Characterization of Low-Temperature (i.e., <65°C) Lipid Transitions in Human Stratum Corneum

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This study aims to characterize human stratum corneum (SC), focusing on those lipid transitions that occur at or below physiologically relevant temperatures. In the past, a lipid transition near 35°C had been thought to be variable and a consequence of superficial sebaceous lipid contamination. However, analysis here indicates that it is widely present, and cannot be attributed to sebum production. We demonstrate that this transition represents a solid-to-fluid phase change for a discrete subset of SC lipids. The reversibility of this transition upon reheating, and its absence in extracted lipid samples imply that these lipids are not uniformly present throughout the SC, but would appear to be differentially distributed in response to terminal differentiation. Further, such an arrangement could involve a close association with other nonlipid (e.g., protein) components. Evidence for a new transition at ~55°C is presented that suggests the loss of crystalline orthorhombic lattice structure. The existence of orthorhombic structure at physiologic temperature is reasoned to involve ceramides and/or free fatty acids. Localization of these lipids at the level of the corneocyte envelope supports a comprehensive picture of water transport across the SC, whereby diffusion occurs primarily via the intercellular lipids. This view, coupled with the hydration-induced changes in lipid disorder observed here provides additional insight into the mechanism by which skin occlusion increases permeability. Summarily, these results i) emphasize the inherent danger of over-interpreting experiments with isolated SC lipids, ii) emphasize the potential advantage(s) of employing several biophysical techniques to study SC structure, and iii) indicate that a full characterization of lipid phase behavior is requisite to our eventual understanding of SC structure and permeability function, particularly those phase transitions that occur near or at normal skin temperature. *Key words: sebum/phase-segregation/lateral-packing/spectroscopy. J Invest Dermatol 103:233–239, 1994*

The permeability properties of the skin are largely governed by its outermost layer, the stratum corneum (SC). The SC consists of keratinized epithelial cells (corneocytes), physically isolated from one another by extracellular lipids. Considerable evidence now exists to show that these lipids, which represent the only continuous structure connecting the external and internal surfaces of SC, constitute the primary route for the passive permeation of many molecules through the skin [1–4]. In the case of water transport, the conformational disorder of the extracellular lipids is directly correlated with SC permeability [5]. This apparent preference for the more convoluted intercellular route is believed to be due, in part, to the relatively low water permeability of the corneocyte lipid envelope [6]. Further, oleic acid (a well-established penetration enhancer) selectively affects only the lipid and not the protein conformational structure of SC [7,8]. All these findings confirm the view that SC lipids are essential to the barrier properties of the skin. It is imperative, therefore, to characterize fully the conformational phase properties of the lipids, as they relate to the integrated structure and function of SC, to facilitate and, ultimately, optimize the delivery of topically applied drugs.

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Abbreviations: DSC, differential scanning microcalorimetry; IR, Fourier transform infrared spectroscopy; OR, orthorhombic; RH, relative humidity; SC, stratum corneum.

Microcalorimetric analysis indicates that, overall, human SC exhibits four endothermic transitions at approximately 35, 65, 80, and 95°C [9,10]. The first three are attributed to lipid-associated conformational changes; the 95°C transition is irreversible, and is believed to reflect protein structural changes within the corneocyte. The presence of the low-temperature transition at 35°C has been described as variable, and is often suggested to originate from sebaceous lipids [10]. Alternatively, x-ray diffraction experiments have suggested that this transition is correlated with changes in the crystal lattice packing of SC lipids [11–13]. Here, we describe a study focusing specifically on just those phase transitions occurring below 65°C. Three major questions are addressed. i) How variable is the appearance of the 35°C transition and to what extent is it affected by hydration? ii) Does this low-temperature transition originate from sebaceous lipids? iii) How does the lattice packing of the alkyl chains of SC lipids vary as a function of temperature and hydration?

MATERIALS AND METHODS

Stratum Corneum Sheets of SC were prepared from human cadaver skin that had been removed from the thigh with a dermatome. The epidermis-dermis sections, which had a nominal thickness of ~350 μm, were placed on filter paper saturated with a 0.5% trypsin (type II, Sigma Chemical Company, St. Louis, MO) in phosphate-buffered saline (pH 7.4). After incubation for approximately 1 h at 37°C, the SC was separated from the underlying tissue, washed with agitation in distilled water, and spread on a wire mesh to dry before storage under a N_2 atmosphere in a desiccator. Prior to use, SC samples were rinsed briefly in cold hexane (5°C) to remove superficial lipids, dry weighed on a microbalance, and subsequently equilibrated to the desired hydration level in an atmosphere of constant relative humidity

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Table I. Composition[a] of the Model Sebum Lipid Mixture

Lipid	Percent (By Weight)
Triglyceride (trioelin)	41.0
Wax ester (oleyl oleate)	25.0
Fatty acid (palmitic acid)	18.0
Cholesterol	1.5
Cholesterol ester (cholesterol oleate)	2.0
Squalene	12.5

[a] Adapted from [19–21].

