

## **Stratum corneum lipids in disorders of cornification. Steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis.**

P M Elias, ... , S Grayson, E H Epstein Jr

*J Clin Invest.* 1984;74(4):1414-1421. <https://doi.org/10.1172/JCI111552>.

### Research Article

The pathological scaling in recessive x-linked ichthyosis is associated with accumulation of abnormal quantities of cholesterol sulfate in stratum corneum (*J. Clin. Invest.* 68:1404-1410, 1981). To determine whether or not cholesterol sulfate accumulates in recessive x-linked ichthyosis as a direct result of the missing enzyme, steroid sulfatase, we quantitated both steroid sulfatase and its substrate, we quantitated both steroid sulfatase and its substrate, cholesterol sulfate, in different epidermal strata, as well as within stratum corneum subcellular fractions obtained from normal human and neonatal mouse epidermis and from patients with recessive x-linked ichthyosis. In normal human and mouse epidermis, steroid sulfatase activity peaked in the stratum granulosum and stratum corneum, and negligible activity was detectable in lower epidermal layers. In contrast, in recessive x-linked ichthyosis epidermis, enzyme levels were virtually undetectable at all levels. In normal human stratum corneum, up to 10 times more steroid sulfatase activity was present in purified peripheral membrane preparations than in the whole tissue. Whereas in normal human epidermis cholesterol sulfate levels were lowest in the basal/spinous layer, and highest in the stratum granulosum, in recessive x-linked ichthyosis the levels were only slightly higher in the lower epidermis, but continued to climb in the stratum corneum. In both normal and in recessive x-linked ichthyosis stratum corneum, cholesterol sulfate appeared primarily within membrane domains, paralleling the pattern [...]

**Find the latest version:**

<https://jci.me/111552/pdf>



## Stratum Corneum Lipids in Disorders of Cornification

### Steroid Sulfatase and Cholesterol Sulfate in Normal Desquamation and the Pathogenesis of Recessive X-linked Ichthyosis

Peter M. Elias, Mary L. Williams, Mary E. Maloney, Jeannette A. Bonifas, Barbara E. Brown, Stephen Grayson, and Ervin H. Epstein, Jr.

*Dermatology Service, Veterans Administration Medical Center and Dermatology Unit, San Francisco General Hospital Medical Center, Departments of Dermatology and Pediatrics, University of California, San Francisco, California 94143*

**A**bstract. The pathological scaling in recessive x-linked ichthyosis is associated with accumulation of abnormal quantities of cholesterol sulfate in stratum corneum (*J. Clin. Invest.* 68:1404-1410, 1981). To determine whether or not cholesterol sulfate accumulates in recessive x-linked ichthyosis as a direct result of the missing enzyme, steroid sulfatase, we quantitated both steroid sulfatase and its substrate, cholesterol sulfate, in different epidermal strata, as well as within stratum corneum subcellular fractions obtained from normal human and neonatal mouse epidermis and from patients with recessive x-linked ichthyosis. In normal human and mouse epidermis, steroid sulfatase activity peaked in the stratum granulosum and stratum corneum, and negligible activity was detectable in lower epidermal layers. In contrast, in recessive x-linked ichthyosis epidermis, enzyme levels were virtually undetectable at all levels. In normal human stratum corneum, up to 10 times more steroid sulfatase activity was present in purified peripheral membrane preparations than in the whole tissue. Whereas in normal human epidermis cholesterol sulfate levels were lowest in the basal/spinous layer, and highest in the stratum granulosum, in recessive x-linked ichthyosis the levels were only slightly higher in the lower epidermis, but continued to climb in the

stratum corneum. In both normal and in recessive x-linked ichthyosis stratum corneum, cholesterol sulfate appeared primarily within membrane domains, paralleling the pattern of steroid sulfatase localization. Finally, the role of excess cholesterol sulfate in the pathogenesis of recessive x-linked ichthyosis was directly tested by topical applications of this substance, which produced visible scaling in hairless mice in parallel to an increased cholesterol sulfate content of the stratum corneum. These results demonstrate an intimate relationship between steroid sulfatase and cholesterol sulfate in normal epidermis: both are concentrated in the outer epidermis (stratum corneum and stratum granulosum), and both are localized to membrane domains. Presumably, as a result of this distribution pattern, continued enzymatic degradation of substrate occurs in normal epidermis, thereby preventing excessive accumulation of cholesterol sulfate. In contrast, in recessive x-linked ichthyosis, degradation of cholesterol sulfate does not occur and cholesterol sulfate accumulates specifically in the stratum corneum, where it produces visible scale.

#### Introduction

Recessive x-linked ichthyosis (RXLI)<sup>1</sup> is an uncommon disorder of cornification which is characterized clinically by excessive scaling due to prolonged stratum corneum retention. Previously characterized by inheritance pattern and clinical presentation (1), this disorder can now be diagnosed biochemically because of the recent discovery that patients with RXLI lack the enzyme steroid sulfatase (2, 3). Presumably, as a consequence

Portions of this work were presented to the Western Regional Meeting, American Federation Clinical Research, Carmel, CA, 1982 (15).

Dr. Williams is a recipient of the VA Clinical Investigator Award and a March of Dimes Basil O'Connor Starter Research Award. Address correspondence to Dr. Elias.

Received for publication 19 April 1983 and in revised form 29 May 1984.

