Subzero Thermal Analysis of Human Stratum Corneum

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Received March 8, 1994; accepted May 30, 1994

The thermal behaviour of human stratum corneum was studied using differential thermal analysis within the temperature range of −130°C to 120°C. Aside from thermal transitions at around 40°C, 70°C, 85°C and 100°C, which have been reported before, a particular transition below 0°C (subzero), at approx. −9°C (264 K), was noticed. This transition was present in the analysis curves of dehydrated as well as hydrated stratum corneum sheets and could be distinguished from the water peak found only in hydrated stratum corneum samples. To further characterize this transition, thermal analysis was performed on stratum corneum sheets: (i) after lipid extraction, (ii) after pretreatment of propylene glycol and (iii) after pretreatment of oleic acid/propylene glycol solution. From the results, it was concluded that the subzero transition (−9°C) belongs to low melting lipid components of stratum corneum.

KEY WORDS: human stratum corneum; differential thermal analysis; skin lipid; subzero transition; enhancer.

INTRODUCTION

The skin barrier function is known to reside in the stratum corneum (1,2). One of the techniques to study the physico-chemical properties of the stratum corneum barrier is thermal analysis.

The earliest thermal studies were aimed to investigate the part of skin which provides a barrier to the loss of water (3), and to calculate the amount of water bound in the stratum corneum at various hydration levels (4-7). Later on, thermal analysis was combined with X-ray studies to find correlations between the endothermic transitions and the nature of the components of stratum corneum (8). Since then, investigators have advanced to more explicit interpretations of several particular transitions of stratum corneum (9-16). The thermal transition observed at *40*°C[3] has been ascribed to an orthorhombic to hexagonal phase transition in the lateral lipid packings on the basis of *wide*-angle X-ray diffraction studies by Bouwstra et al (17). Furthermore, from *small*-angle X-ray diffraction data they found that the thermal transition between 65° and 75°C is due to the transformation of the lamellar lipid structure to a disordered structure (18). This supported the assignment of this transition to lipids by Golden et al. after studies using differential scanning calorimetry and infrared spectroscopy (9,10). The transition at *80*°C may arise from gel to liquid transition of lipids, which are associated with proteins, probably in the form of a lipid-protein complex (9-11). The assignment of this transition to an irreversible α-keratin conformational change from Van Duzee (8) was questioned by Goodman and Barry (16). The endothermic transition at *100*°C is ascribed to the denaturation of proteins (8-11). It can be observed only in stratum corneum samples having a high enough water content. Thus, it is generally agreed that lipid and protein components are the primary contributors to the thermal profile of stratum corneum.

The major problem in administering drugs transdermally arises from the barrier capacity of the stratum corneum. This problem may be partially overcome by applying penetration enhancers, which are able to reversibly alter the barrier properties of stratum corneum and thus allow drugs to penetrate through the skin layers and enter the systemic circulation (19,20). Thermal analysis has been used in attempts to understand the mode of action of some enhancers on stratum corneum, such as dimethyl sulphoxide (12), azones (12,13,22), terpenes (14,15), surfactants (16,21), and other compounds (16). In the case of a congeneric series of azones, the thermal analytical data could be nicely correlated with kinetic data, X-ray data and electron microscopic observations (22). For these purposes, most investigators have so far focused the thermal analysis on the temperature range above 0°C. Yet a large number of penetration enhancers have low melting points, and exhibit thermal transitions at temperatures lower than 0°C (subzero), e.g., from preliminary studies it was found that propylene glycol and propylene glycol/oleic acid mixture show endothermic peaks around −99° and −4.5°C, respectively. We postulate that thermal analysis in the subzero range of human stratum corneum, both with and without skin penetration enhancers, may help to further clarify the nature of the interaction between the enhancers and the stratum corneum. The aim of this study was to investigate the thermal behaviour of human stratum corneum within a broad temperature range, from −130°C to 120°C, with special attention for the thermal transitions at the subzero temperature range. In addition, differential thermal analysis was carried out on human stratum corneum at various hydration levels and after pretreatments with enhancers, like oleic acid and propylene glycol.

