile red staining).

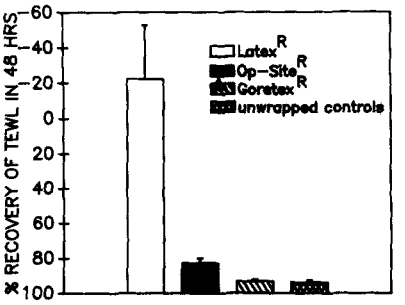

Fig. 3. The effect of membranes on barrier function. Cutaneous barrier function was perturbed by acetone treatment as described in Methods. Approximately 10–20 min after treatment, transepidermal water loss was measured and the animals were then wrapped with a membrane (impermeable, Latex®; vapor-permeable, Op-Site® or Goretex®) or left exposed to air. Forty eight hours later the membranes were removed and transepidermal water loss was determined. Data are expressed as mean ± SEM; n = 4 for all groups.

At both 18 and 24 hr after acetone treatment stratum corneum staining appeared very similar (Figs. 4D and 4E). A bright yellow band of fluorescence was present in the outermost layers with less intense fluorescence in the stratum compactum.

By 48 hr after acetone treatment, nile red stains the stratum corneum to an extent comparable to untreated stratum corneum (see Fig. 5A). These results demonstrate that immediately following acetone treatment, the stratum corneum is depleted of lipid followed by a slow recovery of stainable nonpolar lipids to normal by 48 hr.

To validate the fluorescence studies, we also stained frozen sections of treated and untreated skin with oil red O, a light microscopic indicator of neutral lipids. The staining pattern following acetone treatment with this agent closely resembled that described above with nile red (Figs. 4A–E, 5A-lower panels). Specifically, immediately after acetone treatment there was a paucity of staining in the stratum corneum which again slowly returned to normal over 48 hr. Taken together, these results demonstrate that the recovery of staining in the stratum corneum parallels the time course of barrier recovery as measured by transepidermal water loss.

Acetone treatment followed by occlusion with impermeable membranes. **Fig. 5** depicts nile red- and oil red O-stained sections of mouse skin treated with acetone and then left uncovered for 48 hr (5A), or covered with an impermeable Latex® membrane (5B). As described above the staining characteristics of the stratum corneum left uncovered for 48 hr after acetone treatment were very similar to those observed in controls. However, when barrier function was artificially corrected by occlusion of acetone-treated sites with an impermeable membrane for 48 hr, neither the oil red O- nor the nile red-stained sections demonstrated any evidence of the return of stainable neutral lipids to the stratum corneum (Fig. 5B). By 24 hr following removal of the impermeable membrane, staining in the stratum cor-

