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Research Article

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Rapid Publications

Elevated *n*-Alkanes in Congenital Ichthyosiform Erythroderma

Phenotypic Differentiation of Two Types of Autosomal Recessive Ichthyosis

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Abstract. Previously considered to represent a single genetic disorder, autosomal recessive ichthyosis was examined in clinical and lipid biochemical studies of 18 patients with this condition and instead disclosed to be two distinct diseases. Six patients displayed clinical features of classical lamellar ichthyosis (LI), which is characterized by monomorphous features, including large, dark, platelike scales, severe ectropion, and a uniformly severe, unremitting course. 11 patients displayed clinical features of nonbullous congenital ichthyosiform erythroderma (CIE) characterized by fine white scales, prominent erythroderma, a milder course, and a variable prognosis. CIE could be separated biochemically from LI by the invariable presence of elevated quantities of *n*-alkanes in scale (CIE, 24.8±1.9% vs. LI, 7.2±1.6%, and normal, 6.5±0.9%), which suggested a primary disorder in neutral lipid metabolism. In light of the distinctive clinical features of each, these biochemical studies indicate that autosomal recessive ichthyosis comprises two distinct disorders.

Introduction

The ichthyoses represent a heterogeneous group of acquired and inherited scaling skin disorders which range in severity from the common, mild form, ichthyosis vulgaris, to several severely disfiguring, rarer forms. Based upon their inheritance patterns, clinical features, histopathology, and cell kinetic data, the primary ichthyoses usually are grouped into four major types (1, 2). Both of the dominantly inherited forms, ichthyosis vulgaris and epidermolytic hyperkeratosis (bullous congenital ichthyosiform erythroderma), can be diagnosed by their distinctive clinical and histopathological features, although their underlying metabolic abnormalities remain unknown. Recessive x-linked ichthyosis represents the only form in which the enzymatic defect, steroid sulfatase deficiency, has been identified (3). In contrast, autosomal recessive ichthyosis currently is diagnosed solely by clinical criteria (1, 2). Because patients with this form of ichthyosis exhibit a wide range of clinical involvement, some authorities have lumped this heterogeneous group into a single disorder, lamellar ichthyosis (LI) (1), whereas others recognize two forms, nonbullous congenital ichthyosiform erythroderma (CIE)[1] and LI (Table I) (2). We report here clinical and laboratory studies on 18 patients with autosomal recessive ichthyosis that support the existence of two biochemically distinct disorders.

Methods

Patient populations. Included in this study were 17 patients referred to the University of California Keratinization Clinic (San Francisco) for evaluation and therapy of autosomal recessive ichthyosis. All of the patients reported that the onset of disease had been either at birth or shortly thereafter and demonstrated some degree of erythroderma, generalized cutaneous involvement including the flexures, and an absence of histologic features of the other forms of ichthyosis (2). All patients were instructed to discontinue all topical medications or emollients from sites of scale collection (usually the back or a lower extremity) for at least 4 wk before sampling. Punch biopsy samples for lipid analysis of skin strata were split into separate epidermal layers by gentle trypsinization, as described previously (4).

Lipid biochemistry. Our procedures for lipid extraction and sequential, quantitative thin-layer chromatography (TLC) have been described elsewhere (4–6). To avoid hydrocarbon contamination during preparative

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1. *Abbreviations used in this paper:* CIE, nonbullous congenital ichthyosiform erythroderma; GLC, gas-liquid chromatography; LI, lamellar ichthyosis; TLC, thin-layer chromatography.

procedures we used only spectral-grade, twice-redistilled solvents, rinsed all glassware with solvent before use, and monitored our entire system by running blanks in parallel with clinical samples. When contaminants crept into the system they were quantitatively insignificant and readily differentiated by their characteristic profiles on gas-liquid chromatography (GLC) and GLC-mass spectrometry (5, 6). Commercial high performance TLC plates (Merck AG, Darmstadt, Federal Republic of Germany) were precleaned with chloroform/methanol/water/glacial acetic acid (6), and samples were then fractionated by one-dimensional TLC in petroleum ether/diethyl ether/glacial acetic acid (80:20:1 vol) followed by further separation of the most nonpolar species (sterol/wax esters, squalene, and *n*-alkanes) in petroleum ether alone (4–6). Bands were visible after being sprayed with 1% 8-anilino-1-naphthalene-sulfonic acid under black light. They were excised, and the lipids were extracted from the gel and weighed. Chain lengths of the hydrocarbon fraction were analyzed by glass capillary-GLC (5, 6). Specific compounds were positively identified by co-chromatography against known standards and by GLC-mass spectrometry (5, 6). Because of the small quantities of lipid present in stratified skin biopsy material, hydrocarbon content was determined after lipid extraction by flame-ionization detection (Iatroscan; Newman-Howell Associates, Winchester, England), as previously described (7).

