Decreased Epidermal Lipid Synthesis Accounts for Altered Barrier Function in Aged Mice

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The epidermis of aged mice displays decreased stratum corneum (SC) lipid content and decreased extracellular bilayers, which result in impaired barrier recovery following the solvent treatment or tape stripping. We assessed the role of altered lipid synthesis as the cause of the abnormal barrier and lipid content in aged epidermis, both under basal conditions and in response to acute barrier perturbations. In aged epidermis (≥18 months), synthesis of one of the three key lipid classes (cholesterol) is decreased under basal conditions, and sterologenesis fails to attain the levels reached in young epidermis following comparable acute perturbations. In contrast, fatty acid and sphingolipid synthesis in aged epidermis increase sufficiently to approach the levels attained in stimulated young epidermis. The abnormalities in sterologenesis in aged epidermis are paralleled by a decrease in activity of its rate-limiting enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, under basal conditions, and enzyme activity also fails to increase as much as in young epidermis after barrier disruption. That defective lipid generation contributes to the barrier defect is shown directly by the ability of either a cholesterol-containing mixture of SC lipids or cholesterol alone to enhance barrier recovery. Finally, lipid-induced acceleration of barrier recovery in aged epidermis correlates with repletion of the extracellular spaces with normal lamellar structures. Thus, a deficiency in lipid synthesis, particularly in cholesterologenesis, accounts for the barrier abnormality in aged epidermis. ***Key words: cholesterol synthesis/HMG-CoA reductase/permeability barrier/skin aging. J Invest Dermatol 106: 1064–1069, 1996***

The epidermal permeability barrier resides in the stratum corneum (SC), where hydrophobic lipids are sequestered as multilayered lamellae within the intercellular spaces, which regulate transepidermal water loss (TEWL), corneocyte cohesion, and percutaneous penetration (Williams and Elias, 1987). The barrier in aged epidermis has been largely overlooked, because earlier structural and functional studies described few if any abnormalities in aged epidermis (Montagna, 1965). Yet, the permeation of a variety of molecules is altered across aged epidermis *in vitro* (Montagna, 1965; Harvell and Maibach, 1994). Moreover, aged SC displays a global reduction in lipids, with reduced numbers of extracellular bilayers (Ghadially *et al*, 1994), indicative of less reserve barrier capacity. Furthermore, though the aged permeability barrier functions adequately under basal conditions, when it is challenged acutely, barrier integrity and recovery are impaired in both aged human and murine skin (Ghadially *et al*, 1994). Finally, the barrier abnormality after acute challenges to the epidermis of aged mice is associated with a paucity of lamellar body material at the stratum granulosum-SC interface, and a delay in the return of stainable lipids to the SC interstices (Ghadially *et al*, 1994).

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Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; TEWL, transepidermal water loss.

The extracellular lipids responsible for maintaining barrier homeostasis derive from the secreted contents of the epidermal lamellar body (Odland and Holbrook, 1987). In young epidermis, barrier perturbation results in a homeostatic response that includes: 1) immediate secretion of preformed lamellar bodies; 2) formation and ongoing secretion of rapidly formed, nascent lamellar bodies; and 3) return of lipids to the SC interstices with reformation of the intercellular lamellar bilayers (Grubauer *et al*, 1989; Menon *et al*, 1992). The lamellar body secretory response described above is fueled by increased cholesterol, fatty acid, and ceramide synthesis (Menon *et al*, 1985; Grubauer *et al*, 1987; Holleran *et al*, 1991a), explicable by antecedent increases in: a) activity; b) activation state; c) enzyme mass, and d) mRNA levels of HMG-CoA reductase (Proksch *et al*, 1990; Jackson *et al*, 1992), the rate-limiting enzyme of cholesterologenesis, as well as increases in the activities of acetyl-CoA carboxylase and fatty acid synthase, the rate-limiting enzymes of fatty acid synthesis (Ottey *et al*, 1995). Finally, changes in the activity of serine-palmitoyl transferase, the rate-limiting enzyme of ceramide synthesis, parallel the increase in ceramide synthesis (Holleran *et al*, 1991a). Each of these lipids is specifically required for the barrier, because inhibitors of HMG-CoA reductase, serine-palmitoyl transferase, or acetyl-CoA carboxylase interfere with barrier recovery after acute perturbation (Feingold *et al*, 1990; Holleran *et al*, 1991b; Mao-Qiang *et al*, 1993a). We assessed here the basis for the decreased lipid content and abnormal barrier function in the epidermis of aged mice, both under basal conditions and following acute challenges to the barrier. Finally, we assessed whether the compromised barrier in aged epidermis could be corrected by topical applications of the deficient lipid(s).

