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N-ALKANES IN NORMAL AND PATHOLOGICAL HUMAN SCALE

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SUMMARY: When n-alkanes have been found in mammalian tissues, they have been considered to be solely of exogenous origin, and have not been assigned any normal or pathological function. We have observed n-alkanes regularly in normal stratum corneum (5.5 ± 0.2% total lipid), and found striking accumulations (> 25% total lipid) in some scaling diseases. Although the origin of these n-alkanes is not known, evidence is presented that they do not arise from external contaminants, medications, sebaceous glands, and spontaneous or bacterial degradation The presence of large quantities of n-alkanes in human stratum corneum suggests that they may play a role in normal human epidermal function and in the pathophysiology of some epidermal diseases.

In mammalian stratum corneum, lipids are segregated predominantly in the intercellular spaces where they form broad laminated sheets, accounting for 5-10% of the weight and 10-15% of the volume of this tissue (1). Composed predominantly of neutral lipids and ceramides (2,3), stratum corneum lipids play a central role in the maintenance of the barrier to transcutaneous water loss (2), and possibly in the control of both normal and pathological stratum corneum desquamation (4-7). Although hydrocarbons are found in small quantities in virtually all mammalian tissue (8), biosynthetic pathways for n-alkanes are not known to exist in mammals. When they have been encountered in mammalian skin surface lipids, they have been considered to arise from exogenous sources (9-11). During our studies on the role of lipids in human disorders of keratinization, we found unexpectedly large quantitites of n-alkanes in the scale of all patients with lamellar ichthyosis, a recessively inherited scaling skin disorder, in some patients with other disorders of cornification, and lesser, but significant, levels in normals.

METHODS: Scale was collected from skin sites, left untreated with topical medications for a minimum of 4 weeks, from patients with: lamellar ichthyosis (n=10), epidermolytic hyperkeratosis (n=5), and psoriasis (n=4). Normal control

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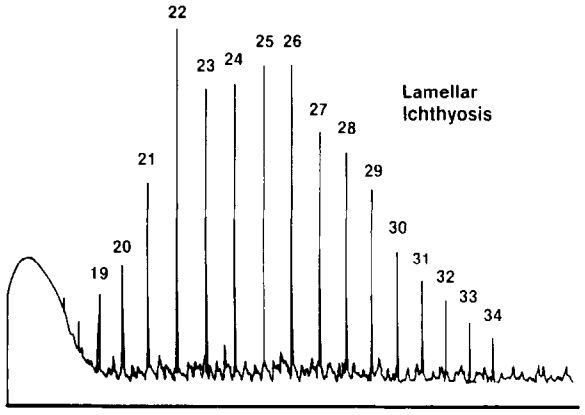

Figure: Gas-liquid chromatograph of n-alkane fraction of lipids extracted from scale from a patient with lamellar ichthyosis. Note the bell-shaped distribution from C19 to C34 with equal contribution by both odd and even chain lengths. The identical pattern, but with lesser quantities, is seen in normal n-alkanes.

scale was obtained post-sunburn (n=4) and following orthopedic cast removal (n=2). In addition, erythrocyte ghosts and/or serum were obtained from two normals and from four patients with lamellar ichthyosis. Details of our methods for lipid extraction and quantitative thin layer chromatography (TLC) have been reported previously (3). To avoid hydrocarbon contamination during preparative procedures we used only spectral grade reagents, pre-rinsed all glassware with chloroform:methanol (1:1), and periodically monitored the system by running solvent blanks in parallel with lipid samples through the entire sequence of extraction and chromatography. Commercial high-performance TLC plates (Merck, Darmstadt) were precleaned with chloroform:methanol:water:glacial acetic acid (60:35:4.5:0.5, vols). Fractionation of stratum corneum lipids in petroleum ether:diethyl ether:glacial acetic acid (80:20:1, vols) by one dimensional TLC results in a single band migrating just behind the solvent front that contains both sterol and wax esters, n-alkanes, and squalene. These fractions were separated further by TLC in petroleum ether alone in in which sterol and wax esters remain at the origin, and squalene and n-alkanes form distinct bands. The chain lengths of the hydrocarbons were determined by glass capillary-gas liquid chromatography (GLC), and their identities established both by co-chromatography against known standards and by glass capillary GLC-mass spectrometry.