(RH). For the differential scanning calorimetry (DSC) and infrared spectroscopy (IR) (CH_2 scissoring band only) experiments, the various samples were hydrated with H_2O. However, for those IR studies that involved analysis of the CH_2 stretching region, D_2O was employed to eliminate spectral perturbation from water-related bands.

Extracted SC Lipids Lipids from the SC were isolated by extraction with a series of chloroform-methanol mixtures according to the method described by Wertz *et al* [13]. The different solvent fractions were combined and evaporated to dryness under N_2. The extracted lipid residue was placed under a vacuum (~63 cm Hg) until a constant weight was achieved, and then stored at −20°C until needed. For DSC and IR analyses, the extracted SC lipids were processed by a method similar to that utilized by White *et al* [14]. A tared lipid residue was first redissolved in an appropriate volume of chloroform-methanol (2:1), and dried under N_2 to a thin film. This film was dispersed in sufficient distilled water to give a final total lipid concentration of 1 mg/ml. This suspension was exposed to several heating/cooling cycles (up to 80°C) to obtain a uniform dispersion. To prepare the sample for IR, 300 μl of this dispersion were deposited on the ZnS window and partially dried in a $CaSO_4$ desiccator. Subsequently, the lipid film was equilibrated to the desired hydration level using either D_2O or H_2O as indicated above.

Sebaceous Lipids A mixture of the different lipid classes typically observed in sebum (Table I) was dissolved in chloroform-methanol (2:1). The individual lipids, including triolein, oleyl oleate, palmitic acid, and cholesterol oleate, were purchased from Sigma Chemical Company (St. Louis, MO). A small volume of this solution was layered onto the apical surface of SC and allowed to evaporate to dryness prior to placement in a chamber maintained at 85% RH. The amount of total sebaceous lipid added to the surface was such that it represented 1% of the initial SC dry weight. Following equilibration to the desired hydration level, the SC was examined by DSC.

Infrared Spectroscopy Infrared spectra were obtained using a Nicolet Fourier transform instrument (Nicolet Instruments, Inc., Madison, WI) equipped with a liquid N_2-cooled MCT-B detector. The samples were sandwiched between two ZnS windows mounted in a specially designed heating/cooling cell. This cell was connected to a low-temperature water circulator containing a 50:50 mixture of ethylene glycol and water. The water bath sample temperature was controlled by a separate computer interfaced to the Nicolet workstation. Using a thermocouple, which was in direct contact with the sample to monitor the temperature, spectra were collected at 2 (±0.1)°C increments, at a rate of 20°C/h. For each temperature, 200 scans were co-added and transformed with a Happ-Genzel apodization function to yield a final resolution of 2 cm^{-1}. The frequency peak position of the CH_2 stretching band was determined using a previously described and validated [15] center of gravity algorithm to reduce the uncertainty to less than 0.02 cm^{-1}. Individual bands in the CH_2 scissoring region were monitored by sequentially subtracting each spectrum from the preceding one (which was obtained at a lower temperature). In the latter case, the frequencies of the ensuing peaks from the CH_2 scissoring region were determined with Nicolet software, which uses a polynomial least-squares method to provide a precision of better than 0.1 cm^{-1} [16].

Differential Scanning Calorimetry Sections of prehydrated and/or pretreated SC weighing 15–25 mg were scanned with an ultrasensitive MC-2 calorimeter (Microcal, Amherst, MA) from 10 to 110°C, at 36°C/h. This instrument has an inherent sensitivity of greater than 0.05 mcal/min, which translates to an effective sensitivity of ~0.1 kcal/mol for SC lipids. Changes in heat capacity were measured at digital increments every 0.05°C, and the transition temperatures were determined graphically from computer-generated plots of the resulting data.

High-Performance Thin-Layer Chromatography Precoated silica gel high-performance thin-layer chromatographic (HP-TLC) plates, 10 × 20 cm (E. Merck, Darmstadt, Germany) were prewashed by submersion in methanol for 5 min, and then dried at 100°C for 15 min before use. SC lipids (200 μg) or standards (2 μg) were applied in 5 μl of chloroform:methanol (2:1). Sequential development of the plates was used to achieve separation of all lipid classes. The chromatographic protocol was modified from the method of Hedberg *et al* [17]: i) hexane:toluene (1:1, to 17 cm), and ii) hexane-diethyl ether-glacial acetic acid (70:30:1, twice to 10 cm). Finally, visualization was accomplished by spraying with 10% (w/v) cupric sulfate in 8% phoshoric acid, and heating to 160°C in a vacuum oven for 10 min.