MATERIALS AND METHODS

Materials Hairless mice outbred CrL:SKHI (hr\hr) BR (Charles River Laboratories, Wilmington, MA) comprised the senescent murine model (Ghadially *et al*, 1994). Young control (also Charles River, hr\hr) mice, between 6 and 10 weeks of age, and aged mice, 18–24 months old, were compared. Aged mice were checked regularly for microbial diseases and tumors; animals that showed evidence of either systemic illness or tumor development were not studied. ^3H_2O, [^{14}C]acetate, [^{14}C]oleic acid, [^{14}C]cholesterol, [^{14}C]HMG-CoA, [^{3}H] mevalonic acid, and [^{3}H]mevalonolactone were all from Amersham (Arlington Heights, IL). Acetone was purchased from Fisher Scientific (Fairlawn, NJ). Linoleic acid, palmitic acid, stearic acid, ceramides III and IV, and cholesterol were from Sigma Chemical Co. (St. Louis, MO).

Acute Barrier Perturbation Acetone was gently applied to the flanks of male hairless mice for 1–5 min, or successive cellophane tape strippings were utilized (3M) six to eight times to disrupt the barrier (Menon *et al*, 1985; Holleran *et al*, 1991a). Transepidermal water loss (TEWL) was measured immediately after treatment using a Meeco electrolytic water analyzer (Warrington, PA) (Menon *et al*, 1985). Animals were studied when TEWL rates exceeded 4.0 mg/cm^2/h (normal <0.3 mg/cm^2/h).

Lipid Biosynthesis *in Vivo* Detailed descriptions of our radioisotope incorporation methods have been published elsewhere (Menon *et al*, 1985; Grubauer *et al*, 1987; Proksch *et al*, 1990). Briefly, 1 h after barrier disruption the animals were injected intraperitoneally with tritiated water (20 mCi/mouse) and killed 3 h later. Solvent- and saline-treated skin was removed from the carcass, weighed (accuracy ± 1 mg), separated by heat treatment (60°C, 60 s) into epidermis and dermis, and saponified overnight (Grubauer *et al*, 1987). After addition of [^{14}C]oleic acid and [^{14}C]cholesterol, the nonsaponifiable lipids were extracted with petroleum ether, fractionated by thin-layer chromatography, and quantitated by liquid scintillation spectrometry (Holleran *et al*, 1991a). Fatty acids were extracted with petroleum ether after further acidification, dried, dissolved in chloroform, and an aliquot was counted by liquid scintillation spectrometry. For determination of sphingolipid synthesis, ^3H_2O was administered as above, epidermal sheets were extracted with Bligh-Dyer solution (Bligh and Dyer, 1959), and the total lipid extracts were separated by high performance thin-layer chromatography (Holleran *et al*, 1991a,b). Lipids were identified by co-chromatography against known standards, counted, and the data were corrected according to the extraction efficiency of C^{14} internal standards. Total sphingolipid incorporation comprised the label in ceramide, glycosphingolipid, sphingomyelin, and sphingoid base fractions.

Lipid Biosynthesis in Organ Cultured Epidermis Full-thickness skin samples were obtained from young and aged mice. Subcutaneous fat was scraped off full-thickness murine skin and 1–2-cm^2 skin pieces were floated on DME-H21 with 5% fetal calf serum plus antibiotics in 100-mm Petri dishes at 37°C. The organ cultures were incubated with [^{14}C]acetate (50 mCi/mmol, 20 μCi/ml) for 3 h, and epidermal sheets were obtained as described above. Lipids were saponified in 45% ethanolic KOH, acidified, extracted, and fractionated, and biosynthetic activity was quantitated, as above.

Isolation of Epidermal Microsomes and Enzyme Assays Epidermal sheets were obtained from young versus aged mice before and 6 h after barrier disruption with tape stripping. Briefly, whole skin was excised, incubated at 37°C for 45 min in phosphate-buffered saline (calcium/magnesium-free) containing Dispase (1.25 U/ml). The epidermis was removed with a scalpel blade, weighed, minced, and stored at −70°C. For assessment of HMG-CoA reductase activity, microsomes were isolated from the epidermal sheets (Proksch *et al*, 1990). Briefly, epidermal sheets (0.05–0.1 g each), obtained from the flanks of hairless mice, were homogenized and centrifuged at 16,000*g* for 15 min at 4°C. The supernatant was removed, the pellet was recentrifuged, and the final supernatant was centrifuged at 100,000*g* for 1 h at 4°C to pellet microsomes. The microsomal pellet was washed and subsequently resuspended in storage buffer (50 μl) containing 50 mM HEPES (pH 7.5), 5 mM ethylenediammine tetraacetic acid, 5 mM dithiothreitol, and 20% glycerol (v/v), and stored at −70°C until use. Protein determination (Bio-Rad Laboratories, Richmond, CA) gave values of between 0.5 and 2.0 mg/ml of microsomal protein. HMG-CoA reductase was assayed as described previously (Proksch *et al*, 1990).