Results

Clinical features of the two diseases. The distinguishing clinical features of the two groups of patients are summarized in Table I. Six patients, including one sibship, exhibited a uniformly severe disorder characterized by extensive, thick, dark platelike scales with severe facial involvement and ectropion but with a mild underlying erythroderma, which conformed with earlier descriptions of LI (1, 2). The remaining 11 patients, including one sibship, conformed with prior descriptions of CIE, displaying a variable erythroderma, fine, whiter scales, and variable ectropion (1, 2). Despite the variability in the CIE group, none of these patients demonstrated the thick, dark scales on the face and trunk that characterized the LI group (Table I).

Stratum corneum lipid biochemical abnormalities. Despite the wide variation in severity of the disease in the patients with CIE, all of them demonstrated a dramatic increase in the *n*-alkane content of scale (Fig. 1, *a* and *b*, Fig. 2, and Table II). This increase in *n*-alkanes was accompanied by a significant decrease in the free fatty acid/triglyceride fraction but not in other lipid fractions. The apparent decrease in the sterol/wax ester fraction shown in Fig. 1 *b* is due to a variable contribution of sebaceous gland lipid (wax ester) which depends on the age of the patient and the body site. When the sterol/wax ester fraction was hydrolyzed and rechromatographed (4), no significant differences were observed in the sterol ester fraction between normal subjects (4.7±0.6% total lipid; $n = 6$) and CIE patients (4.8±0.9% total lipids; $n = 7$). The chain-length distribution of alkanes in CIE scale was similar to that in normal scale: both exhibited a bell-shaped distribution of both odd and even chain length, with fully-saturated hydrocarbons ranging from C19 to C34, with a peak at C24–26 (Fig. 3) (5, 6). The prominent C28 peak in normal scale was demonstrated by mass spectrometry to represent squalene. The prominent long-chain peak has been identified as a 35:6 silicon-oil laboratory contaminant (6). The quantities of *n*-alkanes in CIE patients did not overlap with those in either normal subjects or in the six patients with LI (Fig. 2, Table II), reliably distinguishing CIE from LI. Whereas the CIE group displayed a striking increase in *n*-alkanes, the LI group instead displayed elevated sphingolipids and free sterols (Table II).

Table I. Distinguishing Clinical Features of LI and CIE

Feature	CIE	LI
Scale	Fine, white	Thick, dark, platelike
Erythroderma	Variable	Mild
Ectropion	Variable	Severe
Course	Variable	Unremitting

Studies to exclude exogenous origin of hydrocarbons. Several additional experiments were performed to exclude spurious sources of the *n*-alkanes in the CIE patients: (*a*) Laboratory blanks yielded quantities of *n*-alkanes that were too minute to account for the recovered weights. Moreover, contaminant hydrocarbons displayed qualitative differences from stratum corneum *n*-alkanes on gas-liquid chromatography (see above, reference 5). Moreover, when we extracted a small quantity (25 mg) vs. a larger quantity (120 mg) of scale in parallel from one patient and used identical quantities of solvents and glassware, the two samples demonstrated virtually identical total lipid and *n*-alkane content. (*b*) To control for airborne or topically applied contaminants we tape-stripped the arm of one patient to a moist, bleeding base, signifying the complete removal of stratum corneum. This area was then occluded with polyethelene wrap and left untreated with topical emollients, and the covering was removed only long enough for scale collection every 1–5 d over the next 3 wk. Since the alkane composition of fresh regenerated scale was remarkably constant during this period (Table III), it could not be attributed to either casual airborne or cross contamination from topical sources. (*c*) To exclude the surreptitious use of alkane-containing emollients, we compared the lipid content of scale from untreated sites vs. sites deliberately treated twice daily for 1 mo with high (Eucerin; Beiersdorf, Stamford, CT) and low (lanolin) alkane-containing emollients. Emollient use per se could be readily identified by the almost twofold increase in lipid content of stratum corneum (milligrams lipid per milligram scale [dry weight], 18.0±0.5 in emollient treated [$n = 7$] vs. 11.4±1.1 untreated [$n = 3$] [$P < 0.001$]). Since the total lipid content of CIE scale studied here did not exceed normal (Table II), exogenous medications are unlikely to be the source of the alkane abnormality. (*d*) Bacterial or spontaneous degradation of scale lipids was excluded because the *n*-alkane content of lipids obtained from aliquots of scale frozen in liquid nitrogen immediately vs. samples allowed to incubate at 37°C in 100% humidity for several days did not differ (5). Moreover, regular treatment of one site with the antibacterial