MATERIALS AND METHODS

1. Preparation of the Stratum Corneum Samples

1.1. Isolation of Human Stratum Corneum

Human breast or abdominal skin obtained by surgical operation was processed immediately upon arrival on the day of the surgery. After the removal of subcutaneous fat, the skin was dermatomed to a thickness of approximately 200 μm. The stratum corneum was separated from the epidermis after digestion with a 0.1% Trypsin Type III (from bovine pancreas, Sigma Chemicals, St. Louis, USA) solution in phosphate buffered saline (PBS, pH 7.4) for 24 hours at 4°C and 1 hour at 37°C. The salts used to make PBS were

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[3] Throughout this paper, the transition temperatures above 0°C are presented as an average of values reported by the authors and other investigators.

0724-8741/94/1100-1610$07.00/0

supplied by Merck, Darmstadt, Germany. Thereafter the stratum corneum sheet was washed with 0.1% Trypsin Inhibitor Type II (from soybean, Sigma Chemicals, St. Louis, USA) solution in distilled water and subsequently with distilled water. The sheet was then dried and stored above silica gel in nitrogen atmosphere at room temperature.

1.2. Extraction of Lipid from Stratum Corneum

Stratum corneum sheets (from 1.1.) were weighed and then submerged and shaken continuously in a solution of chloroform : methanol (2 : 1 volume) for 48 hours at room temperature. The sheets were then dried at room temperature, and stored above silica gel in nitrogen atmosphere. The delipidised and dried samples were weighed again before and after further dehydration above phosphorous pentoxide. Chloroform, methanol and phosphorus pentoxide were obtained from J.T. Baker B.V., Deventer, The Netherlands.

1.3. Dehydration of Stratum Corneum

Stratum corneum sheets (from 1.1.) were weighed before and after they were placed for 24 hours at 50°C in a closed vessel above phosphorus pentoxide.

1.4. Hydration of Stratum Corneum

Stratum corneum sheets (from 1.1.) were equilibrated for 24 hours at room temperature in a closed vessel above 27% sodium bromide solution (in distilled water). Sodium bromide was supplied by Merck, Darmstadt, Germany.

1.5. Pretreatment with Propylene Glycol

Pretreatment was carried out by immersing stratum corneum sheets, either dehydrated (from 1.3.) or hydrated (from 1.4.), in propylene glycol for 24 hours at 32°C. Then the stratum corneum sheets were dried by pressing it between 2 pieces of wire-netting wrapped with tissue-paper repeatedly until the sheets did not wet the paper anymore. Propylene glycol was purchased from J.T. Baker B.V., Deventer, The Netherlands.

1.6. Pretreatment with Oleic Acid 5% (0.16 M) in Propylene Glycol

Pretreatment was carried out by immersing stratum corneum sheets, either dehydrated (from 1.3.) or hydrated (from 1.4.), in a solution of 0.16 *M* oleic acid in propylene glycol for 24 hours at 32°C. Then the stratum corneum sheets were dried by pressing it between 2 pieces of wire-netting wrapped with tissue-paper repeatedly until the sheets did not wet the paper anymore. Oleic acid was purchased from Brocacef B.V., Maarssen, The Netherlands.

2. Differential Thermal Analysis

Samples, each weighing 10-30 mg, were placed into medium pressure stainless steel crucibles made by Mettler, Greifensee, Switzerland, and hermetically sealed to avoid water evaporation during the analysis. Differential thermal analysis was performed using Mettler TA 3000 Thermal Analysis System with a Low Temperature Cell, with an empty pan as reference. Samples were subjected to the following thermal analysis cycle: cooling from 20° to −130°C, then equilibrating isothermally for at least 5 minutes, followed by heating from −130° to 120°C. The rate for both cooling and heating was 2 °C/min. The isothermal equilibration time to achieve a stable condition at −130°C was based on preliminary studies. The transition temperatures were determined by taking the maximum endothermic values of the peaks observed on the heating curves. The heating curves were constructed by plotting the heat flow values, which have been normalized using sample weight (as mW/mg), against temperatures.