The Journal of Clinical Investigation, Inc.  
Volume 74, October 1984, 1414-1421

1. *Abbreviations used in this paper:* DHEAS, dehydroepiandrosterone sulfate; DMSO, dimethyl sulfoxide; RXLI, recessive x-linked ichthyosis; SS, steroid sulfatase; TLC, thin-layer chromatography.

of steroid sulfatase deficiency in skin and other tissues (4–6), one substrate, cholesterol sulfate, accumulates in red blood cells (7), serum (7, 8), and stratum corneum (9). In contrast, sulfated steroid hormones, such as dehydroepiandrosterone sulfate, that are also substrates of this enzyme *in vitro*, do not accumulate in either blood or urine (8, 10).

Yet, despite this array of new biochemical data, the pathogenesis of the scaling abnormality in RXLI is still not understood. Because of the unique localization of lipids to the intercellular regions of the stratum corneum (11), we have suspected that lipids may regulate stratum corneum cohesion and desquamation (12). Thus, the scaling abnormality in RXLI might be attributable to accumulation of cholesterol sulfate within stratum corneum cell membrane domains (13, 14). Here, we report (a) that both the enzyme and its substrate are concentrated within the outer epidermis in normal human and rodent epidermis, and that within these layers, they are localized to membrane regions (15); and (b) that cholesterol sulfate itself, when repeatedly applied to normal stratum corneum, produces scale (16). These findings may provide a subcellular basis not only for disease expression, but also for the process of normal desquamation (14).

## Methods

*Preparations of human and neonatal mouse epidermal cell layers.* Normal human skin ( $n = 8$ ) was obtained from fresh autopsy (abdomen) or fresh surgical (abdominoplasty/mammoplasty) full-thickness specimens. By floating specimens dermis-side downward on culture fluid that contained 10 mg/ml of a crude preparation of staphylococcal epidermolytic toxin for 2 h at 37°C, we obtained homogeneous, undamaged sheets of stratum corneum and stratum granulosum (17). Further incubations of sheets over 0.5% trypsin in phosphate-buffered saline (PBS), pH 7.4, for 2 h at 37°C provided homogeneous populations of stratum corneum and stratum granulosum. Trypsinization was halted by the addition of excess soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, MO), and the granular cells were dislodged by vortexing followed by filtering three times through several layers of cheesecloth. Basal and spinous cells were liberated by gently scraping the freshly denuded surface of toxin-split full-thickness skin specimens with a 15 surgical blade. The homogeneity of each epidermal cell layer—stratum corneum, stratum granulosum, and stratum basale/spinosum—was assessed by phase and electron microscopy (11). Nucleated cells were absent in stratum corneum preparations after trypsinization, and conversely, a complete absence of cornified cells was noted in granular cell pellets. Since basal/spinous layer preparations contained neither collagen nor flattened squames, these preparations apparently represented cells that were derived solely from the lower epidermis.

Five patients with RXLI, documented by prior enzymatic assay (5), consented to having 1-cm<sup>2</sup> shave biopsies performed from clinically involved sites on the trunk comparable to controls, *i.e.*, back or abdomen, followed by *in vitro* splitting as described above for normals. Parallel studies were performed in neonatal mouse epidermis, which possesses a comparable spectrum of epidermal lipids to human epidermis (11, 12). The subsequent steps for preparation of stratum granulosum and stratum basale/spinosum followed those described above for human epidermis.

*Development of an animal model.* To test the hypothesis that cholesterol sulfate within the stratum corneum provokes excessive scale, we applied cholesterol sulfate (Research Plus, Bayonne, NJ), dehydroepiandrosterone sulfate (DHEAS, Sigma Chemical Co.) dissolved in dimethyl sulfoxide (DMSO), and cholesterol (Sigma Chemical Co.) dissolved in DMSO/chloroform (50:50, vol) to the backs of hairless mice (Hr/Hr, Jackson Labs, Bar Harbor, ME). Some animals received trace amounts of <sup>3</sup>H-cholesterol sulfate in addition to cold cholesterol sulfate that was synthesized from <sup>3</sup>H-cholesterol (New England Nuclear, Boston, MA) and purified as described elsewhere (16). A total of 1.0–2.0 mg of each chemical or vehicle alone was applied daily to a circumscribed 1-cm<sup>2</sup> area. Animals were housed individually and wore a specially constructed collar to insure that they did not lick themselves or other animals. Biopsies were obtained at 0, 1, 3, and 7 wk, and processed for light microscopy followed by hematoxylin and eosin staining, or for lipid extraction and fractionation (see below).

*Preparation of subcellular fractions.* Recently, a new protocol has been developed for the purification of membrane preparations from both human and neonatal mouse stratum corneum, which traps most of the stratum corneum lipids in the intercellular spaces (18). Briefly summarized, the freshly prepared stratum corneum sheets were snap-frozen in aluminum foil under liquid nitrogen and pulverized into a fine powder on a submerged aluminum block with a precooled hammer. The resultant powder was resuspended in 20–30 ml of 0.03 M Tris-HCl buffer, pH 8.8, and homogenized further by passage through a Stansted cell disrupter (Stansted Fluid Power Corp., Ltd., Stansted, Essex, United Kingdom) at 20–30,000 pounds per square inch. This homogenate was centrifuged at 25,000 *g* for 20 min and the supernatant saved. The pellet was then suspended in 0.005% subtilisin (protease VIII, Sigma Chemical Co.) in 20–30 ml of 0.03 M Tris-HCl buffer and stirred for 1 h at room temperature. This procedure digests away all of the corneocytes' cytoplasmic filaments and matrix, leaving a homogeneous population of unperturbed membrane couplets that contains trapped intercellular lipids (18). The membranes were then pelleted at 30,000 *g* and the supernatants saved.