Lipid Applications to Aged Skin The barrier was disrupted in aged versus young hairless mice by repeated applications of cellophane tape to one flank, as above. In one protocol, immediately after TEWL rates ≥ 4.0 mg/cm^2—either a 1:1:1:1 molar mixture (10 μl of 1.2% lipid applied to a 0.5 cm^2 area) of cholesterol, ceramide, linoleic, and palmitic acid; topical cholesterol (0.4%) alone, or vehicle alone (propylene glycol:ethanol, 7:3 vol/vol), was applied topically to a 5-cm^2 area of skin. In the second protocol, aged skin was treated with either topical cholesterol (0.4%; four applications over 48 h) or the propylene glycol:ethanol vehicle alone. In this protocol, stratum corneum integrity was measured by sequential tape strippings (number of strippings required to attain TEWL rates ≥ 4 mg/cm^2-h (Ghadially *et al*, 1994). Neither the equimolar lipid mixture nor cholesterol alone accelerate barrier recovery in young mice, but both allow normal or near-normal recovery rates (Mao-Qiang *et al*, 1993b). TEWL over treated areas was measured at different time points after barrier disruption, as above. Because of experiment-to-experiment variations in baseline TEWL levels among different cohorts of animals, data are expressed as percent barrier recovery; i.e., 0% at the beginning of each experiment, immediately after acetone treatment.

Electron Microscopy of Lipid-treated Aged Skin Mice were treated twice daily for 48 h with either the equimolar lipid mixture, cholesterol, or vehicle (see above). Biopsies were obtained the morning after the last treatment. Approximately 1 mm^3 samples were fixed in half-strength Karnovsky's fixative overnight, washed in 0.1 M sodium cacodylate buffer, and post-fixed in 0.5% ruthenium tetroxide in 1.5% potassium ferrocyanide followed by ethanol dehydration and embedding in an Epon-epoxy mixture (Hou *et al*, 1991). Ultrathin sections were contrasted further with lead citrate and viewed in a Zeiss 10A electron microscope, operated at 60 kV. Micrographs of lipid- versus vehicle-treated samples were photographed randomly by an uninvolved observer and interpreted blindly by the authors.

Statistical significance was determined using the Student's two-tailed t test.

RESULTS

Senescent Mice Display Abnormalities in Permeability Barrier Homeostasis To assess barrier function in the cohort of senescent animals utilized for these metabolism studies, we initially measured barrier function at various time points after barrier disruption with tape stripping. As reported previously (Ghadially *et al*, 1994), barrier recovery was delayed in comparison to a cohort of young mice of the same sex and strain (e.g., only 18.7% vs. 60.8% recovery [±SEM] by 24 h in aged versus young animals, respectively; $p < 0.001$).

Aged Murine Epidermis Displays Abnormalities in Lipid Synthesis *in Vivo* Whereas our prior studies described a global decrease in the lipid content of aged murine SC (Ghadially *et al*, 1994), we first asked whether this decrease could be attributed to decreased epidermal lipid synthesis. Whereas the rates of total lipid synthesis appeared lower in aged versus young epidermis under basal conditions, the differences did not achieve statistical significance (**Table I**; $p < 0.1$). The basal rates of cholesterol synthesis, however, were significantly lower in aged than in young epidermis (**Fig 1*A***; $p < 0.005$). Although the rates of fatty acid synthesis (total saponifiable lipids) appeared to be slightly reduced under basal conditions, the differences again did not achieve statistical significance (**Fig 1*B***). Likewise, the basal rates of sphingolipid synthesis were also slightly reduced in aged versus young epidermis, but again the differences were not significant (**Fig 1*C***).

Following tape stripping, both young and aged epidermis displayed increased lipid synthesis (**Table I**; $p < 0.01$); however, the absolute levels of synthesis were significantly lower in aged than in young epidermis after comparable barrier insults (**Table I**; $p < 0.01$). As described previously (Menon *et al*, 1985; Grubauer *et al*, 1987; Holleran *et al*, 1991a), young epidermis displayed significant increases in cholesterol, fatty acid, and sphingolipid synthesis after barrier disruption (**Fig 1*A–C***). Aged epidermis also displayed a significant increase in cholesterol and sphingolipid synthesis (**Fig 1*A,C***; $p < 0.01$), but the increase in fatty acid synthesis did not achieve significance (**Fig 1*B***; $p < 0.1$). Both young and aged epidermis displayed comparable percentage increases in the synthesis of all three key lipids (**Fig 2**). Despite the burst in epidermal lipid synthesis in aged epidermis, the increases in cholesterol and fatty acid synthesis in aged epidermis did not reach the absolute levels attained in young epidermis after comparable barrier insults (**Fig 1*A,B***; $p < 0.01$). In contrast, the rates of sphingolipid synthesis in aged epidermis approached those in young epidermis; i.e., sphingolipid synthesis after tape stripping was not significantly different in aged versus young animals (**Fig 1*C***; $p < 0.1$). These studies

Table I. Total Epidermal Lipid Synthesis in Aged Versus Young Mice[a]

