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# Differential scanning calorimetry of dimethylsulphoxide-treated human stratum corneum

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## Summary

Differential scanning calorimetry has been employed to investigate the thermal properties of human stratum corneum. The influence of hydration and DMSO concentration on the interstitial lipid, lipoprotein and protein peaks has been investigated. Hydration between 10% and 60% water had no discernible effect on these endotherms. The influence of DMSO was most pronounced on the protein peak with a concentration dependence observed for the lipoprotein peak for DMSO > 60%. The $T_m$ for the intercellular lipid peak increased linearly as a function of DMSO concentration up to 90%. Neat DMSO abolished the lipid endotherm.

## Introduction

For the majority of compounds the principal barrier to percutaneous absorption is in the stratum corneum. This tissue can be considered morphologically and functionally to represent a two-compartment system comprised of anucleate corneocytes largely composed of fibrous protein networks and the intercellular matrix predominantly composed of neutral lipids.

Morphological and cytochemical observations suggest that intercellular lipids undergo substantial alterations in composition immediately after secretion (Elias, 1983). Phospholipids and sterols, the lipids of cellular membranes, predominate in stratum basale/spinosum. The phospholipid contribution begins to decrease in stratum granulosum and forms an extremely minor component of stratum corneum. Instead, sterols and other neutral lipids (triglycerides, free fatty acids, and hydrocarbons) constitute 60–70% of the lipids of stratum corneum. The bulk of the remaining lipid is sphingolipid. The preponderance of neutral lipids, bearing long-chain saturated fatty acids is well-suited for the provision of the hydrophobic barrier to water loss within the stratum corneum (Lampe et al., 1983). It has been suggested that sphingolipids may provide the polar moieties required in the absence of phospholipids for maintenance of the lipid bilayers in the intercorneocyte spaces of stratum corneum. Biochemical investigation of the different strata of epidermis revealed that they span from C12 to C26, with C16–C18 representing the major species. The major fatty acids were C16 : 0 and C18 : 0; among monoenoic

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acids, C18:1 were always present in greater amounts than C16:1. The amount of higher fatty acid, though present in lower layers of epidermis, is hardly detectable in stratum corneum.

The thin section morphology and freeze-fracture characteristics of stratum corneum reveal that intercellular spaces of stratum corneum enclose membrane bilayers. Phospholipids are vital molecular entities for such bilayer lamellar constitution in biological membranes. During differentiation and cornification phospholipids virtually disappear from the spectrum of stratum corneum lipids (Lampe et al., 1983; Landmann, 1984), and are supplanted by neutral lipids and sphingolipids. Other reports on lipid composition of stratum corneum demonstrate that the glycolipid/ceramide ratio diminishes during differentiation and glycolipids are absent in the outer stratum corneum (Lampe et al., 1983; Downing et al., 1987).

The stratum corneum lipids responsible for the epidermal water barrier consist principally of ceramides, cholesterol, cholesteryl sulphate and free fatty acids, which are probably arranged in multiple intercellular lamellae having a crystalline array of the straight and predominantly saturated lipid chains. It is also proposed that acylglucosylceramides (glucosylceramides esterified to linoleic acid) serve to hold together the adjacent bilayers and prevent the formation of aqueous phases between bilayers (Wertz and Downing, 1983).

Over the last two decades, thermal analysis techniques have been employed to investigate the physical properties of stratum corneum (Wilkes et al., 1973). High sensitivity differential scanning calorimetry (DSC) studies of mammalian stratum corneum has revealed several transitions probably attributable to lipid and protein components in the temperature range of 25–120 °C (van Duzee, 1975; Rehfeld and Elias, 1982). Hydration of stratum corneum altered the thermogram with sharp and reversible transitions at 40 °C (variable), 75 °C attributed to lipid melting and the irreversible transitions at 85 °C and 107 °C, attributed to protein denaturation (van Duzee, 1975; Knutson et al., 1985; Golden et al., 1986).

The DSC thermogram of the lipid extracts and protein residue from hairless mouse, porcine and human stratum corneum confirmed that the transitions below 80 °C are primarily ascribable to lipid transitions, while those above 90 °C were associated with protein transitions, probably due to conversion from $\alpha$ to $\beta$ keratin (van Duzee, 1975; Golden et al., 1986). Thermograms of porcine and human stratum corneum revealed a difference of 5 °C in their lower transition temperature (below 80 °C). Almost the same differences persisted in the transitions of their lipid extracts in the same temperature region under the same experimental conditions (Knutson et al., 1985). DSC and Fourier transform infra-red spectroscopy (FTIR) studies established that midpoints of thermal transitions (below 80 °C) decreased to a limiting value while their peak heights increased with increasing level of hydration of skin samples (Knutson et al., 1985; Golden et al., 1986; Goodman and Barry, 1986).