*Assessment of steroid sulfatase (SS) activity.* The whole cell, broken cell, and supernatant fractions were homogenized in iced 0.014 M Tris-HCl buffer, pH 8.0, using a Polytron PT-10 (Brinkmann Instruments Inc., Westbury, NY) with one or two 10-s bursts at maximal speed, and the enzyme assays were performed on the Miranol-extracted fractions by measurement of desulfation of <sup>3</sup>H-DHEAS as previously described (8). All incubations were performed in duplicate. Enzyme activity was expressed as picomoles of <sup>3</sup>H-DHEAS converted to benzene-soluble <sup>3</sup>H-DHEAS per hour incubation per milligram protein. Protein quantity was measured by the Lowry method, by hydrolysis in 6 N HCl, and analysis in an amino acid analyzer (19). The results were comparable by either method.

*Cholesterol sulfate assay.* The cholesterol sulfate content of portions of the stratum basale/spinosum, stratum granulosum, and the stratum corneum fractions from eight normals and five RXLI patients was assessed by sequential, quantitative thin-layer chromatography (TLC) as described recently (9, 11). Because of its limited lipid solubility, cholesterol sulfate is found not only in the organic phase, but also in the aqueous-methanol phase unless 0.1 M KCl is added to the extraction mixture, which drives all of the cholesterol sulfate into the lipid-containing infranatants (9). After fractionation of lipid extracts in tetrahydrofuran/methylal/methanol:4 M ammonium hydroxide (60:30:10:4, vol), the cholesterol sulfate-containing fraction from the normal skin samples, visualized by black light fluorescence after spraying with the fluorophore, 8-anilino-1-naphthalene sulfonic acid,

was excised, extracted in Bligh-Dyer solvents (19, 20) that contained 0.1 M KCl, and measured colorimetrically by the Franey-Amador method (21). This method accurately detects quantities of cholesterol sulfate in the 25–1,000  $\mu\text{g}$  range. The assignment of the cholesterol sulfate fraction was confirmed by co-chromatography against the authentic compound in three solvent systems, and by mass spectrometry after solvolysis (Fig. 1) (9). We found no evidence of other sulfated sterols such as DHEAS, cholecalciferol sulfate, or 7-dehydrocholesterol sulfate, in this fraction.

To quantitate cholesterol sulfate in lipid extracts from the small biopsy samples that were obtained from the four RXLI patients, we employed a new microchromatographic technique that uses siliconized quartz rods instead of TLC plates (22). The lipid extracts were dried down and resuspended in warm chloroform/methanol (1:2) to assure complete solubilization of cholesterol sulfate. The final lipid concentration was  $\sim 20 \mu\text{g}/\mu\text{l}$ . 1  $\mu\text{l}$  or less was spotted on each Chromarod and the rod was then developed in tetrahydrofuran/methylal/methanol:4 M  $\text{NH}_4\text{OH}$  (60:30:15:4, vol). The rods were then heated for 30 s at  $100^\circ\text{C}$  to dry off remaining solvent, and then run through a flame ionization detector in the Iatroscan TH10-Mark III-TLC analyzer (Ancal Inc., Los Osos, CA) using an air flow of 2,000 ml/min (atmospheric air) hydrogen flow of 160 ml/min (high purity hydrogen), and scanning speed of 2.39 s/cm. Detector response data were collected and integrated with a computing integrator model SP 4100 (Spectra-

Physics Inc., Mountain View, CA). We have previously shown that the agreement between TLC and Iatroscan is excellent for the high concentrations of cholesterol sulfate that were encountered in RXLI (23). Lipid weights were expressed as either percentage of total recovered lipid or per milligram protein (19).

## Results

**Steroid sulfatase activity.** Because the various cell layers and subcellular fractions were exposed to different incubation conditions, e.g., epidermolytic toxin and proteolytic enzymes (trypsin, subtilisin), the impact of these conditions on SS activity was assessed in human placental microsomes, human leukocyte extracts, and stratum granulosum homogenates. After 30-min, 1-h, and 2-h incubations with trypsin, subtilisin, or epidermolytic toxin, we noted no diminution of enzyme activity (data not shown).

**Stratification of SS in epidermis (tables I–III).** In both human and neonatal mouse epidermis, little SS activity was present in the stratum basale/spinosum fraction (Tables I and III). However, in both the human and mouse, a sudden and dramatic increase in activity appeared in the stratum granu-