Young	Basal	24.8 ± 1.9	
		p<.01	
	Tape Stripped	33.1 ± 3.2	p <0.1
Old	Basal	20.5 ± 1.0	
		p<.01	p <0.01
	Tape Stripped	25.7 ± 1.5	

[a] Lipid synthesis was assessed under basal conditions and during the interval from between 1–4 h after barrier disruption (3 h after ^3H_2O injection) in cohorts (n = 7 each) of aged (> 18 months) vs. young (< 6–10 wk) mice. Data are for ^3H_2O incorporation into total saponifiable and nonsaponifiable lipids (μmol/g ± SEM).

demonstrate first, that aged epidermis displays a diminution in cholesterol synthesis under basal conditions. Second, a gel epidermis display a comparable increase in the synthesis of all three key lipids, but the absolute levels do not attain those in young epidermis.

Lipid Synthesis Is Also Decreased in Organ Cultured, Aged Epidermis We next evaluated whether the decreased lipid synthesis rates, demonstrated *in vivo* in aging epidermis, also were demonstrable *in vitro*. The synthesis of cholesterol, total nonsaponifiable lipids, and fatty acids was compared in freshly obtained aged versus young organ cultures of full-thickness skin, maintained under identical conditions. The differences in lipid synthesis, described above for aged versus young epidermis, were retained in organ-cultured skin samples **(Fig 3)**. Both cholesterol and total nonsaponifiable lipid synthesis displayed a 30–50% decrease, while the decrease in fatty acid synthesis in aged epidermis again was somewhat less (≈20–25%), and did not achieve statistical significance. These *in vitro* results demonstrate a defect in basal lipid synthesis, and particularly in cholesterologenesis in aged epidermis, consistent with the *in vivo* results.

The Changes in Cholesterol Synthesis in Aged Epidermis Are Due to Decreased HMGCoA Reductase Activity We next asked whether the abnormalities in cholesterol synthesis could be ascribed to changes in HMG-CoA reductase activity. Total HMG-CoA reductase activity was decreased by about 40% in aged versus young epidermis under basal conditions **(Fig 4**; $p < 0.05$), a decrease comparable to the overall decrease in cholesterol and total nonsaponifiable lipid synthesis (c.f., **Figs 1*A*, 3*A*, 3*B*)**. We next assessed whether HMG-CoA reductase levels in aged epidermis respond to barrier disruption. HMG-CoA reductase activity increased by over 100% in aged epidermis 6 h after barrier disruption, but the absolute levels of enzyme activity again did not attain the levels reached in treated, young epidermis **(Fig 4**; $p < 0.01$). These studies show that the abnormalities in cholesterol synthesis in aged epidermis can be accounted for by reduced HMG-CoA reductase activity.

Exogenous Stratum Corneum Lipids Normalize Barrier Recovery in Aged Epidermis To test directly whether decreased lipid generation contributes to the altered barrier in aged epidermis, we first applied an equimolar mixture of physiological lipids (cholesterol:ceramide:linoleic acid:palmitic acid) immediately after tape stripping. Such an equimolar mixture normalizes, but does not accelerate, recovery in young murine skin (Mao-Qiang *et al*, 1993). Barrier recovery was much slower in aged mice after tape-stripping (TEWL rates ≥ 4 mg/cm^2/h) plus vehicle treatment alone than in comparably treated young mice **(Fig 5)**. In contrast, a single application of the physiological lipid mixture immediately following barrier disruption, approximating the lipid distribution, molar ratio, and concentration present in young murine stratum corneum (10 ml of 1.2% lipid applied to a 0.5-cm^2 area), accelerated barrier recovery in aged epidermis at 6 and 24 h **(Fig 5**; $p < 0.05$). Because the most pronounced synthetic abnormality in aged epidermis was reduced cholesterol generation, we next asked whether topical cholesterol alone would accelerate recovery rates and/or improve SC integrity in aged mice. Prior studies have shown that cholesterol applications alone do not accelerate barrier recovery in young mice (Mao-Qiang *et al*, 1993b). SC integrity (i.e., number of strippings required to abrogate the barrier), did not change significantly following pretreatment with cholesterol (data not shown). As seen in **Table II**, application of topical cholesterol after barrier abrogation accelerated barrier recovery in aged epidermis both 3 and 6 h after application ($p < 0.05$ at both time points). By 24 and 48 h, however, the differences were no longer significantly different **(Table II**; 48 h data not shown). These results show that both equimolar lipid mixtures of SC lipids and topical cholesterol alone accelerate barrier recovery in aged epidermis.