Knutson et al. (1985) suggested a relationship between the flux of lipophilic solutes and lipid fluidity. The enhanced permeation of lipophilic molecules by long-chain unsaturated fatty acids with carbon chain lengths similar to those of the lipid domain was reported by Golden et al. (1987). Recently Saleh et al. (1987) suggested that enhancement in permeation of alkanols corresponded to changes in the 80 °C lipid endotherm.

Dimethylsulphoxide (DMSO) is one of the most widely studied skin penetration enhancers. Its effects have been examined in vitro by techniques ranging from traditional permeability experiments (e.g. Saleh et al., 1987) to calorimetric investigations (e.g. Goodman and Barry, 1986) which are the subject of the current reported studies.

## Materials and Methods

Trypsin from bovine pancreas and DMSO were purchased from Sigma (U.K.). Fresh deionized water was used as an aqueous phase. Tris(hydroxymethyl) methylamine and salts were of AnalaR quality.

*Preparation of stratum corneal samples.* Human abdominal skin was obtained from autopsy and frozen in polythene bags at −20 °C until needed. Prior to use the frozen samples were thawed and brought to room temperature. The skin sample

was carefully defatted and dermatomed to 350 $\mu$m thickness. The epidermis was separated from dermis by heating the dermatomed skin at 60 °C enclosed in aluminium foil for 2 min followed by mechanical removal. The stratum corneum samples were removed from the epidermis by digestion for 1 h in a 0.1% trypsin solution (0.15 M NaCl and 0.05 M Tris, pH 7.4). Control of both time and temperature was essential during the process as a temperature of 65 °C and heating for 2 min could result in denaturation of proteins or in incomplete separation of the epidermis. The trypsinized epidermis was vortexed to obtain a sample of stratum corneum free from all visible epidermal residuals. The samples thus obtained were washed with several changes of water.

*Drying and hydration of stratum corneum samples.* The wet samples were spread on thin wire-mesh and dried over anhydrous $CaSO_4$ for 48 h in desiccators, followed by washing for 1 min with cold (−4 °C) hexane to remove surface lipids. Hexane washing time had a profound effect on the multiplicity and reproducibility of stratum corneum thermogram peaks (van Duzee, 1975). Hexane was removed in vacuum for 1 h and the samples weighed before hydration. A relative humidity of 95.7% was maintained by saturated salt solutions of barium chloride or potassium sulphate at 20 °C.

*Treatment of stratum corneum samples with DMSO.* A dry, hexane washed sample was divided into 5 portions of appropriate weight. One sample was kept as a control (saline incubated) and the remaining 4 were pretreated with different concentrations of DMSO by incubation in either 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% v/v DMSO–saline binary mixtures for 24 h at 37 ± 0.1 °C. The incubated samples were rinsed with deionized water for 5 min and dried over anhydrous $CaSO_4$ for 48 h. From every pretreated and the single control sample, 14 ± 0.1 mg of membrane was hydrated to 40% (0.4 mg/mg of dry skin) at 20 °C and 95.7% relative humidity.

*Thermal analysis of stratum corneum.* 14 ± 0.1 mg of dry, hydrated or DMSO-treated stratum corneum in the form of a suitable 'disc' was placed in a 50 $\mu$l capacity aluminium pan capable of withstanding 20 p.s.i. internal pressure.

A differential scanning calorimeter (Perkin-Elmer DSC-2) was calibrated with indium as standard, m.p. 156.6 °C. The stratum corneum sample pan was placed in the sample holder and an empty sealed pan was placed in the reference holder. All samples were scanned at 10 °C/min and at 0.5 mCal full sensitivity over 30–105 °C. The head of the instrument was cooled with an ice–salt mixture if required. The $T_c$ (temperature of the onset of transition) and $T_m$ (temperature of the maximum amplitude of peak transition) were determined.