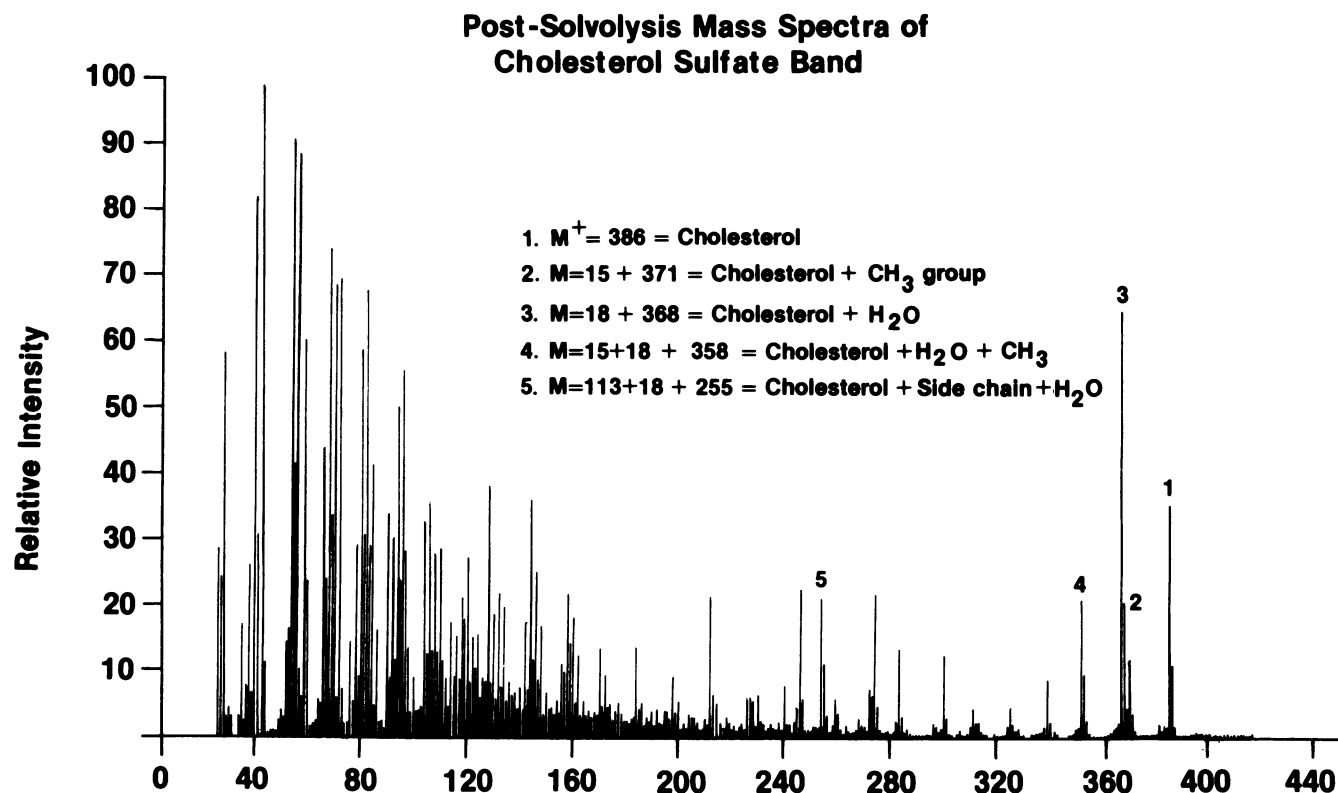


Figure 1. Mass spectrometry analysis of cholesterol sulfate (post-solvolysis) band from prior TLC fractionation of normal human stratum corneum. Note appearance of characteristic  $M^+ = 388$  of

cholesterol and various sterol fragments. There is no evidence of DHEAS, cholecalciferol, or 7-dehydrocholesterol in these samples.

Table I. Stratification of Steroid Sulfatase Activity in Normal and RXLI Epidermis (cpm/mg/h)

Fraction	Normal (exp. #)*					RXLI
	1	2	3	4	5	
Stratum corneum	54,917	38,671	63,493	50,483	42,328	572
Stratum granulosum‡	20,218	170,000	77,000	130,000	92,000	0
Stratum spinosum/ basale	3,859	11,182	20,000	6,565	10,862	0

\* Variability from experiment to experiment could be related to several factors: different ratios of male/female animals; different starting ages of animals; and different lengths of storage at  $-20^{\circ}\text{C}$  before assay.

‡ Protein quantities were less than the lower limits of accurate quantitation by our assay ( $30\ \mu\text{g}/\text{ml}$ ). These values assume protein concentrations of  $30\ \mu\text{g}/\text{ml}$  and therefore are minimum values for specific activity of the enzyme.

losum and stratum corneum (Tables I and III). In both human and neonatal mouse, SS activity in the stratum granulosum and stratum corneum was approximately equal (Tables I and III). In contrast, no enzyme activity was found in any epidermal layer from the RXLI patient (Table I).

*Subcellular localization of SS in stratum corneum.* In both normal human and mouse stratum corneum, the bulk of enzyme activity (4- to 12-fold excess) appeared in the peripheral membrane preparations (Table II, mouse data not shown). In comparison, supernatants generally contained <10% of the activity found in the purified membranes (Table II). In contrast, neither RXLI whole stratum corneum nor RXLI stratum corneum membranes displayed enzyme activity (Tables I and II).

*Cholesterol sulfate levels and localization in normal and RXLI epidermis.* Whereas the cholesterol sulfate content of normal human stratum corneum rarely exceeds 5% of the total lipid (9), an apparent gradient occurred during maturation from the inner to the outer epidermis (Table IV). The levels present in the stratum granulosum were significantly higher than those found in the lower epidermis and consistently higher than stratum corneum levels, although the difference did not achieve statistical significance (Table IV). In RXLI viable epidermal cell layers, cholesterol sulfate levels were only slightly higher than normal viable epidermal cell layers (Table IV). But in contrast to normals, the levels in RXLI continued to rise into the stratum corneum (Fig. 2, Table IV), where

Table II. Localization of Steroid Sulfatase in Normal and RXLI Stratum Corneum (cpm/mg/h)

Fraction	Normal (exp. #)			RXLI*
	1	2	3	
Whole stratum corneum	9,964	27,117	8,300	572
Peripheral membranes	106,773	91,924	77,806	0
Supernatants	3,917	6,097	—	20

\* Several other RXLI preparations also showed negligible activity.