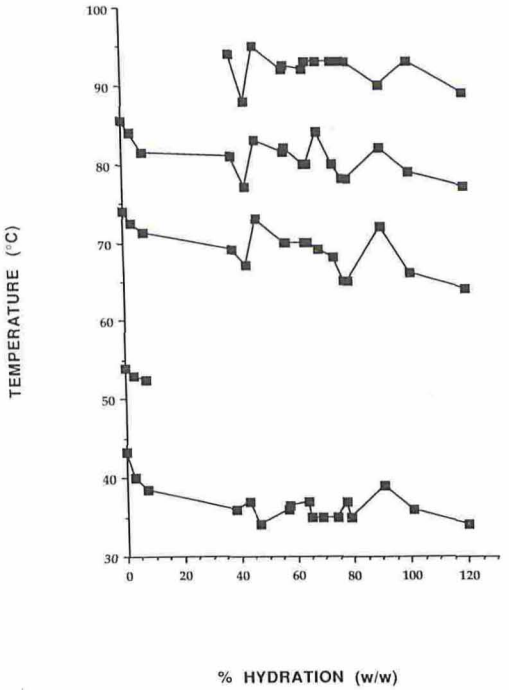

Figure 1. Calorimetric transition temperatures for the different endotherms measured for human SC as a function of hydration. Each data point represents an individual sample hydrated to the indicated level.

RESULTS

The effect of hydration on the transition temperature of the 35°C endotherm of human SC (together with the higher temperature peaks) as measured by differential scanning microcalorimetry (DSC) is shown in Fig 1. At hydration states above ~20% (g water/g dry SC × 100), the transition temperature was essentially constant with an average value of 35.9°C (n = 12, SE = 0.5). Upon reheating (data not shown), the mean temperature was slightly lower (34.2°C), but was not statistically different ($\alpha = 0.05$). As the SC was dehydrated below 20%, the temperature of the lowest transition progressively increased, reaching 43°C in the dry state. This dependence of transition temperature upon hydration agrees with that described previously for human SC [9]. Based on the lack of interaction with urea and its thermal reversibility, van Duzee deduced this rapid change in the SC excess heat capacity at 35°C to be lipid-based in origin. This assignment is totally consistent with the hydration-induced decrease and leveling behavior found for transitions from other *in vitro* lipid-water systems [18]. However, contrary to earlier reports for human SC [10], this transition was observed in nearly all of the individual specimens that were examined (17 of 19); and, although the area under this peak was substantially less than the higher temperature lipid transitions, it nevertheless accounted for 7–10% of the total measured enthalpy associated with the four peaks at 35, 55, 65, and 80°C.

Figure 2 presents DSC profiles of human SC from five independent donors. Scans $2a_i$ (dry) and $2a_{ii}$ (hydrated at 95% RH) were samples prepared from the same skin; scans $2b$–e (hydrated at 95–100% RH) were from four different sources. In $2a_i$, a previously

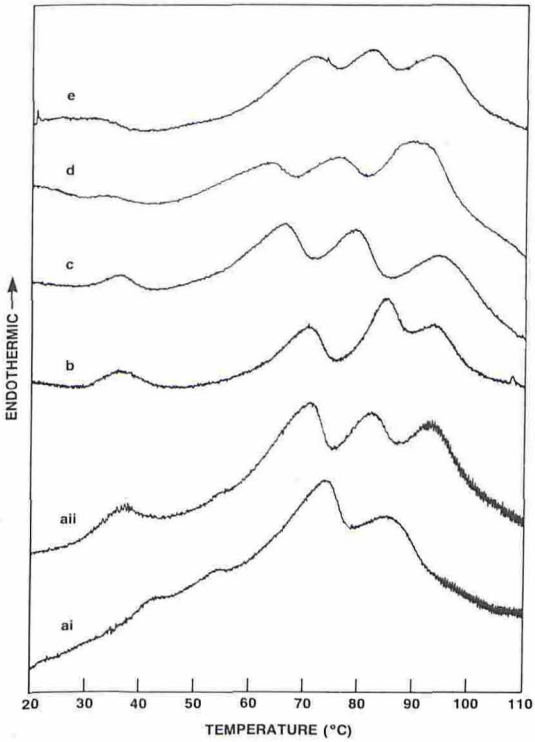

Figure 2. Representative differential calorimetric profiles of five independent human SC samples. Scans $2a_i$ and $2a_{ii}$ were from the same human source at different hydration levels (a_i dry, a_{ii} hydrated). Samples $2b-e$ (hydrated) were from different sources.

undetected transition was observed at ~55°C that persisted with reheating (data not shown). This small endothermic peak, however, was partially obscured after hydration ($2a_{ii}$), and was not explicit in the other four hydrated samples ($2b-e$) representing, at most, only a small shoulder on the much larger lipid endotherm at 70°C. In general, observation of a transition near 55°C by DSC was quite variable occuring in one-fifth of the samples examined, and at low hydration close to the sensitivity limit of the instrument. An analogous DSC peak near 50°C has also been detected in our laboratory using porcine SC at hydration levels less than 17% (unpublished data). Although the calorimetric data are equivocal, results obtained by IR (discussed below) corroborated its existence.