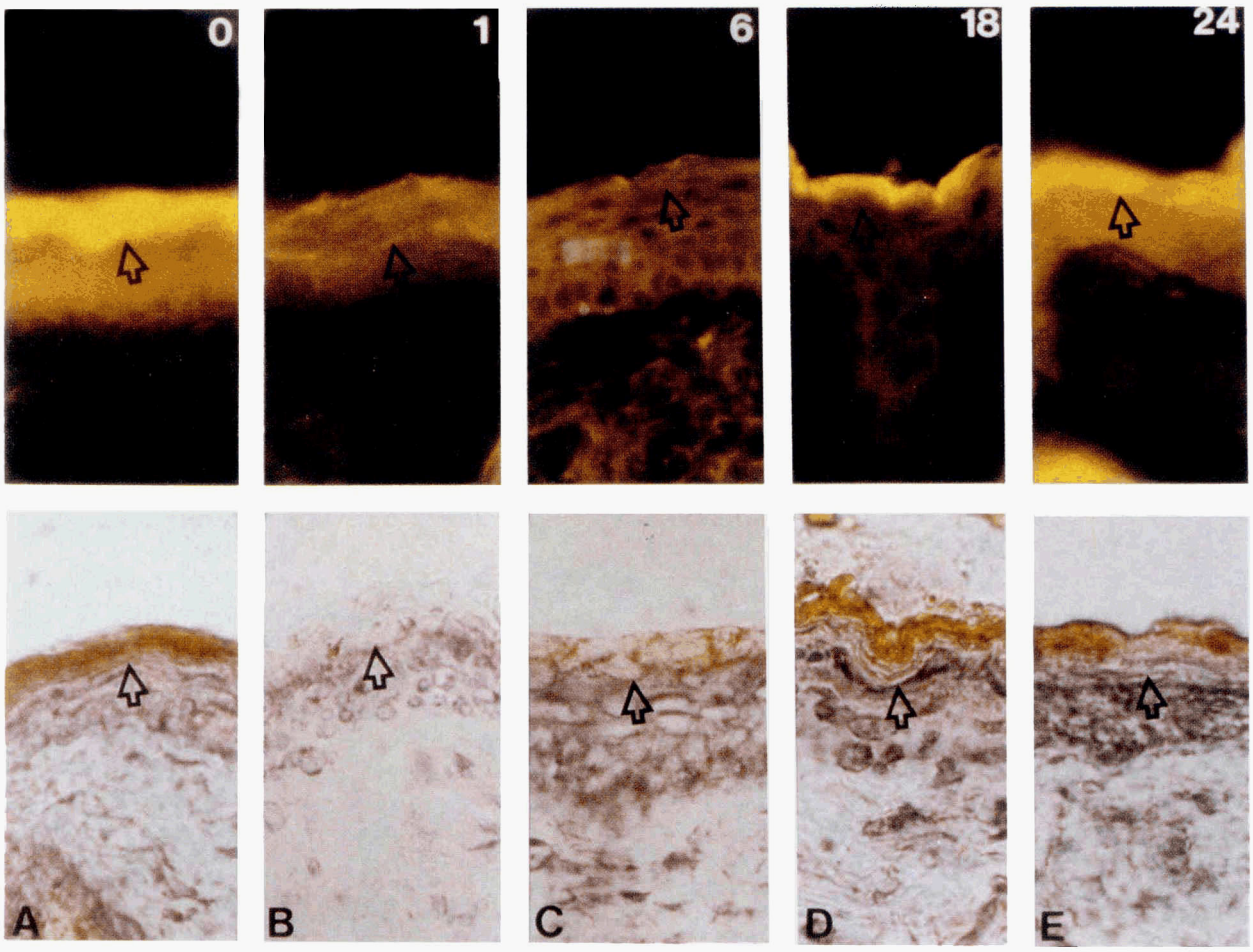

Fig. 4. Recovery of lipid staining after acetone treatment; upper panels, nile red staining; lower panels, oil red O staining. Panel A: prior to acetone treatment; note that both dyes reveal highly positive staining in the stratum corneum. Panel B: 1 hr after acetone treatment; note that no staining is visible in the stratum corneum. Panel C: 6 hr after acetone treatment; note that there is a slight return of staining in the stratum corneum. Panel D: 18 hr after acetone treatment; note that the outer sheets of stratum corneum are intensively stained. Panel E: 24 hr after acetone treatment; note that the quantity of staining is just slightly less than observed in the untreated sections (panel A). Magnification × 400.

neum was similar to that observed in controls (data not shown). Thus, by preventing water transit, the impermeable membrane blocks the return of stainable lipids into the stratum corneum.

Acetone treatment followed by occlusion with a vapor-permeable membrane. In contrast to occlusive membranes, when acetone-treated animals were covered with a vapor-permeable membrane (Op-Site® or Goretex®) for 48 hr, both oil red O and nile red staining demonstrated a complete return of stainable neutral lipids to the stratum corneum by 48 hr (Fig. 5, C and D). These results indicate that when water transit is allowed to proceed, the lipid content of the stratum corneum recovers at the normal rate.

Lipid analysis

To confirm the histochemical observations described above, we next compared the quantity of lipid in untreated stratum corneum, stratum corneum treated with acetone and left uncovered for 24 hr, and stratum corneum treated with acetone and occluded with an impermeable membrane for 24 hr. As shown in **Fig. 6** the quantity of lipid was similar in control stratum corneum and acetone-treated stratum corneum that was left uncovered. In contrast, the total lipid in the stratum corneum was reduced by approximately 50% in the animals treated with acetone and occluded with an impermeable membrane. Of note, the lipid distribution in the extracts of the three groups was similar; i.e., the various proportions of sterol esters, free sterols, fatty acids, triglycerides, and ceramides among the various groups did not differ significantly (data not shown). These studies support the results of histochemical staining by demonstrating that occlusion with an impermeable membrane impedes the return of lipid in the stratum corneum of acetone-treated animals.