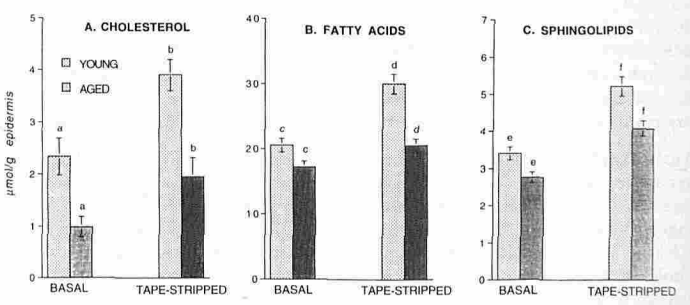

Figure 1. Lipid Synthesis Is Altered in Aged Epidermis Under Basal and Stimulated Conditions. The flanks of aged (> 18 months) versus young (6–10 wk) hairless mice (n = 7 each) were tape-stripped repeatedly until TEWL rates ≥ 4 mg/cm^2/h. One hour after barrier disruption, treated versus untreated animals in both age groups were injected with ^3H_2O, and animals were sacrificed 3 h later. See text for details of extraction and separation of lipid classes. *a* vs. *a*, p 0.005; *b* vs. *b*, $p < 0.001$; *c* vs. *c*, NS; *d* vs. *d*, $p < 0.05$; *e* vs. *e*, NS; *f* vs. *f*, NS; *a* vs. *b* young, $p < 0.005$; *a* vs. *b* aged, $p < 0.05$; *c* vs. *d* young, $p < 0.05$; *c* vs. *d* aged, NS; *e* vs. *f* young, 0.05; *e* vs. *f* aged, $p < 0.01$.

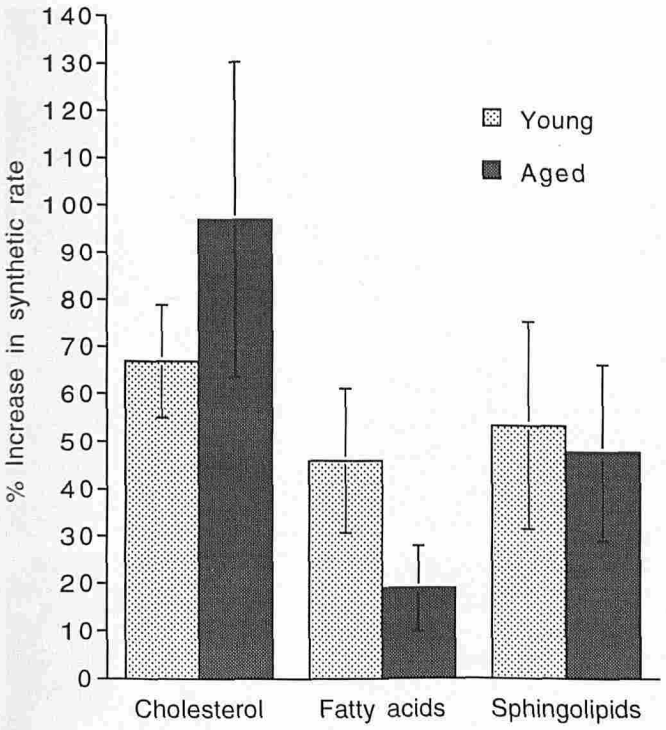

Figure 2. Lipid Synthesis Is Stimulated Comparably in Young Versus Aged Mice After Barrier Disruption (data are from **Fig 1**). The differences in the extent of the increase for each lipid in aged versus young epidermis does not achieve statistical significance.

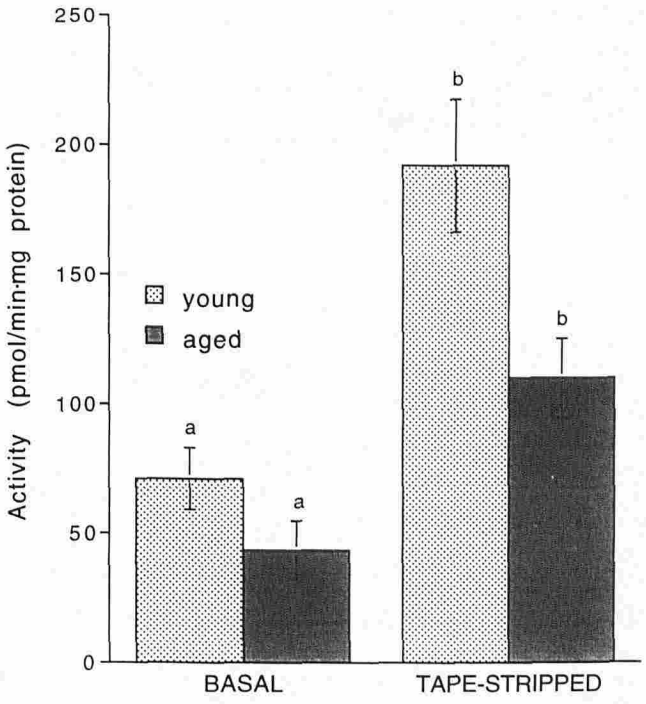

Figure 4. Abnormalities in Cholesterol Synthesis in Aged Epidermis Are Paralleled by Changes in HMG-CoA Reductase Activity. Microsomes were isolated from freshly obtained epidermal sheets, under basal conditions and 6 h after barrier disruption by tape stripping, and HMG-CoA reductase activity was measured as described previously (Proksch *et al*, 1990). (*a* vs. *a*; $p < 0.05$; *b* vs. *b*; $p < 0.005$). n = 6; mean ± SEM.