Figure 3 shows the calorimetric profile of human SC before and after treatment with a sebum-like lipid mixture (for composition, see Table I). The amount of sebum lipid added was 1% of the dry SC weight, corresponding to ~10% of the endogenous SC total lipid weight. The initial scans (3*a*, control; 3*c*, treated) revealed a small (1°–2°C) increase in the temperatures of the 37 and 68°C endotherms, with no effect on the 80°C peak. The transition at 80°C, however, did appear to be sharpened following the addition of the model sebum mixture. With treatment, there was no significant change in the enthalpies of the endotherms at 37, 68, and 80°C. Upon reheating (3*b,d,* control and treated, respectively), the 68° and 80°C peaks coalesced to a single endotherm at 63–65°C, whereas the 37°C transition remained with no change in its transition temperature. Further, for the treated SC, the transition temperature, apparent cooperativity, and enthalpy of the single peak near 65°C were slightly increased. These results suggest that the addition of sebum lipids did not have a discernible effect on the lowest temperature transition, even after reheating, which should have further facilitated the incorporation (and any impact) of the lipid material.

Figure 4 illustrates the relative lipid composition of human SC, as determined by HP-TLC, and includes standards that are representative of human sebum. Squalene, in particular, is an excellent marker

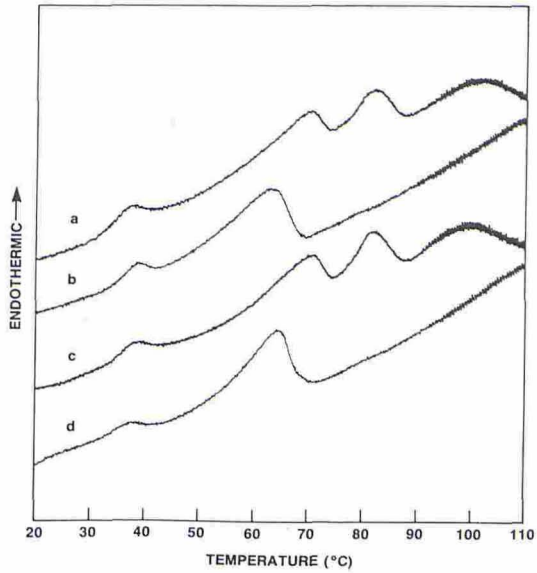

Figure 3. DSC scans of human SC treated with the sebum mixture identified in Table I. Tracings 3*a,c* are the first heats of the control and treated samples, respectively; 3*b,d* are the results upon reheating.

of sebaceous lipids given its largely invariant presence in sebum samples, independent of either the anatomical site or age of the donor [19,20]. The relative quantities of the standards and SC lipid loaded onto the HP-TLC plate were such that the ensuing visual development allowed squalene and wax ester(s) to be detected at a sensitivity of less than 1% of the unknown SC lipid sample. In other words, the lack of detectable bands corresponding to the standards

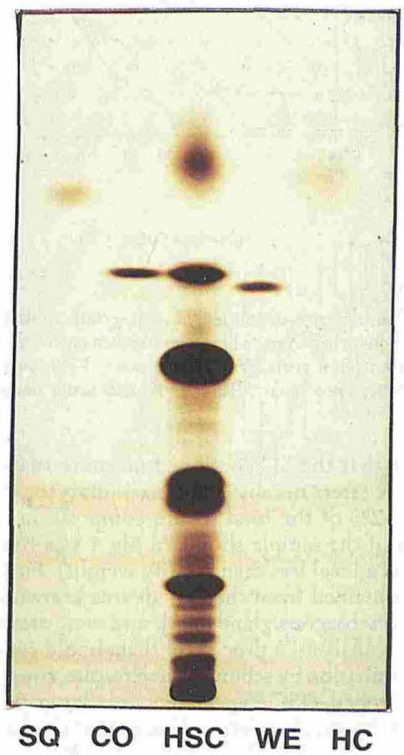

Figure 4. Composition of human SC nonpolar lipids compared to sebaceous lipid standards (i.e., squalene and wax esters), as determined by HP-TLC. SQ, squalene; CO, cholesterol oleate; HSC, human stratum corneum extracted lipids; WE, wax esters; HC, hydrocarbons.