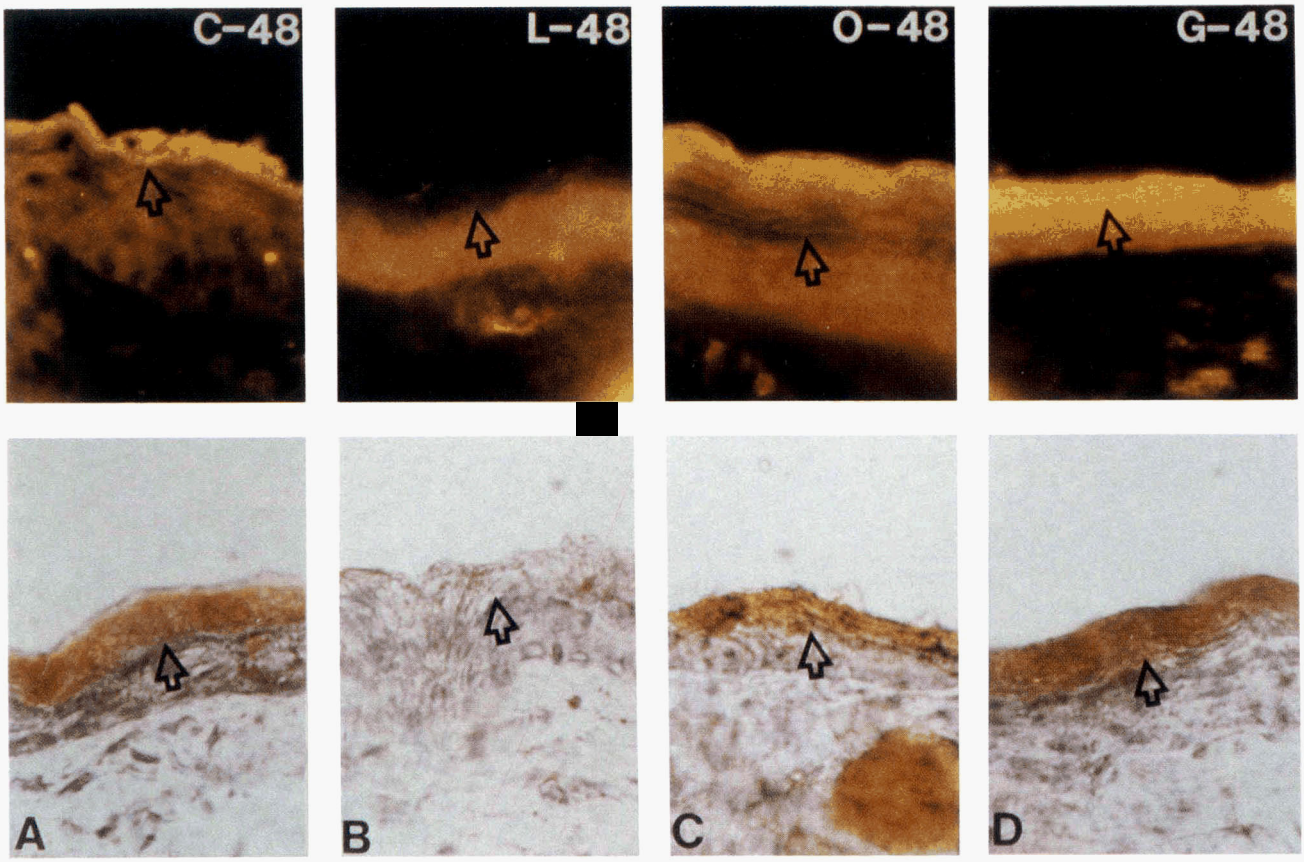

Fig. 5. Effect of membrane on the recovery of barrier structure after acetone treatment; upper panel, nile red staining; lower panel, oil red O staining. Barrier function was disrupted by acetone treatment and 48 hr later skin samples were obtained and stained as described in Methods. Panel A: uncovered skin; note that the appearance and degree of staining in the stratum corneum is identical to that observed in untreated specimens (Fig. 3A). Panel B: skin covered with a vapor-impermeable membrane; note that the lipid staining is virtually absent in the stratum corneum. Panel C: skin covered with a vapor-permeable membrane (Op-Site®); note that lipid staining in the stratum corneum is similar to controls (Fig. 3A) and to uncovered sections (Fig. 4A). Panel D: skin covered with a vapor-permeable membrane (Goretex®); note that lipid staining in the stratum corneum is similar to controls (Fig. 3A) and to uncovered sections (Fig. 4A). Magnification × 400.