The analysis were classified in three series:

Series 1 consists of analysis of human stratum corneum presented in the order of the hydration level: (1A) after lipid extraction followed by dehydration above phosphorus pentoxide (procedure 1.2.); (1B) after *dehydration* above phosphorus pentoxide (1.3.); (1C) after *dehydration* above silica gel (1.1.) and (1D) after *hydration* above sodium bromide 27% solution (1.4.)

Series 2 consists of analysis of on (2A) propylene glycol (alone), (2B) human stratum corneum after *dehydration* above phosphorus pentoxide (1.3.) followed by pretreatment with propylene glycol (1.5.), and (2C) human stratum corneum after *hydration* above sodium bromide 27% solution (from 1.4.) followed by pretreatment with propylene glycol (1.5.).

Series 3 consists of analysis on (3A) oleic acid 0.16 *M* in propylene glycol solution, (3B) human stratum corneum after *dehydration* above phosphorus pentoxide (1.3.) followed by pretreatment with oleic acid 0.16 *M* in propylene glycol (1.6.), and (3C) human stratum corneum after *hydration* above sodium bromide 27% solution (1.4.) followed by pretreatment with oleic acid 0.16 *M* in propylene glycol (1.6.).

RESULTS

Average weight-changes, number of samples and transition peaks of samples for each series are compiled in Table I. The number of each series corresponds to the number of the curve representative for that series. The endothermic transitions presented in that Table were only those, which take place below 0°C, since the present study focused on those transitions and the transitions observed above 0°C did not differ from those reported by many investigators. Nevertheless, all transitions will be taken into consideration in the sections following.

Series 1

Figure 1 shows the thermal profiles of stratum corneum from Series 1. Curve 1D was obtained from the analysis of human stratum corneum after hydration above a sodium bromide solution. The hydration procedure added about 20% to the original weight of the stratum corneum sheet (after isolation) as indicated in Table I. In the temperature range *above* zero degree centigrade this curve contains some endothermal transitions, i.e. at 40°, 70°, 85° and 100°C, which have been reported in many publications (3,8-16) and referred to by Goodman and Barry (12,16) as *T1*, *T2*, *T3* and *T4*, respectively. These transitions, except the one at 100°C, can be found in curves 1C, representing human stratum corneum after dehydration above silica gel. The absence of the transition at 100°C in this curve has been related to the irre-

Table I. Characteristics and Subzero Transitions of Each Experimental Series

Series	Sample; Treatment	Number of Sample	Percentual Weight Change[a]	Subzero transitions (°C)			
				1	2	3	4
1A	HSC[b]; delipidised, dehydrated	2	−41.2	—	—	—	—
1B	HSC; dehydrated (above P_2O_5)	3	−2.9 ± 1.8	—	—	−8.7 ± 1.2	—
1C	HSC; dehydrated (above silica gel)	3	—	—	—	−8.8 ± 1.7	—
1D	HSC; hydrated (above NaBr 27%)	5	+22.3 ± 3.1	—	−18.1 ± 3.2	−10.3 ± 1.2	—
2A	Propylene glycol (PG)	4	—	−100.1 ± 0.1	—	—	—
2B	HSC; dehydrated, PG-treated	2	+109.6 ± 20.0	−99.7 ± 0.0	—	—	—
2C	HSC; hydrated, PG-treated	3	+96.2 ± 23.7	−99.2 ± 0.6	—	−7.8 ± 2.1	—
3A	0.16 *M* Oleic acid in PG (OA/PG)	2	—	−99.8 ± 0.6	—	—	−4.5 ± 0.3
3B	HSC; dehydrated, OA/PG-treated	2	+153.9 ± 9.4	−98.3 ± 0.0	—	−12.4 ± 0.1	−5.6 ± 1.4
3C	HSC; hydrated, OA/PG-treated	2	+199.6 ± 60.3	−99.4 ± 0.9	—	−12.2 ± 0.6	−4.2 ± 1.1

All values ± SD.