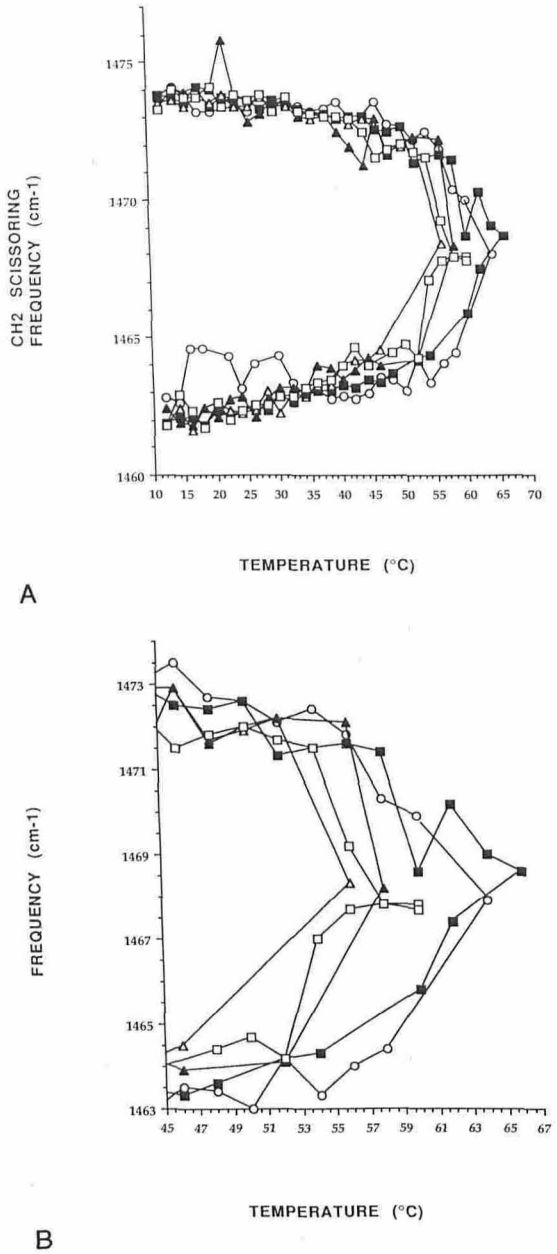

Figure 5. *A*, Temperature-dependent, factor group splitting of the infrared-active, CH_2 scissoring mode of human stratum corneum as a function of different hydration (*open circle*, 0%; *closed square*, 17%; *open triangle*, 33%; *closed triangle*, 70%; *open square*, 300%). *B*, the same data plotted on an expanded scale.

would indicate that the SC contained no more than 1% of either squalene or wax ester. Because these two lipids together generally comprise 32–42% of the total sebum composition [21], it can be concluded that if the sample shown in Fig 4 was contaminated by sebum, it was at a level less than 2% (by weight). Further, these SC samples were obtained from the leg, an area known to be sparsely populated with sebaceous glands [22], and were washed with cold hexane prior to analysis, a procedure that should remove most superficial contamination by sebum. These results, combined with the previously described DSC experiment involving pretreatment of SC with sebum lipids, therefore indicated that the 35°C lipid transition cannot be simplistically attributed to sebum contamination.

The temperature-dependent, IR factor group splitting of the alkyl chain CH_2 scissoring mode of human SC, as a function of hydration, is shown in Fig 5. Two distinct bands (1462 and 1473 cm^{-1}) were observed from 10–45°C, before their rapid collapse to a single value (1468 cm^{-1}) between 55–65°C. Notably, this change (as viewed in the rescaled Fig 5*B*) occurred at progressively lower temperatures as the hydration state of the SC was increased. The IR measurement of the CH_2 vibrations from the scissoring region provides an extremely sensitive means of monitoring qualitative changes in the lattice packing of alkyl lipids [23,24]. Factor group splitting of the CH_2 band at 1468 cm^{-1} is indicative of a tightly-packed, orthorhombic (OR) crystalline structure, in contrast to the loosely associated molecular arrangement usually found in the gel phase lipids that primarily accompany hexagonal subcells.

The pattern of the factor group splitting observed for human SC indicated that a fraction of the intercellular lipids was aligned in OR subcells. Although such splitting in this IR spectral region is quite representative of OR structure in the SC, it is not possible, at this time, to quantitate the percentage of the total lipids that are configured in this crystalline state. Nonetheless, the rate at which the two bands from SC approached each other, and the initial degree of separation, suggested that alkyl chains longer than C_{22} were involved [23]. Independent evidence for the existence of OR structures has been found with x-ray diffraction using hairless mouse [14], neonatal rat [12], and human SC [11,25], in addition to recent IR analyses of porcine SC§ [26]. Based on the IR data reported here for human SC, the OR structure rapidly disappeared between 45–65°C, prior to the main lipid thermal transitions. This temperature range did not coincide with the 35°C endotherm, but did approximate the apparent lipid transition observed by DSC at 55°C. Consequently, the thermally induced alteration in the lateral packing of the SC lipids observed by IR argues that the calorimetric transition at 55°C was not artifactual, and that lattice changes were not linked to the 35°C transition. This latter conclusion was supported by additional IR data from the CH_2 stretching region (see below), which revealed no significant change in frequency at 55°C, a characteristic of lipid systems manifesting OR subcells [24].