Table III. Steroid Sulfatase Activity in Different Layers of Neonatal Mouse Epidermis (cpm/mg/h)

Layer	Experiment*				
	1	2	3	4	5
Stratum corneum	76,600	10,000	52,262	12,844	17,000
Stratum granulosum	15,600	1,100	22,189	17,532	30,000
Stratum spinosum/basale	—	154	1,806	1,312	646

\* Variability from experiment to experiment could be related to several factors: different ratios of male/female animals; different starting ages of animals; or different lengths of storage at  $-20^{\circ}\text{C}$  before assay.

they were 3–5 times normal whether expressed in terms of lipid weight percent or protein weight. As with other stratum corneum lipids (17), 60–80% of the cholesterol sulfate in both normal and RXLI stratum corneum appeared in membrane domains (Fig. 3), while the remaining cholesterol sulfate was lost into the supernatants during tissue preparation (18). Based upon the known, fivefold increased content of cholesterol sulfate in stratum corneum membranes over whole stratum corneum, the concentration of cholesterol sulfate in RXLI membranes was calculated to be  $\sim 1.6\ \mu\text{g}/\mu\text{g}$  protein, or  $\sim 25$  times that of whole stratum granulosum.

*Application of topical cholesterol sulfate to hairless mice.* To directly test the hypothesis that the accumulation of excess cholesterol sulfate in the stratum corneum is associated with pathological scaling, we applied cholesterol sulfate to circumscribed areas on the backs of hairless mice. When cholesterol sulfate, at concentrations  $>2.5\ \text{mg}/\text{ml}$  ( $0.02\ \text{ml}$  total daily dose) was applied, fine scaling appeared between 7 and 14 d. Doubling or quadrupling the dose produced scaling in 5–7 d. Inclusion of equimolar concentrations of cholesterol prevented scaling, and scaling could not be produced by another substrate of steroid sulfatase, DHEAS, nor by either the straight DMSO or DMSO/chloroform (50:50, vol) vehicles alone. With prolonged treatment ( $>2\ \text{wk}$ ), scaling eventually disappeared, apparently as a result of enzyme induction (16).

Clinical scaling was accompanied by histologic evidence of a direct effect on the stratum corneum (Fig. 4): the thickness was increased almost threefold over normals ( $10.3 \pm 1.1$  vs.  $3.6 \pm 0.5\ \mu\text{m}$ ,  $P < 0.01$ ). However, there was no evidence of acanthosis, increased thymidine labeling (mitotic index), or alteration of transepidermal water loss, which would be indicative of an effect on the viable epidermis (16). Moreover, when trace quantities of  $^3\text{H}$ -cholesterol sulfate were added, either alone or with cold substrate, 97% of recovered label was retained in the stratum corneum, and only 3% reached the viable epidermis (data not shown).

That abnormal scaling is dependent on cholesterol sulfate accumulation is also shown by the data in Table V: comparison of cholesterol sulfate content in lipid extracts from cholesterol sulfate-treated vs. vehicle-treated control sites demonstrated that scale appears in treated animals just as the cholesterol

Table IV. Cholesterol Sulfate Content of Human Epidermal Fractions (% Total lipid $\pm$ SEM)

Layer	Normal		RXLI*	
	Lipid weight (%)	Lipid weight/ $\mu$ g protein	Lipid weight (%)	Lipid weight/ $\mu$ g protein
Stratum corneum	3.4 $\pm$ 0.5 (n = 8) $\ddagger$	0.09	11.2 $\pm$ 1.1 (n = 5) $\ddagger$	0.32
Stratum granulosum	5.2 $\pm$ 1.4 (n = 7) $\ddagger$	0.05	5.5 $\pm$ 0.9 (n = 5) $\ddagger$	0.06
Stratum spinosum/basale	2.6 $\pm$ 1.0 (n = 5) $\ddagger$	0.04	5.5 $\pm$ 0.4 (n = 3) $\ddagger$	0.10

\* Shave biopsies from five patients with RXLI were split into distinct layers with the staphylococcal epidermolytic toxin and trypsin (see Methods). After solvent extractions, cholesterol sulfate was quantitated by microchromatography on silica-coated quartz rods (23).  $\ddagger$  Results of stratum granulosum vs. spinosum/basale in normal epidermis and of stratum corneum vs. stratum granulosum or stratum spinosum/basale in RXLI, are statistically significant ( $P < 0.01$ ,  $< 0.05$ , respectively). However, differences between normal stratum corneum and stratum granulosum do not achieve significance ( $P < 0.1$ ).

sulfate content doubles levels encountered in control stratum corneum. Shorter application times produce intermediate cholesterol sulfate levels without evidence scale (Table V). Cholesterol content was lower in treated scaling sites than untreated or treated nonscaling sites, but these differences were not significant.

## Discussion

Previous studies have demonstrated that absence of the enzyme, steroid sulfatase, in RXLI is associated with accumulation of cholesterol sulfate in erythrocytes (7), serum (8), and stratum corneum (9). The possibility that the increased cholesterol sulfate in RXLI stratum corneum results from passive accumulation of circulating cholesterol sulfate seems unlikely, since the concentration of cholesterol sulfate in serum and erythrocyte membranes in RXLI are an order of magnitude less than the levels encountered in normal epidermis, and two orders of

magnitude lower than those found in RXLI stratum corneum (references 7 and 9, this study). Moreover, the relatively low quantities of cholesterol sulfate in lower epidermis in comparison to upper epidermal strata is consistent with active upper epidermal synthesis of cholesterol sulfate, rather than passive accumulation from the circulation. This likelihood is supported further by the observation that porcine skin ranks second only