[a] Defined as: (post-treatment weight − pre-treatment weight)/pre-treatment weight × 100%; pre-treatment condition corresponds to HSC dehydrated above silica gel (as in 1C).

[b] HSC: Human stratum corneum.

versible denaturation of proteins or intercellular keratin (12), which is dependent on the water content of the stratum corneum (8-9); at high water contents a peak was observed, while at low water contents the whole peak disappeared (11,16). Curve 1B, which is originated from human stratum corneum after dehydration above phosphorus pentoxide, also does not contain the peak at 100°C.

Curve 1B, 1C and 1D, representing both dehydrated and hydrated stratum corneum, obviously show that *below* 0°C there are several endothermic transitions. All three samples have one particular transition in common, which is located at around −9°C (*cf.* Table I), the evaluation of which follows in the discussion session. A variation of the subzero peak shape and position was observed from the analysis of *hydrated* stratum corneum samples (prepared following procedure 1.4.) collected from several donors. Curve 1D in Figure 1 is the representative of this series. The position of the peak

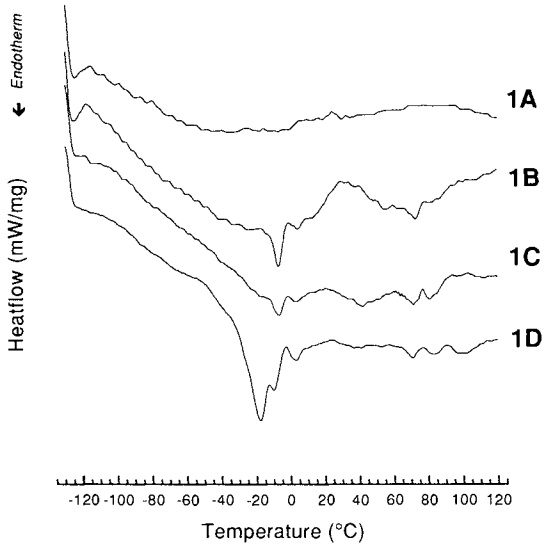

Fig. 1. Thermal profiles of human stratum corneum after (**1A**) lipid extraction followed by *dehydration* above P_2O_5; (**1B**) after *dehydration* above P_2O_5; (**1C**) *dehydration* above silica gel; and (**1D**) *hydration* above 27% NaBr solution.

at around −18°C varied between samples in the range from −21° to −12°C. But in all cases, it has a higher magnitude than the consistent peak at −9°C. In two out of five samples, the smaller peak (at −9°C) was obscured by the stronger one (at −18°C). In two other samples both peaks were clearly distinguishable, and in one sample the small peak could be detected as a shoulder at the stronger peak. The strong peak at −18°C can be assigned to additional amount of water embodied in the *hydrated* sample, which was lacking in other samples from the same series.

Curve 1A is obtained from the stratum corneum sheets, which have undergone lipid extraction following the similar procedure as described by other investigators (8,10). The difference was, after the extraction, we *dehydrated* the stratum corneum sheets once more above phosphorus pentoxide. This may explain why we could not see the peaks at the temperature range of 85° to 100°C, which had been reported (8,10). However, Golden et al did reheat their extracted sample and reported the disappearance of any peaks upon the analysis (10). On the other hand, the absence of lipid peaks at 40° and 70°C is verified. Furthermore, after lipid extraction and dehydration of stratum corneum, the subzero peak at −9°C is not detectable.