Shown in Fig 6 are the thermally induced (10–60°C) changes in the CH_2 stretching frequency of intact human SC plotted as a function of hydration (6*A*, single SC donor) and reheating (6*B*, n = 4 independent SC donors). Corresponding reheat data (n = 3 independent SC donors) for the extracted lipid samples are depicted in Fig 7. For SC, the frequency increased with temperature and showed a small, but definite, inflection point between 35° and 45°C. Under dry conditions, the midpoint was estimated to be 45°C, decreasing to a constant value of ~35°C as the hydration level was increased. This behavior closely mimicked the calorimetric results described above and elsewhere [9,10] suggesting i) that the 35°C inflection observed by IR corresponded to the same endothermic process measured by DSC, and ii) that this transition is not related to lattice packing variations (which would not produce measureable changes in the CH_2 stretching frequency [24]). With SC obtained from the same individual source, the frequency was increased by 0.3 cm^{-1} as the hydration was varied from 23 to 300% (w/w) at 32°C, which is close to physiologic. However, this relatively small change was obfuscated by intersample variation when the data were averaged. Unlike the results for the intact SC, no definitive evidence of a thermal transition at 35°C (Fig 7) was observed by IR in the lipids isolated from SC by chloroform : methanol extraction.

The CH_2 symmetric stretching frequency is sensitive to trans/gauche isomerization along the alkyl chain [27], and has been used to monitor gel-to-liquid crystalline transformations in phospholipids [28] as well as the main lipid transitions in SC [29]. The frequency assumes a characteristic value dependent upon the conformational state of the lipid alkyl chains. In homogenous phospholipid systems, for example, values between 2848 and 2850 cm^{-1} are representative of a hexagonal-type gel bilayer structure, whereas

§ Ongpipattanakul B, Francoeur ML, Potts RO: Polymorphism in stratum corneum lipids. *Biochim Biophys Acta* 1190:115–122, 1994.

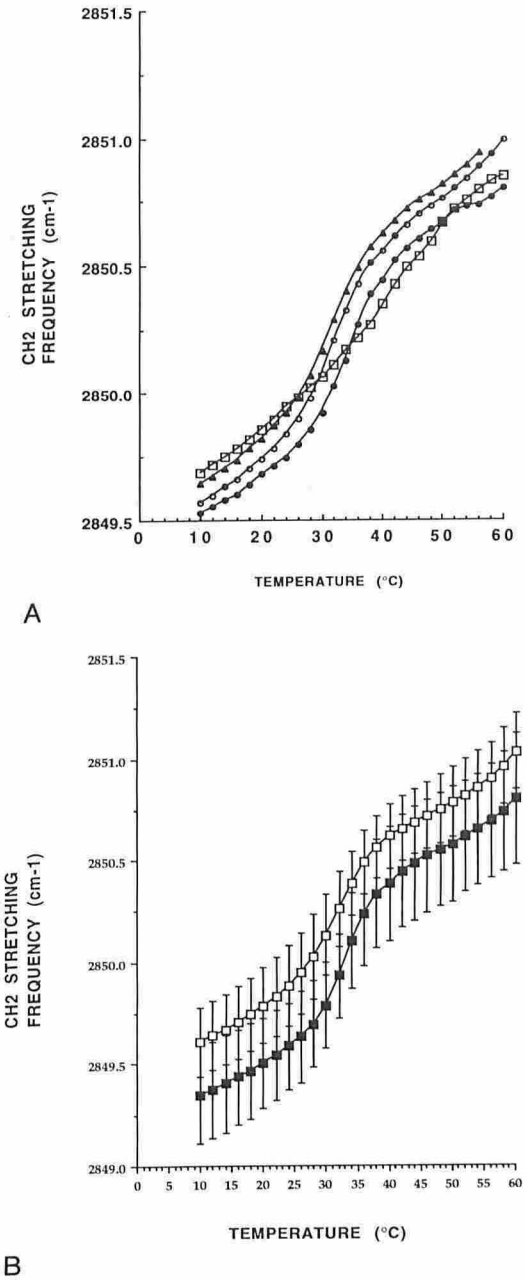

Figure 6. Thermally induced changes in CH_2 symmetric stretching frequency of intact human SC measured by infrared spectroscopy. *A)* First heat as a function of SC hydration (*open square*, 4%; *closed circle*, 23%; *open circle*, 100%; *closed triangle*, 300%). *B)* Average values for first *(closed square)* and second *(open square)* heats at constant hydration (95% RH). *Error bars,* SD.

frequencies of 2852 to 2854 cm^{-1} are found for liquid-crystalline phases [30]. At 10°C, the CH_2 stretching band from human SC had a (hydration-dependent) frequency between 2849.5 and 2849.7 cm^{-1} consistent with predominantly *trans*-configured alkyl chains. Above the inflection, the frequency approached 2851 cm^{-1}, a value that is intermediate between the gel and liquid-crystalline states. Although the absolute change in frequency for a given physical state is reliant on several system-specific factors (e.g., chain length) the presence of a definitive inflection at ~35°C nevertheless implied the existence of a mixed population of both fluid and solid SC lipids in the normal physiologic temperature range (32–37°C). Further, the small increase in the CH_2 stretching frequency after SC hydration may be significant if it defines a large increase in lipid disorder limited to a specific subset of lipids, rather than a small fluidity change manifested for all SC lipids.