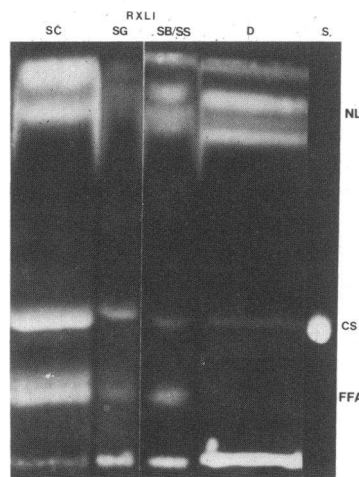


Figure 2. Representative TLC fractionation of cholesterol sulfate in epidermal strata from a patient with RXLI. Note increased prominence of the cholesterol sulfate (CS) fraction during outward progression from the dermis (D), basal/spinosum layer (SB/SS), granular layer (SG), to the stratum corneum (SC). S, standards; FFA, free fatty acids; and NL, neutral lipids.

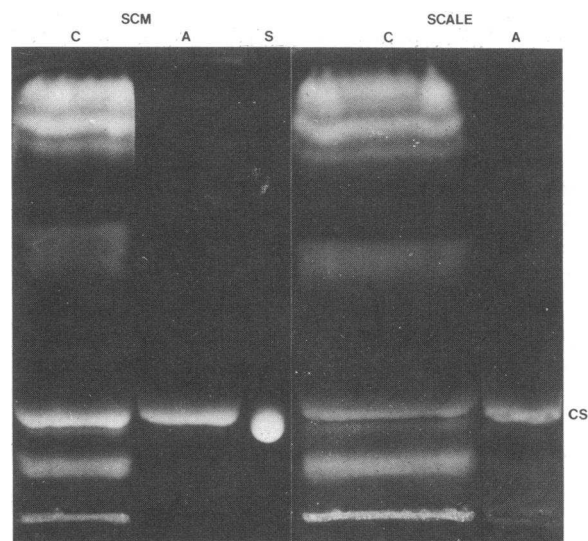


Figure 3. Cholesterol sulfate localization in solvent extracts of whole scale vs. stratum corneum membranes (SCM) in normal epidermis. Whereas the chloroform phase (C) contains the full array of stratum corneum lipids, only cholesterol sulfate appears in the aqueous phases (A) in substantial quantities. Note that there appears to be at least as much cholesterol sulfate (CS) in membrane preparations as in whole scale. The differences in staining intensities do not reflect differences in total applied lipid, since the amounts of chloroform and aqueous phase samples applied were equivalent. S, standard.

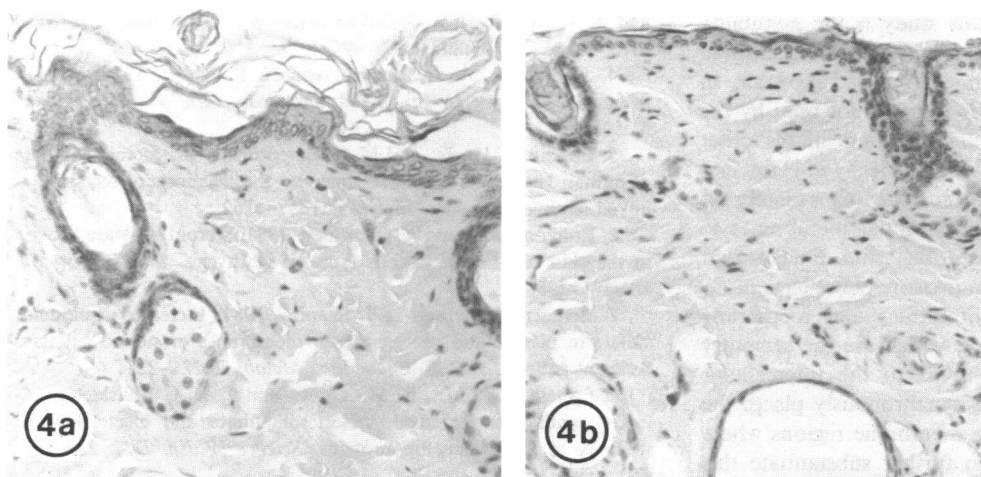


Figure 4. Histology of cholesterol sulfate-treated (a) vs. vehicle-treated (b) hairless mouse skin. Whereas the stratum corneum appears much thicker in cholesterol sulfate-treated skin, the nucleated cell layers and dermis appear to be unchanged; there is no apparent increase in the number of mitotic figures in CS-treated skin, as well. (a) and (b),  $\times 2,250$ .

to liver in cholesterol sulfate synthesis (24). Finally, we have provided preliminary evidence for a cholesterol sulfurylation pathway in both human and mouse epidermis (25).

The studies reported here provide strong evidence for a direct relationship between SS deficiency, cholesterol sulfate accumulation, and disease expression in RXLI: (a) Both the enzyme and its substrate in normal mammalian epidermis are concentrated in the outer epidermis. (b) In normal stratum corneum, the localization of enzyme and substrate to peripheral membrane domains presumably permits continued enzymatic degradation of substrate, as reflected by the lower cholesterol sulfate content of the stratum corneum in comparison to the stratum granulosum. (c) In RXLI, absence of SS activity leads to accumulation of cholesterol sulfate in the stratum corneum, and specifically in cell membranes where it could exert important influences on intercellular cohesion. (d) An additional, important piece of evidence linking enzyme, substrate, and disease expression consists of the scale produced in hairless mice by topical applications of cholesterol sulfate. Evidence is presented here that scale results directly from accumulation of cholesterol sulfate within the stratum corneum (Table V). The

likelihood that cholesterol sulfate produces its effect directly on the stratum corneum is supported further by the observation that >97% of each daily, applied dose of labeled cholesterol sulfate remained in the stratum corneum (Eliás, P. M., and B. E. Brown, unpublished observations).