Series 2

Figure 2 contains the curves representing series 2. The dominant component in this series is propylene glycol. This compound freezes at −59°C (23). However, in our experiments, on heating from −130°C it exhibited only one endothermic transition at low temperature, i.e. at −100°C, and within the temperature range of −130° to 120°C, no other significant transition was present, as shown on curve 2A in Figure 2. The −100°C peak persisted even in the presence of other substances, such as oleic acid, water in small amounts, or when propylene glycol was applied to stratum corneum. On the curve 2B in the same figure no subzero transition in the *dehydrated* stratum corneum was visible, while a small peak at −8°C was noticed on the curve 2C from the *hydrated* stratum corneum. This peak was located very close to the peak observed in the stratum corneum samples not treated

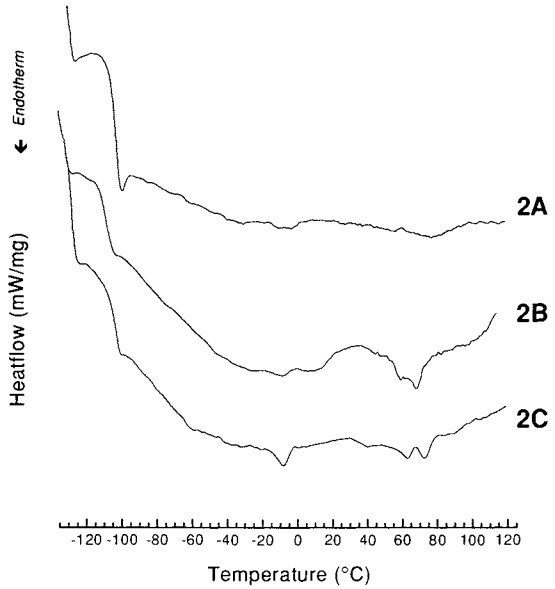

Fig. 2. Thermal profiles of (2A) pure propylene glycol (PG); (2B) human stratum corneum after *dehydration* above P_2O_5 followed by pretreatment with PG; and (2C) human stratum corneum after *hydration* above 27% NaBr solution followed by pretreatment with PG.

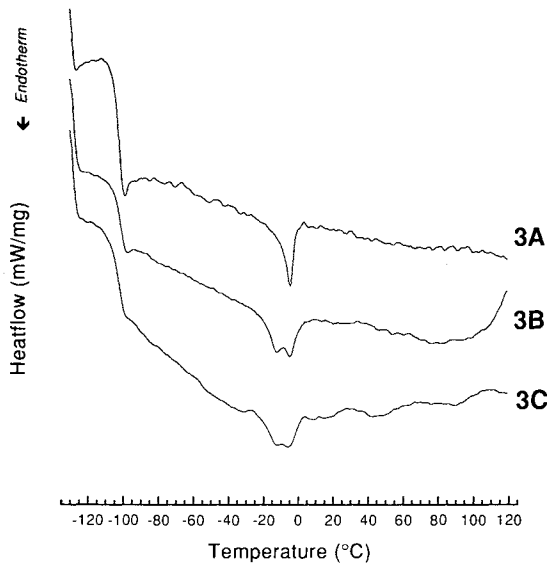

Fig. 3. Thermal profiles of (3A) oleic acid 0.16 *M* in propylene glycol (OA/PG); (3B) human stratum corneum after *dehydration* above P_2O_5 followed by pretreatment with OA/PG; and (3C) human stratum corneum after *hydration* above 27% NaBr solution followed by pretreatment with OA/PG.

with propylene glycol (see curves 1B, 1C and 1D from Figure 1). In the higher temperature region, the transition at 100°C disappeared and the peaks at 70° and 85°C, which are observed in samples untreated with propylene glycol, were shifted to lower temperatures, i.e. 62° and 72°C respectively. This is in agreement with previous studies (11,13).