DISCUSSION

From the results presented here, the transition observed at ~35°C cannot be attributed to superficial contamination by sebaceous material, or to variations in the lattice packing. The lack of thermal correspondence between the 35°C transition and changes in lipid packing observed here by IR are quite interesting when compared to results obtained with x-ray spectroscopy. A diffraction line at 0.37 nm has been found in studies of neonatal rat [12], hairless mouse [14], and human [11] SC that disappears on heating to ~40°C. The loss of this line and the persistence of another diffraction band at 0.41 nm indicates a change from OR to hexagonal packing. This transition occurs 10–15°C below that indicated by IR. Given that x-ray diffraction is a direct and proved method of measuring crystalline structure, the observation of OR packing at ~25°C is quite definitive. However, IR is also a quite reliable method for monitoring lattice structure, and correlates well with theoretical calculations and x-ray results obtained for simple, homogenous phospholipid systems. Consequently, it is likely that these two methods reflect true structural subtleties, possibly related to the heterogenous nature of SC. Because x-ray diffraction absolutely requires a repetitive pattern to produce a band (IR does not), it is plausible that human SC either i) undergoes a thermally induced disorientation somewhere between 40 and 60°C with respect to the long-range alignment of the OR lipids, or ii) that there are subpopulations of OR lipids that sequentially lose their structure upon heating; the latter group would not be detectable by x-ray techniques. Additional IR and x-ray diffraction studies are clearly necessary (with identical samples) to understand better these apparent discrepancies, and reveal their significance relative to the structure and function of the SC.

Nonetheless, the source of the 35°C calorimetric transition can be addressed with some certainty. Spectral results from the CH_2 stretching region indicate a significant thermal inflection point at ~35°C. Because an abrupt increase in frequency would not be anticipated for an orthorhombic-to-hexagonal change, it is unlikely that this low temperature transition is related to variations in SC lattice packing. Given the observed CH_2 stretching frequency of 2850.5 cm^{-1} at 50°C (a value intermediate between those characteristic of homogenous solid and fluid phases), we suggest that this

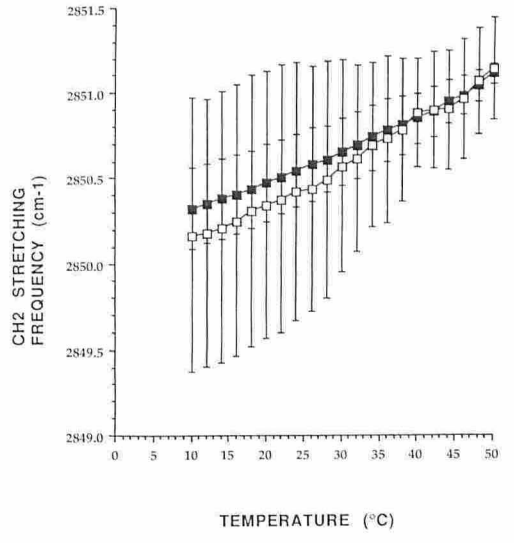

Figure 7. Average CH_2 symmetric stretching frequency of stratum corneum lipids, isolated by chloroform : methanol extraction, plotted as a function of temperature (*closed square,* first heat; *open square,* second heat) at constant hydration (95% RH). *Error bars,* SD.

transition reflects a solid-to-fluid phase change for a discrete subset of SC lipids. In fact, additional supportive evidence for the coexistence of fluid and solid phases in hairless mouse SC have been obtained with x-ray diffraction [14], and with IR and nuclear magnetic resonance studies using porcine SC lipids [31].

Although the precise composition and positional coordinates of such fluid lipids cannot yet be specified, it is instructive to consider whether these lipids are physically segregated or phase separated in the SC. In the former, the presence of fluid lipids could be secondary to keratinocyte terminal differentiation during which profound compositional and structural changes are known to occur throughout the SC. Elias and Menon [32] have suggested that lipid catabolism contributes to the heterogeneity of SC lipids, perhaps defining its barrier and desquamation properties. Furthermore, Bommannan *et al* [33] and others¶ have found that lipid alkyl chain fluidity is greatest near the SC surface, in agreement with the idea that fluid lipids are physically segregated during the process of differentiation. In contrast, the implications of phase-separation are disparate in that the fluid and solid lipids would be more uniformly distributed regardless of position in the SC. The coexistence of uniformly distributed, phase-separated fluid and solid lipids would be predicated on mutual immiscibility due to imbalances in their hydrophobic and hydrophilic interactions. In our opinion, the apparent absence of a 35°C phase transition in the extracted lipids (Fig 7) argues that physical segregation is more probable.