De novo lipid biosynthesis

As described in previous studies from our laboratory (8–10), the incorporation of tritiated water into sterols and fatty acids in the epidermis is stimulated by barrier disruption, which in turn is prevented by covering with an impermeable membrane (8, 9). In contrast, when acetone-treated skin was covered with a vapor-permeable rather than occlusive membrane, the incorporation of tritiated water into sterols and fatty acids in the epidermis was significantly increased in comparison to the saline-treated flank (**Table 1**). The relatively small increase in epidermal lipid synthesis observed in this experiment can be attributed to the mild disturbance in barrier function that occurs in animals covered with a vapor-permeable membrane. As discussed above, the vapor-permeable membrane provides a partial barrier, and in prior studies (8) we have observed a correlation between the degree of barrier abrogation and the intensity of synthetic activity. Thus, these results show that epidermal lipid synthesis is stimulated under circumstances where excessive water loss occurs.

DISCUSSION

The intercellular lipids of the stratum corneum, by forming broad, intercellular laminae of relatively nonpolar lipids, have been implicated as the major regulators of the epidermal permeability barrier (1–5). Prior studies of the permeability barrier have focused primarily on descriptions of the structure or lipid content of normal or perturbed barriers. In contrast, both in our recent studies and in the present work we have employed a metabolic approach, choosing to link perturbations in barrier function with modulations in structure and lipid synthesis. By utilizing various animal models, we have demonstrated that when the cutaneous permeability barrier is disrupted, as determined by elevated transepidermal water

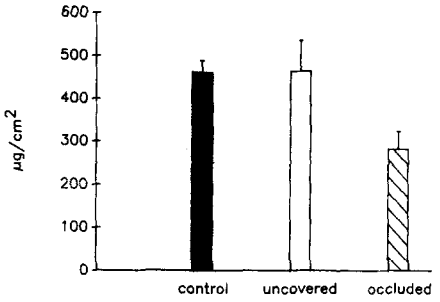

Fig. 6. Quantity of lipid in the stratum corneum. Stratum corneum sheets were isolated and the total quantity of lipid was determined in samples from control animals, animals wiped with acetone and left exposed to air for 24 hr, and animals wiped with acetone and then occluded with an impermeable Latex® membrane for 24 hr. Data are expressed as mean ± SEM; n = 4 for all groups; control versus occluded, $P < 0.02$; *uncovered versus occluded*, $P < 0.05$.

loss, both sterol and fatty acid biosynthesis in the epidermis are increased (8, 9, 16). Additionally, as barrier function recovers, epidermal lipid synthesis returns toward normal. Furthermore, the degree of stimulation of lipid synthesis correlates with the extent of barrier disruption. Most importantly, if one artificially restores barrier function with a vapor-impermeable membrane, the burst in epidermal lipid synthesis that occurs in response to perturbations in barrier function is prevented (8, 9, 16). In the present study we have shown that occlusion with a vapor-permeable membrane, which prevents the transport of compounds larger than water, does not prevent the increase in epidermal lipid synthesis following barrier perturbation (Table 1).

These observations suggest that the increase in epidermal lipid synthesis following disruption of the barrier is due to increased water transit. Although it could be argued that the increase in synthesis is a nonspecific phenomenon related to epidermal injury, this is unlikely for a number of reasons. First and most importantly, the increase in lipid synthesis can be prevented by an impermeable membrane (8, 9). If the increase were due to a nonspecific injury, occlusion should not completely blunt this response. Second, other methods that perturb barrier function also cause an increase in epidermal lipid synthesis (SDS treatment, essential fatty acid-deficient diet) (8, 9, 16). Moreover, in these models the increase in epidermal lipid synthesis also can be prevented or reversed by occlusion with an impermeable membrane (8, 9, 16). Furthermore, in the essential fatty acid-deficient model, lipid synthesis normalizes with occlusion even in the face of a progressive deficiency state accompanied by epidermal hyperplasia (16). These observations taken together led us to hypothesize that the increase in epidermal lipid synthesis is related to perturbations in barrier function rather than nonspecific injury.