Series 3

Series 3 specifically compares the thermal behaviour of skin penetration enhancer oleic acid in propylene glycol (resulted in curve 3A in Figure 3), with the thermal behaviour of the *dehydrated* and *hydrated* stratum corneum treated with those compounds (curves 3B and 3C, respectively). The thermal profile of the mixture oleic acid in propylene glycol (curve 3A) exhibited two endotherms: (a) one at −100°C, which is recognizable as arising from propylene glycol (*cf.* curve 2A, Fig. 2), and (b) one at around −5°C, which is most likely due to oleic acid.

Both curves 3B and 3C have subzero transitions at −100°C and −5°C in common with curve 3A. However, they both have an additional transition around −12°C. Above 0°C there is no significant peak observed. The peaks of curve 3B, originated from *dehydrated* stratum corneum sheet pretreated with oleic acid in propylene glycol, were better resolved than those found at curve 3C, the thermal profile of the *hydrated*, oleic acid/propylene glycol treated sample. It is important to note, however, that the position of the peaks remained unchanged with the increase of hydration level from case 3B to 3C. Upon second-run analysis, i.e. the same samples after the first analysis—still in the hermetic sealed pans—were cooled again to −130° and then reheated to 120°C, the peaks at −12° and −5°C were replaced by a single peak at −13°C (±*SD* 1°) as shown in Figure 4 curve 3B 2nd run (*dehydrated* samples) and curve 3C 2nd run (*hydrated* samples).

DISCUSSION

Assignment of Subzero Endothermic Transitions

All endothermic transitions in the range of −20°C to 0°C so far reported for stratum corneum have been related to the presence of water, particularly bound water (4-7). Thermal

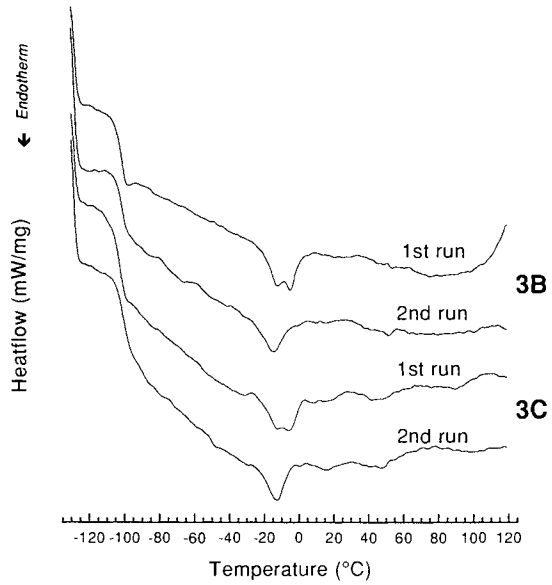

Fig. 4. Thermal profiles of human stratum corneum after *dehydration* above P_2O_5 followed by pretreatment with OA/PG (3B), and after *hydration* above 27% NaBr solution followed by pretreatment with OA/PG (3C). Second runs represent the reheat profiles of the same samples.

experiments for measuring the amount of bound water in stratum corneum have generally been performed according to the following procedure: drying the stratum corneum sheet to a "zero" water content, hydrating it to a certain water content, and then analyzing it. Walkley assigned the minimum water content to samples, which had undergone drying over a molecular sieve (4), but more recent papers assigned "zero" water content values to samples stored over phosphorus pentoxide (5-6) or silica gel (7) for 24 hours under reduced pressure. Inoue et al (5) reported the subzero transitions of stratum corneum containing 26 weight% bound water (with respect to dry weight) at −14° and −9°C. Imokawa et al (6) observed single peaks between −6° and −2°C in the range of 17% to 60% water content while investigating isolated stratum corneum lipids, and multiple peaks between −17° and −6°C from intact human stratum corneum sheet within the range of 30% to 90% water content. They found that lowering the water content decreased the magnitude of the peak (calculated as the transition enthalpy). The same phenomenon has also been observed by Takenouchi et al (7). From the data presented it is also apparent that lowering the water content decreased the peak temperature. This was not explored further in those papers. In fact, the data from previous investigators always displayed more than one peak of different magnitudes in the subzero temperature range −20° to 0°C (5-7), from which they generally assigned the peak with high magnitude to water. The origin of peaks other than water peak has never been discussed.