RESULTS AND DISCUSSION: The total lipid content of pathologic and normal scale did not differ (Table I). In both normal and pathological scale lipids, n-alkanes displayed a bell-shaped distribution of both odd-and even-chained material from C19 to C33 (Figure). Whereas n-alkanes represented 5.5 $\pm$ 0.2% of the total lipid in normals, in pathological scale this fraction represented as much as 35% of the total lipid. Although the most consistently observed increase in n-alkanes occurred in scale from lamellar ichthyosis (24.9 $\pm$ 0.7%, $p<0.001$), some patients with the other hyperproliferative scaling disorders also exhibited increased n-alkanes (Table I). Pathological scale samples that were high in n-alkanes

TABLE I: TOTAL LIPIDS AND N-ALKANES IN NORMAL AND PATHOLOGICAL OUTER STRATUM CORNEUM

Condition	% Total Lipid* (Mean ±-SEM)	% n-Alkanes of Total Lipid (Mean ± SEM)	P**
Normal (n=6)	10.5 ± 1.4	5.5 ± 0.2	-
Lamellar ichthyosis (n=10)	10.7 ± 0.6	24.9 ± 0.7	< 0.001
Epidermolytic hyperkeratosis (n=5)	11.0 ± 0.9	20.3 ± 8.0	> 0.05
Psoriasis (n=4)	10.1 ± 0.9	7.8 ± 4.5	> 0.1

*Lipid weight recovery/dry weight of scale.

**Two-tailed Student's test.

exhibited decreased triglyceride content (Table II), suggesting that esterified fatty acids may be precursors for scale n-alkanes . Other epidermal lipid fractions were unchanged. Despite the high levels of n-alkanes found in the scale of lamellar ichthyosis, the quantities found in serum and erythrocyte membrane preparations of these patients did not differ from normal (data not presented). This indicates that the increased n-alkane content of pathological epidermis does not only reflect circulating levels.

In view of the widespread occurrence of hydrocarbons in the environment, several additional experiments were performed to exclude spurious sources of these n-alkanes: 1) Laboratory blanks yielded quantities of n-alkanes that were too minute to account for the recovered weights. Moreover, they displayed qualitative differences from stratum corneum n-alkanes on gas liquid chromatography, with a predominance of shorter chain lengths and prominent peaks at C22 and/or C35:6; 2). As an additional check on laboratory-introduced hydrocarbons, we extracted a small quantity (25 mg) and a larger quantity (120 mg) of scale from one patient in parallel using identical quantities of solvents and glassware. Exogenously introduced hydrocarbons would have resulted in greater lipid weight recovery (mg lipid/mg scale) and a higher proportion of n-alkanes (% total lipid) from the smaller sample. However, the two samples demonstrated virtually identical total lipid and n-alkane content. 3) To consider the possible contributior

TABLE II: WEIGHT DISTRIBUTION OF MAJOR LIPID SPECIES IN NORMAL VS PATHOLOGICAL SCALE IN HIGH IN N-ALKANES*

FRACTION	NORMAL (n=6)	PATHOLOGICAL (n=15)	SIGNIFICANCE*
n-Alkanes	5.4 ± 0.8	26.0 ± 1.9	p<0.001
Free Fatty Acids	15.0 ± 1.2	8.5 ± 1.3	N.S.
Triglycerides	11.6 ± 1.1	7.5 ± 0.8	p<0.05
Free Sterols	15.4 ± 1.0	15.7 ± 1.4	N.S.
Sterol Esters	4.7 ± 0.6	4.8 ± 0.9	N.S.
Wax Esters	8.3 ± 1.6	4.1 ± 2.5+	N.S.
Sphingolipids	25.5 ± 1.0	23.2 ± 1.3	N.S.

*Expressed as % total lipid; two-tailed Student's test

+(n=7)