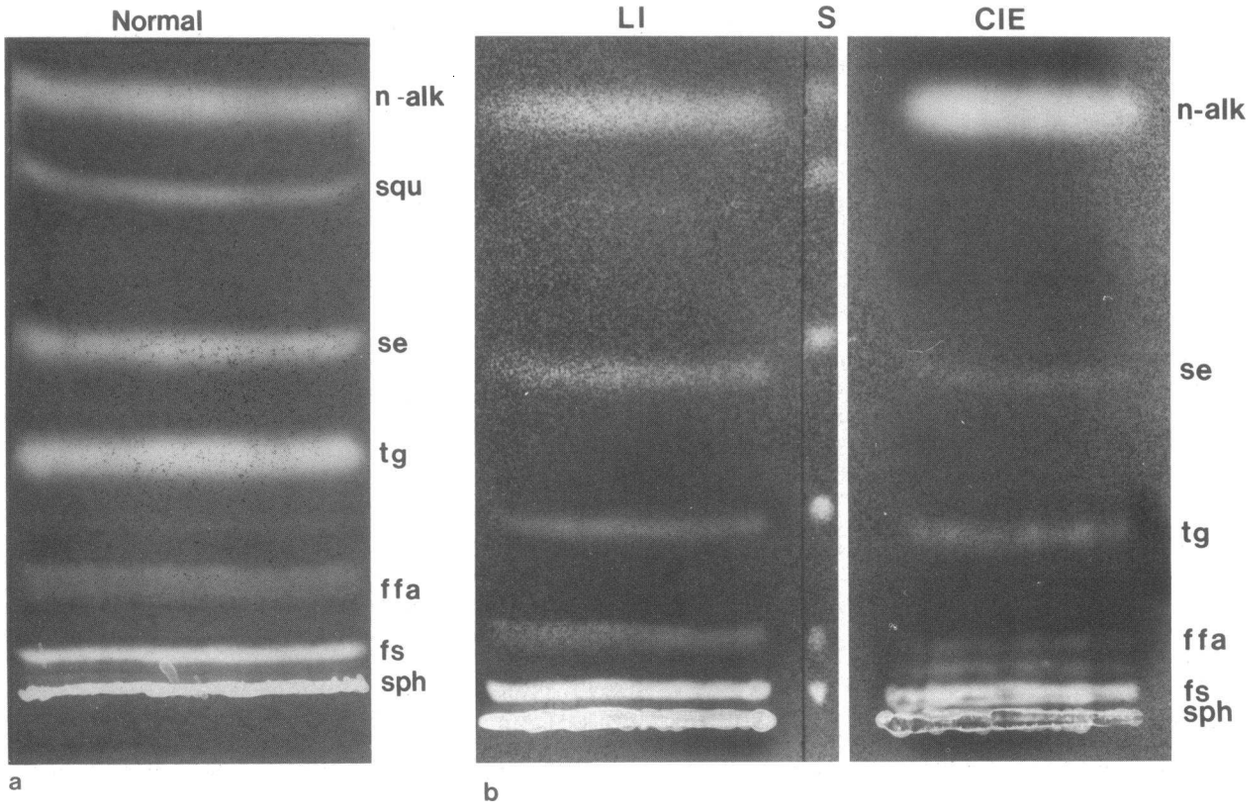

Figure 1. TLC profile of scale lipids from a normal subject (*a*), and from one patient with CIE and one patient with LI (*b*). Note the prominence of the *n*-alkanes (n-alk) vs. the relative decrease in triglycerides (tg) and free fatty acids (ffa) in CIE (compare Table II). The increased free sterol (fs), and sphingolipid (sph) species that characterize LI are not visualized well in this solvent system (see text). The prominence of the wax and sterol ester (se) band is not observed consistently and signifies primarily lipid of sebaceous gland origin (see text). squ, squalene. S, standards.