Exogenous Lipids Normalize SC Membrane Structure in Aged Mice To ascertain the mechanism whereby topical lipids improve barrier homeostasis in aged epidermis, we next compared SC membrane structures in aged epidermis after repeated (4 ×) applications of lipids versus vehicle to aged skin. Application of either the equimolar lipid mixture or cholesterol alone resulted in increased quantities of normal-appearing lamellar bilayers in the SC extracellular spaces **(Fig 6*A–C*)**. In contrast, vehicle-treated samples displayed extensive domains with a paucity of extracellular lamellae **(Fig 6*D*)**, comparable to the appearance of SC in untreated, aged epidermis (not shown; see Ghadially *et al*, 1994). These results show that the improvement in barrier morphology following lipid applications can be attributed to enhanced formation of extracellular lamellar bilayers.

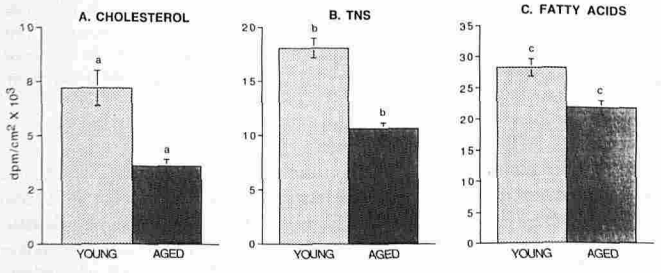

Figure 3. Lipid Synthesis Is Abnormal in Aged Murine Epidermis *in Vitro*. Full thickness skin samples from young and aged mice were incubated with [^{14}C]acetate for 3 h, and the lipids were extracted, saponificated, and fractionated as described in the text. (*a* vs. *a*, *b* vs. *b*; $p < 0.05$ contrast *c* vs. *c*; $p < 0.1$). n = 3; mean ± SEM.

DISCUSSION

Although the aged barrier functions adequately under basal conditions, when subjected to stress, it displays decreased integrity (ability to withstand graded insults), as well as a delay in barrier recovery (Ghadially *et al*, 1994). These functional changes are associated with a 30–35% global decrease in the content of all three key lipid classes in the murine model, and the biochemical alter-

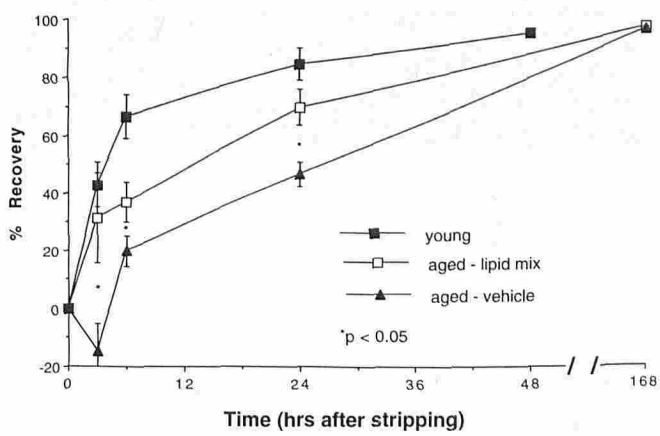

Figure 5. An Equimolar Lipid Mixture Accelerates Barrier Recovery in Aged Epidermis. Aged animals received either one topical application of a 1:1:1:1 mixture of ceramide, cholesterol, linoleic acid, and the nonessential free fatty acid, palmitic acid (final concentration 1.2%), or vehicle alone immediately after barrier disruption by tape stripping. Barrier recovery was assessed by measurement of transepidermal water loss at 0, 3, 6 h, using a Meeco electrolytic water analyzer. n = 6; mean ± SEM.

