Effect of Preheating on the Permeability of Neonatal Rat Stratum Corneum to Alkanols

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We heated flat sheets of neonatal rat stratum corneum for various times at temperatures between 40 and 90°C before determining the permeability coefficient (Kp) of propanol and/or hexanol from water. Below 70°C, Kp remained constant; at 75°C, Kp increased linearly with exposure time; at 80°C and above, there was a large increase in under 2 h, with no further increase on longer heating. There was a 15-fold increase in 6-h Kp between 70°C and 80°C, values being constant above 80°C but at a figure less than for lipid-extracted stratum corneum. Thermal analysis showed that the increase in Kp corresponds to changes in the 80°C lipid endotherm, suggesting that the increased Kp is due to a disordering of the lipid structures.

The effect of treating preheated stratum corneum with dimethylsulfoxide (DMSO) vapor for 16 h was also studied. Below 70°C, Kp was increased five-fold, but between 70 and 80°C this difference was eliminated, so that above 80°C the Kp was the same as with heat treatment alone. We concluded that both heat and DMSO affect the lipid structures of stratum corneum. DMSO produced a small, reversible structural change, while the effect of heat is irreversible and produces a greater degree of disorder in the lipid structures, but the lipid still contributed to the barrier effect of stratum corneum. *J Invest Dermatol 89:430–433, 1987*

In a previous detailed study of the differential thermal analysis (DTA) of neonatal rat stratum corneum [1], one of us has established that there are two endothermic transitions at 71 and 80°C, both caused by lipids. The higher transition was lost after heating to temperatures in excess of 75°C.

Some workers have reported that preheating the stratum corneum increased its permeability. Behl and coworkers [2–4] suggested that scalding or branding at 60°C for less than 1 min could increase the penetration rate of water and low molecular weight alkanols. Other workers, however, have concluded that such a temperature would not alter permeability. Thus, Polano et al [5] showed that heating at 60°C for 16 min did not affect the penetration of methyl nicotinate and Vinson et al [6] demonstrated that, although heating at 70°C for 45 min severely damaged viable dermis and epidermis, it did not alter skin permeability. Allenby et al [7] reported an undamaged barrier function after heating for 1 h at 65°C, but that irreversible structural changes occurred at 71.3°C. Other reports by Behl and co-workers [8–10] confirm a dramatic, irreversible increase in skin permeability following heating in excess of 80°C and Cooper [11] made similar observations at 100°C.

The temperatures of the lipid transitions found on thermal analysis and the changes in permeability on preheating occur in a similar temperature range. In this paper we report an investigation of both the effect of preheating time and temperature and of the effect of subsequent exposure to dimethylsulfoxide (DMSO) vapor on the permeability of neonatal rat stratum corneum to propanol and hexanol.

MATERIALS AND METHODS

Stratum corneum was prepared as described previously [12]. Dry stratum corneum sheet was heated in a humidity oven (Vindon) at 50% RH for various lengths of time at 40, 60, 70, 75, 80, 85, and 90°C. For those heated for 6 h, half the sheets were placed on a cylinder of metal gauze in a desiccator and stored in an atmosphere of saturated DMSO (from a reservoir of liquid DMSO in the base) for 16 h. The metal gauze was used in an attempt to minimize the possibility of extraction by condensing DMSO vapor. A control of unheated stratum corneum was also exposed to DMSO vapor.

Using the method described previously [12], the permeability of the stratum corneum sheets to either radiolabeled n-propanol (Radiochemical Centre, Amersham) or n-hexanol (ICN Pharmaceuticals) was determined. Water was used as the delivery and receptor phases in all experiments. To observe the effects of the exposure to DMSO vapor on the lipid endotherms, we used DTA on 10-mg samples using a Stanton Redcroft 671B instrument heating rate 10°C/min in static air with alumina as reference.

RESULTS AND DISCUSSION

In one series of experiments, in which the effect of the duration and temperature of heating was investigated, we used hexanol as the penetrant. The values for the permeability coefficient obtained

Manuscript received June 19, 1986; accepted for publication March 9, 1987.
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Abbreviations:
DTA: differential thermal analysis
DMSO: dimethylsulfoxide
Kp: permeability coefficient