Implicit also in the concept of physical segregation is that the lipids of intact SC are not required, on average, to exist in their state of minimum free energy. Such a conclusion is reasonable for a biologic system; in this case particularly, considering the extremely heterogenous nature of the SC and that the eventual outcome of differentiation is desquamation. Further, it is conceivable under such conditions that nonlipid components such as intercellular proteins contribute to the *in vivo* structure of SC lipids. The fact that the 35°C transition persists even upon reheating and is absent in extracted lipids implies that phase segregation may be maintained through a close relationship with one or more proteins. At the very least, it is apparent from these results that the use of extracted lipids to map SC lipid phase properties may be extremely misleading.

The presence of OR lipids at normal skin temperatures may also play an important role in skin barrier function. The highly ordered nature of OR lipids requires that the participant alkyl chains be of roughly the same length and orientation to maximize interchain interactions. Introduction of dissimilar molecules (like cholesterol) will rapidly destroy the propensity for such an intimate arrangement. This physiochemical dependence is exemplified by the disappearance of OR structure in phospholipid-based systems after the addition of small molecule perturbants such as anesthetics [28,34,35] and cholesterol [36]. It is striking that the corneocyte lipid envelope both lacks cholesterol [37] and consists mainly of covalently bound ceramides [38]. These two features would make the lipid envelope a good candidate for the site of OR lipids. Because the bound ceramides would be physically constrained by their covalent attachment to the protein envelope, unbound interdigitated free fatty acids may be required to facilitate the close proximity of alkyl chains characteristic of OR structures. Calorimetric evidence for such an arrangement has been shown for delipidized porcine SC [29].

The occurence of such a tightly-arranged lipid structure could account for the relatively low permeability of the corneocyte as proposed by Potts and Francoeur [6], in effect encouraging transepidermal water loss to occur primarily via the tortuous but more permeable extracellular lipids. This hypothesis is consistent with the fact that SC from the palate contains less bound lipid, and is correspondingly more permeable than epidermal SC [37]. *In vitro* human skin cultures grown at the air/liquid interface,** and hairless guinea pig skin,†† similarly exhibit increased water permeation relative to intact human SC, and lack a bound lipid envelope. It is further intriguing that isolated SC lipids exhibit evidence of OR structure, similar to that of intact samples [14,26]. The covalent link between the proteins and the ceramides, which constitute the lipid envelope, may be necessary, therefore, to localize the OR structure to the corneocyte surface.

Relevant to this view of SC permeability is additional insight into how skin transport is increased upon hydration. A recent IR study has found that there was no significant change in the CH_2 stretching frequency as SC hydration was increased [40]. However, in the present study, a small, but significant, increase in CH_2 stretching frequency (Fig 6*A*) occurs as the SC is hydrated to values exceeding 40% (w/w). The importance of this change depends entirely on whether it reflects a large disordering of a small subset of SC lipids, or a small disordering of a larger subset. With that in mind, it is easy to conceive of a number of situations where significant disordering of a small population of SC lipids (namely, bound envelope lipids or those lipids associated with 35°C transition, *etc.*) might readily increase skin permeability. Whatever the source of this effect, it is obvious that more detailed and careful biophysical studies are required to fully characterize the hydration properties of the SC.

In conclusion, we report on the behavior of two lipid phase transitions which may be important to the barrier properties of human SC. The transition at 55°C may represent a complex change in the lattice structure of certain lipids. Based on results from x-ray diffraction, this change could involve multiple lipid subsets or be preceded by a disorientation of long-range alignment for OR subcells. We believe the transition at 35°C reflects a solid-to-fluid phase change for a small subset of human SC lipids. These lipids are most likely physically segregated, differentially distributed as a function of SC depth, and maintained by other nonlipid constituents of the SC under essentially nonequilibrium conditions. Importantly, the existence of a fluid lipid phase near physiologic temperature in human SC may imply a point of intervention whereby exogenous factors such as skin penetration enhancers have a direct effect on skin permeability. Certainly, the penetration enhancer oleic acid is known to interact with SC lipids while, itself, it is fluid [7,8]. To date, most attention has been focused on the main lipid transitions (65° and 80°C) which occur well above normal skin temperature, making conclusions about the structure and function of SC at 32°C (as related to its permeability characteristics) largely inferential. Although these lipids are undoubtedly crucial to barrier properties, it may now be appropriate to more closely examine the phase properties of SC lipids at lower temperatures to ensure a comprehensive understanding of the relationship between lipids and permeability.

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