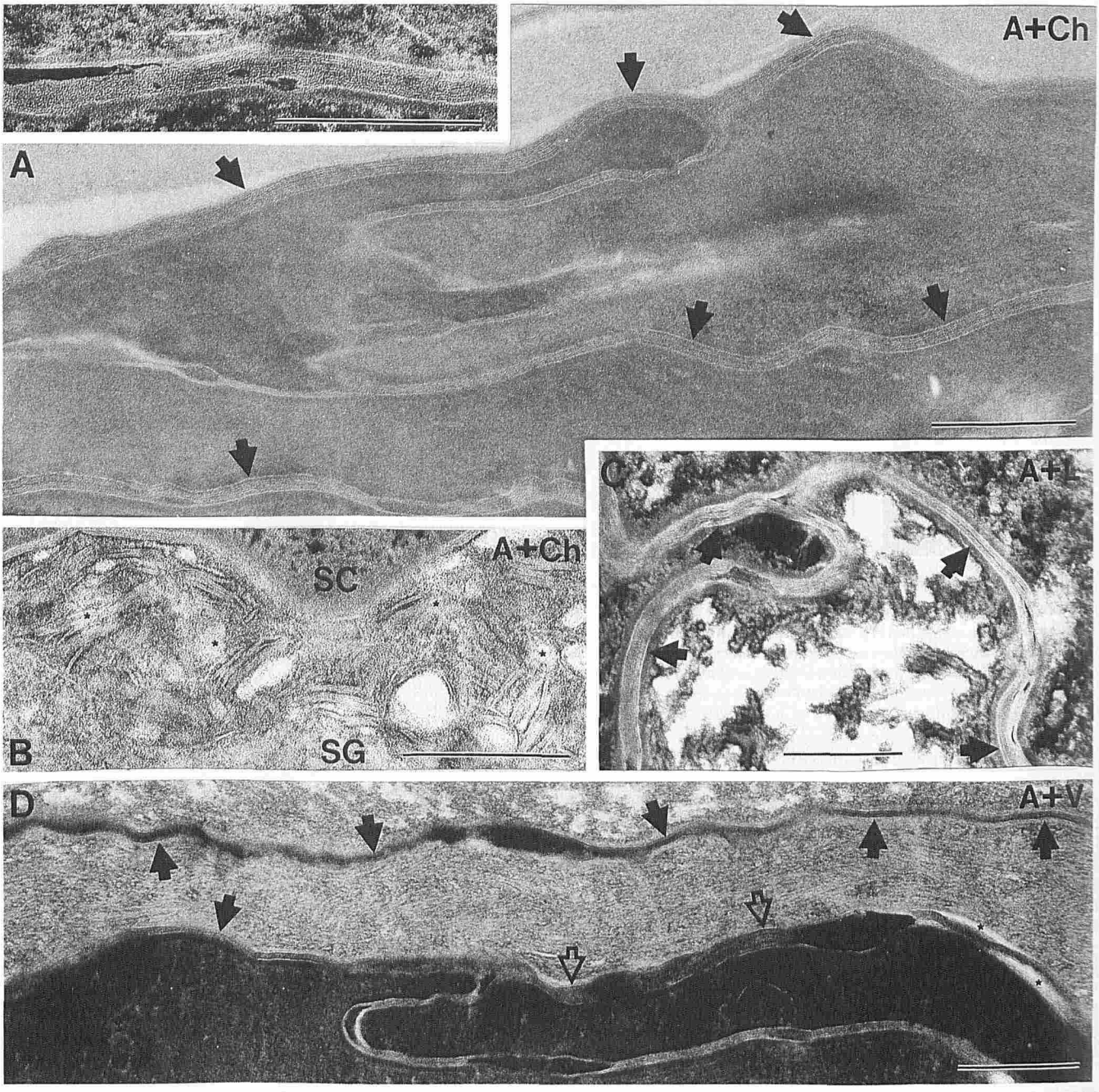

Figure 6. Lipid Repletion Normalizes SC Membrane Structures. *A*, following four separate applications of cholesterol (+ *Ch*) to aged (*A*) skin over 48 h, the SC extracellular domains appear replete with membrane structures, which display normal unit membrane structures (*A*, *arrows* + *insert*). *B*, likewise, in cholesterol-treated (*A* + *Ch*) animals, the stratum granulosum-SC interface is engorged with secreted lamellar body contents (*asterisks*). *C*, moreover, four applications of the equimolar mixture of SC lipids (*A* + *L*) over 48 h generates extracellular domains replete with lamellar bilayers (*arrows*). *D*, in contrast, vehicle (*A* + *V*)-treated sites display extensive extracellular domains devoid of lamellar bilayers (*solid arrows*), although some normal-appearing areas are present (*open arrows*). *Scale bar* = 0.25 μm.

ations are accompanied by decreased SC extracellular lamellae (comparable to aged human epidermis), as well as decreased secreted, lamellar body-derived contents at the SC-stratum granulosum interface (Ghadially *et al*, 1994). The similarities in structure and function in aged human versus murine epidermis further validate the use of senescent hairless mice for studies on permeability barrier homeostasis in aging. Murine epidermis, in general, displays similar stratum corneum membrane ultrastructure (Hou *et al*, 1991; Ghadially *et al*, 1992), lipid content (Schurer and Elias, 1991), and function (e.g., Ghadially *et al*, 1994).