Clearly the most intriguing finding of the present study was the particular *subzero* peak at −9°C peak as observed in curve 1C Figure 1, i.e. the thermal profile of stratum corneum after isolation and dehydration above silica gel (procedure 1.1.). There are generally three possible assignments for this transition; it may represent: (a) water, (b) proteins, or (c) lipid components of human stratum corneum. We shall consider these possibilities one by one.

Although the stratum corneum sheet has actually been considered to have "zero" water content value (7), as the first step we checked whether this transition might belong to **water** by two means: (i) further *dehydration* of the stratum corneum (procedure 1.3.), and (ii) *hydration* of the stratum corneum to a certain level (procedure 1.4.). Table I shows that the dehydration above phosphorus pentoxide for 24 hours at 50°C (series 1B) reduced the water content of stratum corneum to 3 weight% (compared with series 1C). Only one sharp *subzero* peak at around −9°C was observed upon heating from −130° up to 120°C (curve 1B Figure 1). The *hydration* above sodium bromide 27% solution increased the water content by about 20 weight% (Table I) and yielded one more subzero peak at −18°C, while maintaining the −9°C peak (curve 1D Figure 1). The strong peak at −18°C, which was absent in the curves of dry samples (curve 1B and 1C), disclosed the presence of water, as is already stated in the results section. This peak was distinguishable from the peak at −9°C. Hence, we propose that the peak detected at −9°C cannot be attributed to water.

Now, we consider the possibility to assign the subzero −9°C peak to the **proteins** of stratum corneum. Proteins can be denaturated by heating the samples with subsequent equilibration to high temperature (more than 100°C) (8). After heating the samples to 120°C and then cooling down to −130°C, a subsequent heating run resulted in the disappearance of the transition peak normally present at 100°C. However, the peak at −9°C persisted. Hence, we may conclude, that the −9°C peak most likely cannot be assigned to protein components of the stratum corneum. Therefore, we hypothesize that this particular endothermic transition could be assigned to the lipid components of stratum corneum.

To verify this lipid assignment hypothesis, three pretreatments have been carried out on human stratum corneum: (i) lipid extraction, (ii) immersion in propylene glycol and (iii) immersion in oleic acid-propylene glycol mixture, containing 0.16 *M* oleic acid.

After lipid extraction, the peaks at 40°, 70°C and −9°C disappeared (curve 1A Figure 1). Some investigators have already reported the vanishing of the first two peaks following similar extraction procedures and therefore assigned them to the lipid components of stratum corneum (8-10). The fact that the subzero peak at −9°C also disappeared upon lipid extraction provides a strong evidence for assigning it to lipids as well. Interestingly in this respect, from his thermal analysis on the residue of the lipid extract from stratum corneum, Van Duzee pointed out that there is a transition centered around −10°C which, according to his opinion, was due to the melting of water, although he stated that the lipid extract had been "evaporated to dryness" (8). Our hypothesis that this transition may belong to lipid components will in fact give a more reliable explanation for his data.

Effects of Propylene Glycol and Oleic Acid

The treatment of propylene glycol on stratum corneum is known to affect the thermal transitions of the components of this skin layer. The transitions above 0°C from stratum corneum pretreated with propylene glycol have been discussed by Bouwstra et al (11,13). The disappearance of the peak at 100°C was thought to be due to the extraction of water from the protein regions by propylene glycol (11). The protein denaturation would have turned the two clearly separated lipid transition peaks into one peak (9,13).