Alternatively, there is evidence that stratum corneum retention may be due primarily to changes in free sterol content of stratum corneum. First, several drugs that lower serum cholesterol may produce disorders of stratum corneum retention in man (reviewed in reference 12). One of these agents, 20, 25 diazcholesterol, produces an ichthyotic state in hairless mice, with decreased stratum corneum cholesterol content that is correctable by topical cholesterol (26). Secondly, the increased cholesterol sulfate content of RXLI scale is accompanied by a reduction in free sterols (9), and a recent report describes correction of scale in RXLI after topical application of cholesterol (27). We have recently demonstrated that cholesterol sulfate inhibits sterologenes in cultured fibroblasts and keratinocytes by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, the rate-limiting step in cholesterol synthesis (28). The issue of the relative importance of increased cholesterol sulfate or decreased cholesterol in RXLI stratum corneum to the pathogenesis of disease remains to be resolved. However, the demonstration in hairless mice of scaling after topical application of cholesterol sulfate, where little seems to penetrate to the viable epidermis, supports a direct, provocative role for cholesterol sulfate per se.

Still unanswered is the question of localization of disease to the skin in RXLI. One explanation for localization of disease to skin, and for the paucity of extracutaneous organ involvement in RXLI, may be the epidermis' unusually great capacity to generate large quantities of sulfated sterols (24, 25). We have recently hypothesized that a cholesterol sulfate cycle is operative in the epidermis, and that interruption of this sulfation-desulfation cycle leads to the scaling in RXLI (14). Alternatively, there may be other mechanisms for desulfation of sterols in extracutaneous tissue.

Table V. Cholesterol/Cholesterol Sulfate Content of Cholesterol Sulfate-treated Hairless Mouse Stratum Corneum

Ratio	Cholesterol sulfate-treated*		Controls
	Scale evident	Scale not yet evident	
			mean $\pm$ SEM; n = 3
Chol/CSO <sub>4</sub>	8.2	15.6	18.6 $\pm$ 2.1
Chol/mg protein ‡	0.87	1.08	1.03 $\pm$ 0.15
CSO <sub>4</sub> /mg protein ‡	0.11	0.07	0.05 $\pm$ 0.01

\* Mean of two experiments.

‡ Milligram lipid per milligram protein.

Another important aspect of this study is the possibility that steroid sulfatase is an effector of physiologically normal desquamation. Such a link between enzyme and substrate, in relation to normal desquamation, is fortified not only by the parallel concentration of enzyme and substrate in the outer epidermis, but also by their localization specifically to peripheral cell membrane domains in the stratum corneum. This abrupt shift in enzyme localization from intracellular membranes to the cell periphery during the transition from the stratum granulosum to the stratum corneum presumably reflects insertion into the plasma membrane of intracellular membrane fragments, including lamellar bodies, which are the secretory organelle that delivers epidermal lipids to the intercellular spaces (12). This relocation process synchronously places the enzyme in close proximity to those membrane regions where cholesterol sulfate is segregated. To further substantiate this hypothesis it will be necessary to find evidence of progressive desulfation of cholesterol sulfate within the stratum corneum itself. In addition, positive proof for a relationship between desulfation and desquamation requires demonstration of sulfatase activity in stratum corneum in vivo, rather than in detergent extracts, as shown here. If such activity could be demonstrated, then the stratum corneum might yet be shown to possess a broad spectrum of metabolic activities that could govern its own functions, and not to be "dead" as generally accepted.

The molecular mechanism(s) whereby cholesterol sulfate might influence cohesion is still unknown. Two possibilities that are under active consideration include: first, that abnormal lipid thermal transitions would result from an altered intercellular lipid mix (29); or second, that intermolecular crosslinking between cholesterol sulfate moieties on adjacent cell membranes, which are mediated by divalent cations, would increase cohesion (13).

## Acknowledgments

We are grateful to Marilyn A. Lampe and Mara Hincenbergs for performing some of the cholesterol sulfate determinations. Drs. JoAnne Whitney and A. L. Burlingame, Mass Spectrometry Units, University of California School of Medicine, San Francisco, kindly performed the mass spectrometry. Our thanks to Margarita Devincenzi and Rick Frautschi for expertly typing and editing this manuscript.

This work was supported by National Institutes of Health grants AM 19098 and 29908 and by the Medical Research Service, Veterans Administration.