## Results and Discussion

The DSC scans for stratum corneum samples hydrated at 40%, 50% and 60% w/w water are illustrated in Fig. 1. For each level of hydration, 3 endotherms are evident between 70 and 102 °C in agreement with the observations of Golden et al. (1986). Van Duzee (1975) and Barry (1986) have reported the existence of a fourth endotherm at approximately 40 °C. Both Golden et al. (1986) and the authors are of the opinion that the appearance of this endotherm relates to stratum corneum preparation procedures and is ascribable to loosely associated lipids of sebaceous origin. The two lower temperature peaks are reversible; the third at 102 °C is irreversible when hydrated stratum corneum is rescanned after heating to 120 °C (Fig. 2). The reversible peaks are attributable to intercellular lipids and a lipoprotein complex associated with corneocyte membranes (Wertz and Downing 1987), while the third transition at the higher temperature is due to intracellular keratin (Golden et al., 1986). Increasing hydration generally leads to a sharpening of the peaks although no discernible dependence of $T_m$ on hydration level over the range 10–60% was observed. Values of $T_m$ for the intercellular lipid peak of 73.1 ± 1.5; lipoprotein peak 85.4 ± 1.0 and the protein peak of 101.4 ± 2.9 ($n = 18$; ± S.D.) were determined. These values are in good agreement with literature values for the lipoprotein peak, lower than the 75 °C reported for the intercellular lipid peak by van Duzee (1975) and Knutson et al. (1985) and higher than the range of 95–100 °C

quoted by Knutson et al. (1985) for the protein peak.

On rescanning (Fig. 2), the intercellular lipid and lipoprotein peaks shift to a lower temperature with a reduction in the magnitude of the lipoprotein endotherm, which suggests a reduction in the concentration of the lipoprotein complex due perhaps to denaturation of the protein component while the peak shift can be ascribed to increased disordering of the acyl chains consequent to the dissociation of the complex. The third irreversible peak is attributable to proteins (Goodman and Barry, 1986) and is thought to relate to the conversion of $\alpha$ to $\beta$ transformation state of keratin. Protein is implicated in 2 of the 3 observable peaks although it is not known whether it is the same protein in different environments or two chemically different species.

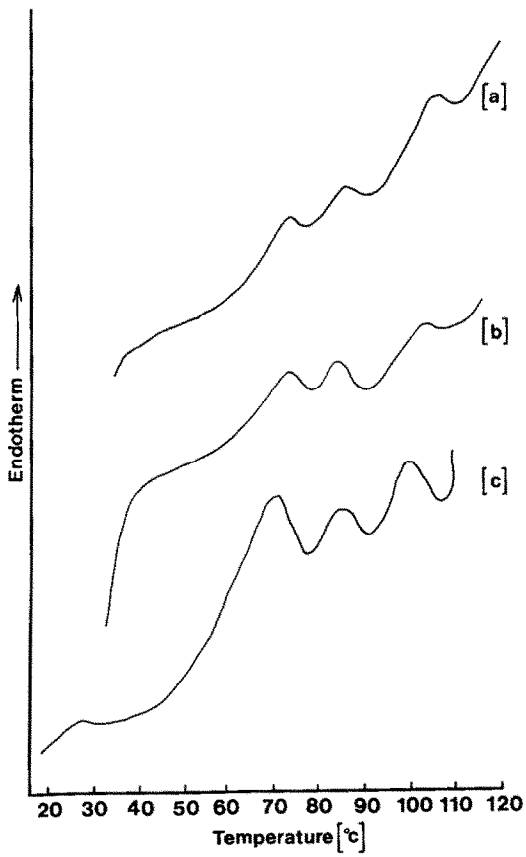


Fig. 1. DSC thermograms of human stratum corneum. Samples hydrated to (a) 40%, (b) 50% and (c) 60% w/w.