In the series of studies presented in this article we have examined whether water flux itself might serve as the regulator of barrier repair. Whereas prior studies examined only the consequences of occlusion on lipid biosynthesis, in this study we correlated barrier function, stratum corneum lipid content, and epidermal lipid biosynthesis with water flux. Moreover, to control for possible nonspecific effects of occlusion per se on barrier repair, and to more definitively pinpoint water loss as the regulatory signal, we compared barrier function, lipid deposition, and lipogenesis in skin occluded with impermeable versus water vapor-permeable films. Acetone treatment results in the removal of stratum corneum lipids as shown in Fig. 4B, which is associated with a marked disturbance in the cutaneous permeability barrier. Over time the lipid in the stratum corneum measured both by histochemical staining and by biochemical analysis returns toward normal (Fig. 4E) so that by 48 hr the quantity of lipid present in the stratum corneum of acetone-treated animals is very similar to that seen in controls (Fig. 4D, Fig. 6). Moreover, the return of barrier function to normal parallels the

TABLE 1. The effect of a vapor-permeable membrane on epidermal lipid biosynthesis

	Transepidermal Water Loss	Cholesterol	Total Nonsaponifiable Lipids	Fatty Acids
	ppm/hr per c²	*μmol ^3H_2O incorporated/3 hr per g*		
Controls (n = 8)	6 ± 0.7	0.76 ± 0.14	1.49 ± 0.21	3.77 ± 0.59
Acetone-treated	479 ± 37[a]	0.91 ± 0.15	2.06 ± 0.18	4.48 ± 0.54
Vapor-permeable membrane (n = 8)	(107 ± 3)[b]			
	$P < 0.0005$	$P < 0.025$	$P < 0.005$	$P < 0.025$

The incorporation of ^3H_2O into cholesterol, total nonsaponifiable lipids, and fatty acids in the epidermis is compared in uncovered saline-treated control flanks and in flanks after barrier disruption with acetone and covering with a vapor-permeable membrane (Op-Site®). Statistical significance was determined using a paired Student's *t*-test. Data are expressed as mean ± SEM; n, number of animals.

[a]Transepidermal water loss in animals prior to the placement of the vapor-permeable membrane.

[b]Transepidermal water loss in animals after the placement of the vapor-permeable membrane.

reappearance of stratum corneum lipids. By 48 hr both barrier structure and function have returned to normal, demonstrating the close relationship between the repletion of stratum corneum lipids and the restoration of normal barrier function. These observations provide further support for the stratum corneum lipids as a major component of the cutaneous permeability barrier (1–5).

As discussed above, during the same period of time that the quantity of lipid in the stratum corneum is increasing, epidermal sterol and fatty acid synthesis increase two- to threefold (9). This increase in synthesis returns towards normal as barrier function is restored. Since prior studies have demonstrated that extracutaneously synthesized lipids do not contribute substantially to the quantity of sterols and fatty acids in the epidermis (8, 9), taken together these results indicate that the reappearance of stratum corneum lipids is associated with an increase in epidermal lipid synthesis. It should not be assumed, however, that new lipid synthesis alone replaces the lipid that is removed from the stratum corneum following acetone wiping. The detailed sequence of events that follows barrier disruption is not known. Barrier disruption could directly stimulate lipid biosynthesis independent of lamellar body secretion or alternatively, based upon abundant evidence that the epidermal lamellar body is the source of the intercellular lipid that constitutes the permeability barrier (2, 3), barrier disruption may trigger the secretion of lamellar bodies. The observed increase in epidermal lipid biosynthesis then might be attributable to replenishment of lamellar bodies. If deposition of lamellar body-derived lipids reconstitutes the barrier, it is interesting that these lipids appear to normalize histochemical staining within 48 hr, while the turnover of stratum corneum is thought to be much longer. Thus, if lamellar body secretion is the explanation for the return of the histochemical staining pattern to normal, then the secreted lipids may be displaced within the stratum corneum interstices at rates different from the turnover of the cellular compartment. Further studies are underway to address these important issues. Nevertheless, these studies clearly demonstrate that an increase in local lipid synthesis is associated with a recovery of barrier structure and function following disruption of the barrier with acetone.