## References

1. Goldsmith, L. A. 1976. The ichthyoses. *Prog. Med. Genet.* 1:185-210.
2. Shapiro, L. J., R. Weiss, D. Webster, and J. T. France. 1978. X-linked ichthyosis due to steroid-sulfatase deficiency. *Lancet.* 1:70-72.
3. Koppe, J. G., A. Marinkovic-Ilsen, Y. Rijken, W. P. de Groot, and A. C. Jobsis. 1978. X-linked ichthyosis. A sulfatase deficiency. *Arch. Dis. Child.* 53:803-806.
4. Kubilus, J., A. J. Tarascio, and H. P. Baden. 1979. Steroid-sulfatase deficiency in sex-linked ichthyosis. *Am. J. Hum. Genet.* 31:50-53.
5. Epstein, E. H., Jr., and M. E. Leventhal. 1981. Steroid sulfatase of human leukocytes and epidermis and the diagnosis of recessive x-linked ichthyosis. *J. Clin. Invest.* 67:1257-1262.
6. Roukoniemi, A., and A. Oikarinen. 1981. Steroid sulfatase activity in the skin biopsies of various types of ichthyosis. *Br. J. Dermatol.* 105:291-295.
7. Berger, E. A., and L. J. Shapiro. 1981. Increased cholesterol sulfate in plasma and red blood cell membranes of steroid sulfatase deficient patients. *J. Clin. Endocrinol. Metab.* 53:221-223.
8. Epstein, E. H., Jr., R. M. Krauss, and C. H. C. Shackleton. X-linked ichthyosis: increased cholesterol sulfate and electrophoretic mobility of low-density lipoproteins. *Science (Wash. DC).* 214:659-660.
9. Williams, M. L., and P. M. Elias. 1981. Stratum corneum lipids in disorders of cornification. I. increased cholesterol sulfate content of stratum corneum in recessive x-linked ichthyosis. *J. Clin. Invest.* 68:1404-1410.
10. Roukoniemi, A., A. Oikarinen, R. Palatsi, and I. Huhtaniemi. 1980. Serum steroid sulphates in ichthyosis. *Br. J. Dermatol.* 103:245-248.
11. Elias, P. M., B. E. Brown, P. O. Fritsch, R. J. Goerke, G. M. Gray, and R. J. White. 1979. Localization and composition of lipids in neonatal mouse stratum granulosum and stratum corneum. *J. Invest. Dermatol.* 73:339-348.
12. Elias, P. M. 1981. Epidermal lipids, membranes, and keratinization. *Int. J. Dermatol.* 20:1-19.
13. Williams, M. L., S. Grayson, J. M. Bonifas, E. H. Epstein, Jr., and P. M. Elias. 1983. Epidermal cholesterol sulfate and steroid sulfatase activity and recessive x-linked ichthyosis. In *The Stratum Corneum*. R. Marks and G. Plewig, editors. Springer-Verlag, New York. 79-85.
14. Epstein, E. H., Jr., M. L. Williams, and P. M. Elias. 1984. The epidermal cholesterol sulfate cycle. *J. Am. Acad. Dermatol.* 10:866-868.
15. Williams, M. L., S. Grayson, P. M. Elias, J. M. Bonifas, and E. H. Epstein, Jr. 1982. Localization of steroid sulfatase and cholesterol sulfate in normal and x-linked ichthyotic epidermis. *Clin. Res.* 30:161A. (Abstr.)
16. Maloney, M. E., M. L. Williams, M. Y. L. Law, and P. M. Elias. 1983. Lipids in pathogenesis of abnormal cornification: induction of scaling with topical cholesterol sulfate in hairless mice. *Clin. Res.* 31:585A. (Abstr.)
17. Elias, P. M., H. Mittermeyer, P. O. Fritsch, G. Tappeiner, and K. Wolff. 1974. Experimental staphylococcal toxic epidermal necrolysis in adult humans and mice. *J. Lab. Clin. Med.* 84:414-424.
18. Grayson, S., and P. M. Elias. 1982. Isolation and lipid biochemical characterization of stratum corneum cell membrane complexes: implications for the cutaneous permeability barrier. *J. Invest. Dermatol.* 78:128-135.
19. Epstein, E. H., Jr., and N. H. Munderloh. 1975. Isolation and characterization of CNBr peptides of human ( $\alpha 1$  (III))<sub>3</sub> collagen and tissue distribution of ( $\alpha 1$  (I))<sub>2</sub>  $\alpha 2$  and ( $\alpha 1$  (III))<sub>3</sub> collagens. *J. Biol. Chem.* 250:9304-9312.
20. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total



lipid extraction and purification. *Can. J. Biochem. Physiol.* 37:911-917.

21. Franey, R. J., and E. Amador. 1968. Serum cholesterol measurement based on ethanol extraction and ferrie chloride-sulfonic acid. *Clin. Chim. Acta.* 21:255-263.

22. Ackman, R. 1981. Flame ionization detection applied to thin layer chromatography on coated glass rods. *Methods Enzymol.* 72:205-252.

23. Brown, B. E., M. L. Williams, and P. M. Elias. 1984. Stratum corenum lipid abnormalities in ichthyosis: detection by a new lipid microanalytical method. *Arch. Dermatol.* 120:204-209.

24. Hochberg, R., S. Ladany, and S. Lieberman. 1974. Cholesterol sulfate: some aspects of its biosynthesis and uptake by tissues from blood. *Endocrinology.* 94:207-213.

25. Barbar, T., J. M. Bonifas, and E. H. Epstein, Jr. 1982. Detection

of cholesterol sulfotransferase in normal epidermis. *Clin. Res.* 30:575A. (Abstr.)

26. Elias, P. M., M. A. Lampe, J.-C. Chung, and M. L. Williams. 1983. Diazacholesterol-induced ichthyosis in the hairless mouse. I. Morphological, histochemical and lipid biochemical characterization of a new animal model. *Lab. Invest.* 48:565-577.

27. Lykkesfeldt, G., and H. Hoyer. 1983. Topical cholesterol treatment of recessive x-linked ichthyosis. *Lancet.* II:1337-1338.

28. Williams, M. L., M. Wiley, and P. M. Elias. 1983. Cholesterol sulfate inhibits cholesterol synthesis in cultured keratinocytes and fibroblasts. *Clin. Res.* 21:268A. (Abstr.)

29. Rehfeld, S. J., and P. M. Elias. 1982. Mammalian stratum corneum contains physiologic lipid thermal transitions. *J. Invest. Dermatol.* 79:1-3.