soap, Betadine (The Purdue Frederick Co., Norwalk, CT), resulted in an increase rather than a decrease in *n*-alkanes (*n*-alkanes: 30.5%, Betadine vs. 20.2%, control). (*e*) The scale *n*-alkanes were also not derived from sebaceous glands because preschool children, patients undergoing treatment with 13-cis retinoic acid, a drug that involutes sebaceous glands, and plantar surfaces devoid of sebaceous glands revealed comparable *n*-alkane contents (26.3, 31.1, and 18.7% of the total lipid, respectively). (*f*) In three CIE patients and four normal subjects, analysis of the *n*-alkane content of individual skin layers revealed *n*-alkanes elevated over normal in stratum corneum and stratum granulosum in CIE, with normal levels in lower epidermal levels (Table IV). This finding rules out not only casual surface contamination but also argues against extracutaneous origin of the *n*-alkanes (see below). The chain length distribution of stratum granulosum alkanes is identical to that of CIE stratum corneum (Fig. 3). (*g*) Finally, the CIE group also displayed a concurrent decrease in triglycerides and free fatty acids (Table II), which further suggests that the *n*-alkanes in CIE are locally generated and may derive from an abnormality in neutral lipid metabolism.

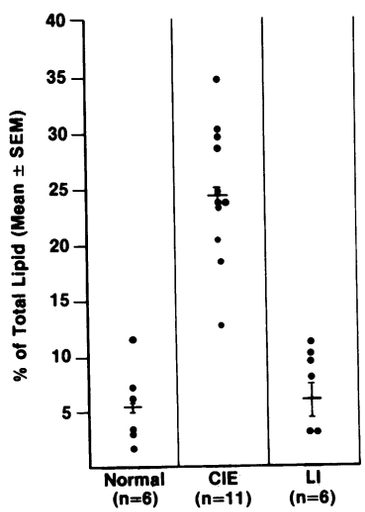

Figure 2. Scattergram of *n*-alkane content of scale from CIE, LI, and normal patients in this study. Note the uniform elevation in all patients with CIE and the lack of overlap with either normals or LI patients.

Discussion

Whereas we (2) and others (8, 9) have suspected the existence of two discrete forms of autosomal recessive ichthyosis based upon clinical features, histology, and prognosis, the absence of

Tc · II. Neutral Lipid Fractionation of Scale from Autosomal Recessive Ichthyosis Patients vs. Normal

	Ichthyosis			
	CIE (n = 11)	LI (n = 6)	Normal (n = 6)	Significance*
Total lipid (*mg lipid/mg scale [dry weight]*)	10.4±0.7	11.1±0.9	10.5±1.4	
Neutral lipids‡				
n-Alkanes	24.8±1.9*	7.2±1.6	5.5±0.2*	$P < 0.001$
Triglycerides plus free fatty acids	15.9±1.6*	25.4±2.1	29.5±2.4*	$P < 0.001$
Free sterols	17.7±1.5	23.6±1.3*	15.6±2.1*	$P < 0.01$
Sphingolipids‡	22.9±1.5	35.7±0.7*	26.0±2.2*	$P < 0.01$
Other lipids‡	18.7	8.1	22.4	

* Significance of difference between CIE or LI and normal. ‡ Percentage of total lipid.

laboratory criteria to distinguish these entities had led to the inclusion of CIE and LI under the diagnostic umbrella of lamellar ichthyosis (10). We now provide evidence that CIE and LI are separate disease entities that can be distinguished reliably by the regular presence of large amounts of *n*-alkanes in the stratum corneum of CIE. Indeed, the first 10 patients with autosomal recessive ichthyosis whom we studied exhibited the alkane abnormality and were reported as having lamellar ichthyosis (5), although they have since been reclassified as having CIE (2); nine of these are included in this report. The next eight patients studied included two with the alkane abnormality and six without. Correlation of the clinical features of these six patients with the lipid biochemistry convinced us that we were dealing with an important phenotypic marker of genetic heterogeneity. The fact that siblings within each group were clinically and biochemically consistent further supports this hypothesis. Because of our great concern that the large amounts of *n*-alkanes in the stratum corneum of CIE were of spurious origin, we performed several experiments that appear to have excluded this possibility. In future studies it also will be necessary to be equally vigilant to ensure that patients are not applying alkane-containing emollients, which are rich sources of alkanes (6).

Both the source of the *n*-alkanes in CIE and their potential role in disease production remain a mystery. Although we have excluded casual contamination, it is generally accepted that mammals do not synthesize these substances (11, 12). On the