Since water loss per se might be the signal for increasing epidermal lipid synthesis, we studied the effect of artificial restoration of the barrier on the recovery of stratum corneum lipids and barrier function following acetone treatment. When acetone-treated animals are covered with a vapor-impermeable Latex membrane that reduces transepidermal water loss to 0, barrier function does not return to normal (Fig. 1). Additionally, as shown in Fig. 5B, 48 hr after acetone treatment, animals covered with a Latex glove display very little stainable lipid in the stratum corneum. Moreover, biochemical analysis, as shown in Fig. 6, revealed that the quantity of lipid in the stratum corneum of animals occluded with an impermeable membrane is about 50% of that observed in control animals or animals left exposed to air. These observations contrast with the results described above in which the recovery of stratum corneum lipids and barrier function is complete by 48 hr following barrier disruption with acetone when animals are left uncovered. Since we have shown in previous studies that the characteristic stimulation of epidermal lipid synthesis that follows disruption of the barrier can be blocked by an impermeable membrane (9), it is likely that inhibition of the usual burst in local lipid synthesis prevents the recovery of stratum corneum lipids, and hence blocks the return of normal barrier structure and function. The fact that removal of the impermeable membrane is followed by a return of both barrier structure and function to normal provides further support for the concept that blunting of the normal lipid biosynthetic response to injury is associated with a delay in the return of normal barrier function.

To ascertain further whether water flux is the signal for repair of the barrier, and to eliminate potential nonspecific effects of occlusion, we determined the effect of vapor-permeable membranes on the recovery of stratum corneum lipid, transepidermal water loss, and epidermal lipid biosynthesis. Although the membranes used in these experiments are permeable to water vapor, they prevent the transport of numerous other larger compounds. In fact, Goretex® is impermeable to liquid water but will allow the transport of water vapor (17). As shown in Table 1, the usual stimulation of epidermal lipid synthesis, following acetone disruption of the barrier, occurs in animals covered with a vapor-permeable membrane.

The fact that two vapor-permeable membranes, OpSite® and Goretex®, did not produce effects similar to the impermeable Latex® membrane suggests that the effects observed with the impermeable membrane are not nonspecific effects of occlusion per se. Additionally, impediments to blood flow cannot explain our observations since our prior studies used a loose-fitting plastic wrap as an impermeable membrane and it also prevented the increase in epidermal lipid synthesis (8). Thus, it is likely that rates of water loss regulate epidermal lipid biosynthesis. Epidermal lipid biosynthesis apparently is attuned to a gradient of water content across the stratum corneum (27). Removal of lipids would result in reduced water content in the stratum corneum (28, 29), leading in turn to increased flux of water from the nucleated cell layers into the stratum corneum. As the lipid content of stratum corneum begins to normalize, its water content would increase proportionally, and this gradient would become less steep. Artificial normalization of the gradient with a vapor-impermeable membrane likewise would normalize this gradient. However, our experiments do not completely rule out other differences in local environment under impermeable versus permeable films. It is possible

that factors other than water loss, such as carbon dioxide or oxygen tension, could in part explain our observations.

Together these observations suggest that restoration of the barrier function leads to the normalization of epidermal lipid synthesis. As shown in Fig. 5, C and D, stratum corneum lipids reappeared by 48 hr after acetone treatment in animals covered with vapor-permeable membranes. Moreover, as shown in Fig. 3, barrier function recovered in parallel with the return of stratum corneum lipids. The observation that transepidermal water loss did not completely normalize by 48 hr presumably reflects the fact that these vapor-permeable membranes provide a partial barrier. The combination of a partial recovery plus a partial exogenous membrane barrier results in the total normalization of barrier function. These findings demonstrate that increased epidermal lipid synthesis occurs when water loss rates are increased and is associated with the recovery of stratum corneum lipids and the restoration of barrier function.

In conclusion, the present study suggests that water transit itself may serve as the signal that stimulates epidermal lipid synthesis following disruption of the barrier. This local increase in epidermal lipid synthesis is associated with the repletion of stratum corneum lipids and restoration of normal barrier function.

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