of topical medications to skin lipids, we analyzed four commonly used emollient-vehicles (Eucerin® cream, petrolatum, anhydrous lanolin, and H-E-B® ointment). Two of these, Eucerin® and petrolatum, contained large quantities of n-alkanes of virtually the same chain lengths and bell-shaped distribution as that found in skin lipids. Yet, the present finding of increased n-alkanes in pathological scale probably cannot be explained by retention of n-alkanes derived from topical medications, since our patients were carefully instructed to discontinue all topical medications and emollients for at least four weeks prior to scale collection. This precaution should have been more than adequate since the time for epidermal transit in normal human skin is 12 to 14 days, and is accelerated to 4 to 5 days in lamellar ichthyosis (12). 4) To exclude surreptitious use of emollients containing n-alkanes, we compared the lipid content of scale from untreated sites vs. sites deliberately treated with emollients twice daily for one month. Emollient use could be readily identified by the two-fold increase in lipid content of stratum corneum (lipid weight percent:18.0 ± 0.5 mg% emollient treated (n=7) vs. 11.4 ± 1.1 mg% untreated (n=3)(p <0.001). Since the lipid weight percent of pathological scale that is high in n-alkanes did not exceed normal in this study, exogenous medications are unlikely to be the source. Furthermore, if n-alkanes are endogenous, then the application of an emolient

TABLE III: LIPID CONTENT OF LAMELLAR ICHTHYOSIS SCALE UNDER DIFFERENT CONDITIONS OF TREATMENT

	CONTROL	EUCERIN®*	LANOLIN*
% Total Lipid (mg lipid/mg dry scale)	9.5%	17.7%	17.0%
% change	--	+86.3%	+78.9%
% n-alkanes of total lipid	20.2	41.1%	6.7%
% change	--	+103.5%	-66.8%

containing solely n-alkanes (Eucerin®) to one site and one containing all human skin lipids except n-alkanes (lanolin) to another site should increase the relative content of n-alkanes in the first instance and reduce, but not abolish them in the second instance. The results in Table III confirm this prediction. 5) Furthermore, bacterial or spontaneous degradation of scale lipids during the period of time between scale collection and lipid extraction was excluded as an exogenous source, because the n-alkane contents of lipids obtained from aliquots of the same scale sample frozen in liquid nitrogen immediately against portions allowed to incubate at either room temperature or at 37°C in 100% humidity for several days prior to extraction did not differ. Moreover, treatment with an antibacterial soap, Betadine® resulted in a slight overall decrease in lipid content (weight % lipid:7.8% Betadine® vs. 9.5% control), with a relative increase in n-alkanes probably (n-alkanes:30.5% Betadine® vs. 20.2% control) due to extraction of more soluble stratum corneum lipids (Betadine® itself does not contain n-alkanes). The failure to diminish n-alkanes with Betadine® treatment provides further evidence against a bacterial sources for n-alkanes in the pathological scale.

n-Alkanes are ubiquitous in nature and are distributed widely throughout mammalian tissues (8). Although previously believed to be neither synthesized nor catabolized by mammals, it is now clear that hydrocarbons, including n-alkanes, can be catabolized to some extent via the cytochrome p-450 system (8,13). Moreover, some preliminary evidence exists for mammalian synthesis of n-alkanes (14).

Prior work on skin n-alkanes has been restricted to analysis of skin surface lipids that derive predominantly from sebaceous glands. In these studies, n-alkanes comprised less than 2% of the total lipid, but possessed a similar chromatographic profile to scale n-alkanes (3,15). The possibility that scale n-alkanes are predominantly of sebaceous gland origin was excluded by lipid analysis of lamellar ichthyosis scale in circumstances where the sebaceous gland contribution should be negligible: 1) preschool children; 2) patients undergoing treatment with 13-cis retinoic acid, a drug that involutes sebaceous glands (16); and 3) plantar surfaces devoid of sebaceous glands. The mean n-alkane content of scale in these instances was 26.3%, 31.1%, and 18.7% of the total lipid, respectively. Further evidence for endogenous origin comes from two sources: first, we have recently found significant quantities of n-alkanes throughout the epidermis in normal human skin (17); and second, the inverse relationship between n-alkanes and triglycerides in pathological scale (Table II) suggests a metabolic interrelationship for these substances.

The observation of n-alkanes in normal and pathological human scale has important implications. First, as normal epidermal constituents, n-alkanes may play a functional role in stratum corneum. This possibility is intriguing in view of the established role for hydrocarbons in plant and insect cuticular barrier function (18,19). Second, n-alkanes have not been previously associated with human disease. The regular occurrence of increased n-alkanes in lamellar ichthyosis stratum corneum, may provide both an important clue into the underlying metabolic defect in this disorder, as well as into the role of lipids in modulating normal and abnormal desquamation, in general. One possible link between the elevated n-alkanes and the rapid epidermal turnover in lamellar ichthyosis (12), is the known capacity for these substances to induce hyper-proliferation after topical application (19).

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