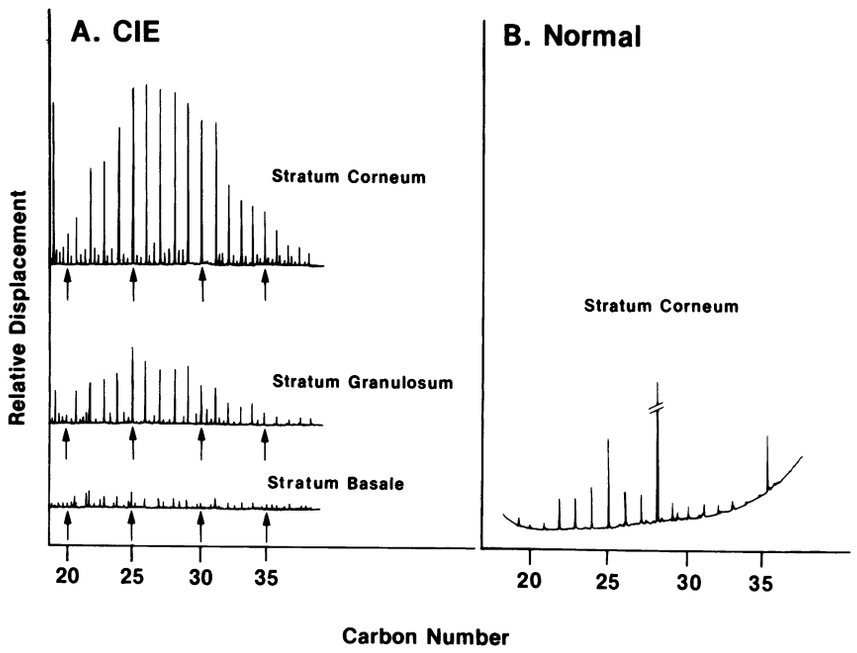

Figure 3. GLC profile of the hydrocarbon fractions from stratum corneum, stratum granulosum, and stratum basale in CIE (*A*) and of stratum corneum in normal (*B*). In CIE stratum corneum and stratum granulosum, a bell-shaped distribution of both odd and even chain length alkanes from C19 to C36 with a peak at C24–26 is demonstrated. Normal stratum corneum alkanes exhibit a similar pattern; the prominence of the C28 peak is due to incomplete TLC removal of squalene from the sample; the peak at C35:6 is attributed to laboratory contaminants (see text).

Table III. Alkane Content of CIE Scale After Tape-Stripping and Continuous Occlusion

Time	Scale	Alkanes
d	*mg*	%
1–2	3.3	31.5
2–4	4.7	27.5
5	3.3	29.4
6–7	5.8	25.4
8–9	19.0	25.9
10–13	16.6	23.5
14–15	2.3	29.9
16–17	2.0	20.7
18–23	2.2	35.5
24–25	3.5	36.0

An area of ~5 cm^2 was tape-stripped to a bleeding base on the arm of a CIE patient. The area was then left untreated and occluded with polyethylene wrap in between scale collections. Scales were weighed, lipids were extracted and weighed, and alkane content was determined by Iatroscan (see Methods). Alkane content is expressed as percentage of total lipid content.

other hand, recent work establishes the ability of mammals to catabolize exogenous alkanes via the cytochrome p450 system (12, 13). Therefore, another possibility might be that *n*-alkanes accumulate from the diet (12), with the high levels in CIE epidermis resulting from a catabolic error (i.e., a functional deficiency or the absence of a component of the cytochrome p450 system) (13). The hypothesis that *n*-alkanes accumulate in the skin from the blood, however, is not supported by the demonstration of near-normal alkane levels in the dermis and lower epidermis in CIE and by the normal content of *n*-alkanes in serum and erythrocytes (Williams, M. L., and P. M. Elias, submitted for publication). Indeed, the distribution of alkanes within CIE epidermal strata and the reciprocal decrease in the fatty acid/triglyceride fraction raises the possibility that these hydrocarbons are locally generated. Recent work that demonstrates alkane biosynthesis in mouse sciatic nerve cell-free preparations lends further support to this possibility (14). Regardless of their source, there is reason to believe that *n*-alkanes may play an important role in disease pathogenesis. Long-chain alkanes (e.g., hexadecane) are known irritants, producing a hyperplastic epidermis when applied topically to experimental animals (15). Thus, both the striking erythroderma and the hyperkeratosis found in CIE may result from the intracutaneous generation and deposition of alkanes, which would then function as endogenous irritants.

Table IV. Occurrence of n-Alkanes in Different Cutaneous Layers in CIE

	n-Alkane content (%)*			
Layer	Case 1	Case 2	Case 3	Normal subjects (±SEM)
Dermis	1.2	ND	2.6	ND
Epidermis				
Strata basale and spinosa	4.7	4.0	4.1	3.9±0.3
Stratum granulosum	10.4	9.8	14.0	3.8±0.8
Stratum corneum	29.6	11.9		6.1±2.6

ND, not done.
* Expressed as percentage of total lipid. Alkanes were determined by flame ionization detection (7).
‡ Reference 4. $n = 4$.

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