The analysis curves from the *hydrated* stratum corneum treated with propylene glycol (curve 2C Figure 2) contained the subzero peak at −9°C, but on the curves of *dehydrated* samples this peak can hardly be noticed (curve 2B). These phenomena can be explained by the property of propylene glycol and by taking into account the nature of lipids of the −9°C peak. Apparently, propylene glycol has more affinity towards water than lipids. In the absence of water, as in the case of *dehydrated* samples, propylene glycol interacts with lipids, especially those with low melting points, causing a depression in their phase transition temperatures. In the *hydrated* stratum corneum samples, i.e. in the presence of water and lipids together, propylene glycol interacts with water in the first place, and proteins, which have a strong affinity towards water. The interaction of propylene glycol with water and proteins is clearly shown by the depression of the transition peaks at 100°C and might also result in the shift of peaks at 70° and 85°C to 63°C and 73°C, respectively. However, there seems not enough excess propylene glycol for reactions with the lipids. Hence, the peak at −9°C is less affected and can be clearly observed.

The treatment of oleic acid/propylene glycol solution on

both *dehydrated* and *hydrated* stratum corneum produced curves 3B and 3C in Figure 3, respectively. As mentioned before, they are almost identical curves. Of particular interest is the appearance of two peaks: (a) at −12°C and (b) −5°C. None of the oleic acid/propylene glycol treated stratum corneum samples showed a peak at −9°C upon heating from −130°C. Since the hydration states of samples resulting in curve 3B and 3C were very different, it is unlikely that the absence of this −9°C peak has anything to do with water. Rather, we suspect that the subzero peak, which in untreated stratum corneum occurs at −9°C, was shifted to −12°C as a result of an interaction between the oleic acid and the stratum corneum lipids. We would like to suggest, that this interaction may well be based on the formation of a eutectic mixture between oleic acid and skin lipids. Such eutectic mixing often occurs in multi-component lipid mixtures, for instance the binary system of fatty acids (24), leading to melting point depression with respect to the melting points of the pure components. Furthermore, in curves 3B and 3C Figure 3 we observe a peak at −5°C. Compared to the transition peak of the oleic acid in propylene glycol on curve 3A Figure 3, the peak at −5°C is most likely related to excess oleic acid in propylene glycol solution having maintained its own transition and most likely therefore being present as a separate oleic acid/propylene glycol phase. Upon subjecting the samples, that produced curves 3B and 3C, to repeated cooling/heating cycle (second-run), it was observed that both subzero peaks at −12° and −5°C tend to unify as one peak at −13°C and could not be distinguishable anymore (Figure 4). This phenomenon was not observed when the samples being *hydrated* (without any further treatments) was analyzed for the second time; the strong water peak at −18°C did not unify with the peak at −9°C.

Lampe et al (25) reported that 20 weight% of the lipid components of human stratum corneum consist of free fatty acids, mainly (in order of quantity) palmitic acid, octadecadienoic acids (e.g. linoleic acid), octadecenoic acids (e.g. oleic acid), stearic acid and hexadecenoic acids (e.g. palmitoleic acid). The remaining lipids comprise fatty acids bound to other compounds. Among the free fatty acids, the unsaturated fatty acids have melting points near by or even below zero centigrade (e.g. linoleic acid and other poly-unsaturated fatty acids). These compounds can undergo thermal transitions at subzero temperatures, especially in the presence of certain substances. A good example is taken from the series 3A, oleic acid in propylene glycol solution. Pure oleic acid solidifies at 4°C to crystalline mass (23). At 0.16 *M* concentration in propylene glycol, however, it shows a thermal transition around −5°C (see Curve 3A Figure 3). In the presence of oleic acid, stratum corneum lipids may endure the same phenomenon, so that their transition undergoes a shift to lower temperature.

As a conclusion, we believe that the particular endothermic transition of stratum corneum at −9°C can be assigned to lipids. As its position is below 0°C we wish to call it a **subzero lipid peak**. The composition of this stratum corneum lipid component has yet to be investigated, although we suspect that it may consist of free fatty acids with low melting point such as oleic acid, linoleic acid, etc, since these compounds have a significant percentage among other lipid components of the stratum corneum.

ACKNOWLEDGMENTS

The authors thank Dr. P. A. Cornwell for valuable discussion.

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