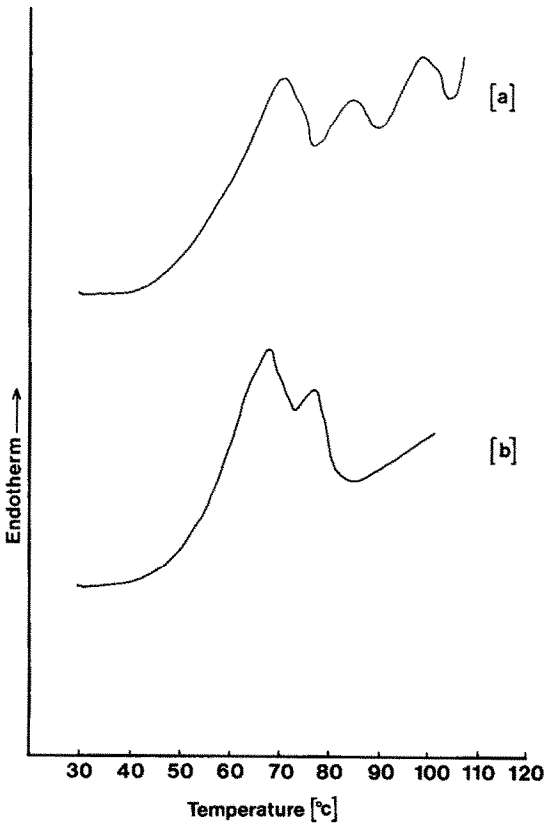


Fig. 2. DSC thermograms of human stratum corneum hydrated to (a) 60% and (b) same sample rescanned.

DMSO pretreatment, followed by hydration to 40% w/w, resulted in changes in the $T_m$ of the intercellular lipid peak and the lipoprotein peak as shown in Fig. 3. The $T_m$ of the lipid endotherm increases linearly with DMSO concentrations up to 90%. The peak decreased in intensity with DMSO concentration and was abolished for neat DMSO. The extent to which this represent DMSO modulation of the structured intercellular lipid as opposed to solvent extraction of component lipids is unclear.

The $T_m$ of the lipoprotein peak in contrast showed no change up to 60% DMSO. A split peak was evident at 70%; higher concentrations thereafter resulted in a rise in $T_m$ with DMSO content with considerable broadening of the endotherm. DMSO has been shown to form a 1 : 2 hydrogen-bonded complex with water (Cowie and Toporowski, 1961) which corresponds to approximately

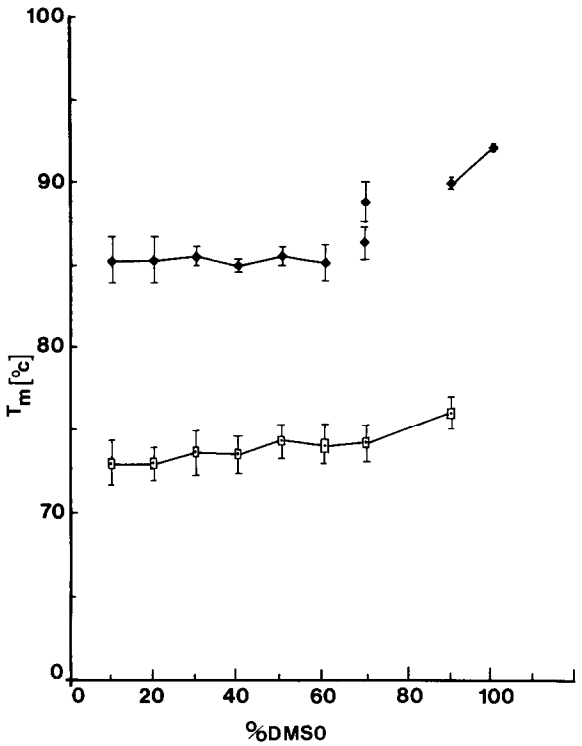


Fig. 3. The influence of DMSO concentration on $T_m$ of the intercellular lipid endotherm (□) and the lipoprotein endotherm (◆). For 70% DMSO there was a splitting of the lipoprotein peak into a doublet.

66% v/v DMSO. The observed changes in $T_m$ of the lipoprotein endotherm occurs around this concentration of DMSO, suggesting the requirement of uncomplexed DMSO molecules to exert the structural changes in the lipoprotein complex.

The protein peak in contrast resulted in fragmentation, reduction in magnitude, broadening and finally the disappearance as the DMSO concentration increases, all features of denaturation of the structured proteins (Elfbaum and Laden, 1968; Scheuplein and Ross, 1970).

As a transdermal penetration enhancer, DMSO exhibits an unusual concentration dependence (Sweeny et al., 1966; Allenby et al., 1969a). It is reported that 60% DMSO is required for a measurable penetration enhancement. The rate rises to a maximum after 3 h then declines for both neat DMSO and its binary mixture containing > 70% DMSO. DMSO application may lead to the delipidisation of stratum corneum (Allenby et al., 1969b). The mechanism of DMSO accelerant action probably arises from the interrelationship between delipidising and protein denaturation properties and delamination of the horny layer by stress resulting from cross-currents of the highly water-interactive DMSO and water (Chandrasekaran et al., 1977).

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