The generation of SC lipids is attributable to high basal rates of lipid synthesis in mammalian epidermis (Feingold, 1991). Whereas we confirmed the robust rates of lipid synthesis in young epidermis again here, we also found that cholesterol synthesis rates are lower in aged murine epidermis in the basal state, measured both *in vivo* and *in vitro*. Prior studies have demonstrated decreased rates of cutaneous cholesterol synthesis with aging (Spady and Dietschy, 1989), but no further localization within the skin was undertaken. Our studies also demonstrate decreased cholesterol synthesis in aged murine epidermis under basal conditions. Both our *in vivo* and

Table II. Topical Cholesterol Accelerates Barrier Recovery in Aged Epidermis[a]

	% Recovery ± SEM		
Time (Hrs)	+Cholesterol	Significance	+Vehicle
3	49.5 ± 4.9	$p < 0.001$	0 ± 5.0
6	62.2 ± 3.4	$p < 0.01$	19.2 ± 4.8
24	66.5 ± 5.2	$p < 0.2$	42.7 ± 8.4

[a] Treated sites in aged animals received four topical applications of either cholesterol or vehicle over 48 hrs prior to tape stripping (see Methods). TEWL was measured 0,3,6, and 24 hrs after tape stripping. Data represent % recovery from an initial 0% in each group; n = 3–4 data points in 3 animals in each group.

in vitro data also suggest that the overall rates of lipid synthesis in aged epidermis are decreased, but the data did not quite achieve statistical significance. Both the fragility of aged animals and their requirement for individual housing for 18 months make the costs of additional experiments prohibitive.

We described previously that cholesterol, fatty acid, and sphingolipid synthesis are stimulated after acute forms of barrier abrogation (Menon *et al*, 1985; Grubauer *et al*, 1987; Holleran *et al*, 1991a). In the current studies, we show again that the synthesis of these lipids increases after barrier abrogation in young murine epidermis. Although a comparable increase in lipid synthesis occurs after barrier abrogation in aged epidermis, the overall increase is insufficient to attain levels in young epidermis. While the percentage increase in lipid synthesis in aged epidermis increases comparably to young epidermis in response to barrier disruption, the absolute rates of lipid synthesis remain lower than in young epidermis. The absolute rates of cholesterol and fatty acid synthesis do not attain those in young epidermis after barrier disruption, while sphingolipid synthesis approaches the levels in young epidermis.

The burst in lipid synthesis in young epidermis following barrier abrogation can be explained by reported, antecedent changes in mRNA, protein content, specific activities, and/or phosphorylation states of the key regulatory enzymes (Proksch *et al*, 1990; Holleran *et al*, 1991a; Jackson *et al*, 1992; Ottey *et al*, 1995). We have shown here that the decrements in basal cholesterol synthesis in aged epidermis can be ascribed to a corresponding decrease in the activity of its rate-limiting enzyme, HMG-CoA reductase. As with total lipid synthesis, after barrier perturbation in both young and aged mice, the absolute levels of HMG-CoA reductase activity in older epidermis remain significantly less than those in stimulated young epidermis. Thus, the changes in epidermal cholesterol synthesis in aged epidermis are paralleled by alterations in the activity of its rate-limiting enzymes.

Recent studies have shown that topical stratum corneum lipids (cholesterol, fatty acid, and ceramide) traverse the stratum corneum and enter the upper layers of the epidermis (Mao-Qiang *et al*, 1993b, 1995). When added as an equimolar mixture, the physiological lipids allow normal barrier recovery, but incomplete mixtures, comprising only one or two of the key lipids, generally delay barrier recovery. Normal versus delayed barrier recovery could be ascribed to alterations in the lamellar body secretory system and its extracellular membrane products (Mao-Qiang *et al*, 1993b). Finally, the topical physiological lipids are processed within the granular cell by a route that bypasses the endoplasmic reticulum and proximal Golgi apparatus (Mao-Qiang *et al*, 1995). Accordingly, we have shown here that both an equimolar lipid mixture and topical cholesterol alone appear to bypass both the defective secretory apparatus and the lipid biosynthetic machinery (these studies) in aged epidermis, largely restoring normal barrier function. Importantly, neither of these two lipid preparations accelerate barrier recovery in young epidermis (Mao-Qiang *et al*, 1993b). Finally, the ability of the exogenous lipids to improve barrier recovery in aged epidermis correlates with the increased generation of extracellular lamellae in lipid-treated aged skin. Thus, the ability of selected SC lipids to improve barrier recovery in aged epidermis confirms the importance of the lipid metabolic defects for the functional abnormality in aged epidermis; i.e., decreased lipid generation is the key defect underlying the permeability barrier abnormalities in aged skin.

In summary, our findings suggest that the decreased lipid synthetic rates in aged epidermis supply sufficient lipids to the stratum corneum interstices to provide an adequate barrier under basal conditions. With damage to the barrier, a decreased capacity of aged epidermis for epidermal lipid synthesis, in general, and cholesterol synthesis in particular, results in an impaired repair response. The clinical implications of these findings include decreased ability of aged epidermis to repair following various types of injury and altered rates of transcutaneous drug delivery across aged epidermis.

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