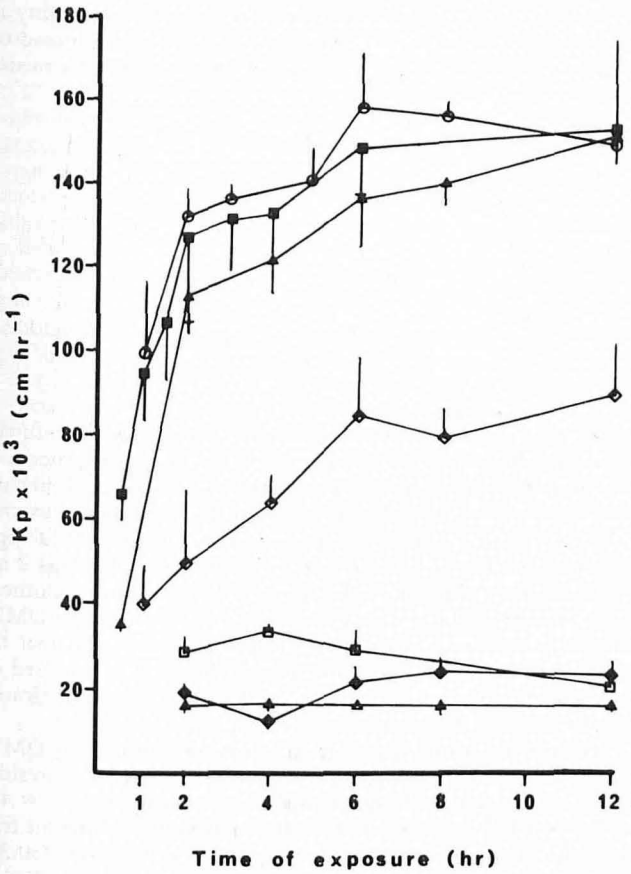

Figure 1. Plots of Kp of hexanol as a function of time of exposure to elevated temperature (*open triangle*, 40°C; *solid diamond*, 60°C; *open square*, 70°C, *open diamond*, 75°C, *solid triangle*, 80°C, *open circle*, 85°C, *solid square*, 90°C).

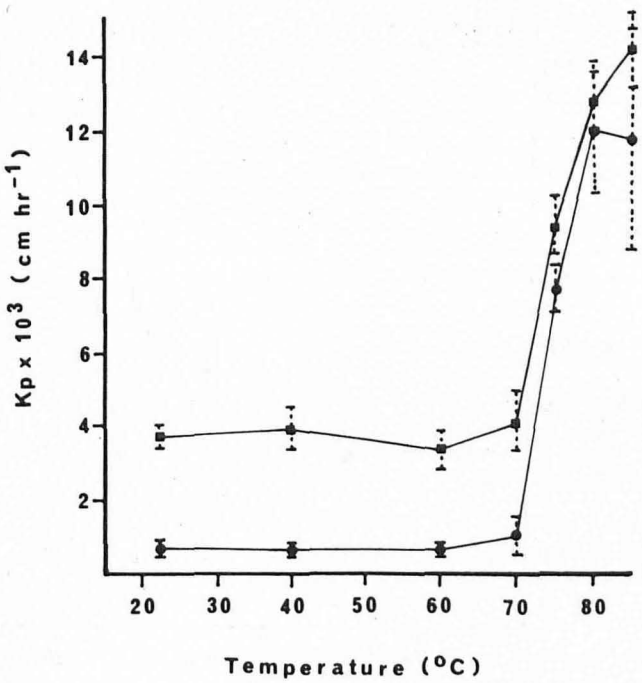

Figure 2. Plots of Kp of propanol as a function of the temperature of exposure of neonatal rat stratum corneum to heat for 6 h. *solid circle*, heat alone; *solid square*, heat plus exposure to DMSO vapor.

are plotted against heating time in Fig 1. From this data it can be seen that there are three different patterns of behavior: (*i*) at 40, 60, and 70°C, where there is virtually no change in the stratum corneum permeability with duration of heating; (*ii*) at 75°C, where there is a continuing increase in permeability, with the length of heat treatment up to the 12-h duration of the experiment; (*iii*) at 80, 85, and 90°C, where there is a rapid increase in permeability over the first two hours, followed by a levelling off as heating is extended. The profiles of (*iii*) appear to be asymptotic to a value of permeability coefficient of about 150×10^{-3} cm/h.

These results agree with most of the published work, although direct comparison is not always valid because of differences in experimental detail. Full thickness human [5] and hairless mouse [2–4] skin was found to give increased permeability when heated at 60°C for 30 to 60 s, but no further increase when exposed for up to 16 min.

In the present work, a 1.9-fold increase, relative to untreated stratum corneum, was found after heating at 60°C for 1 h. Heating at 70°C produced a small increase in permeability (2.7-fold after 1 h, relative to control), which is comparable with the effect of preheating full-thickness hairless mouse skin at 80°C [8–10] on the permeability of water, phenol, and n-alkanols. These workers also reported that heating at 80°C and higher for 1 min, using a scalding technique, produced an exaggerated increase in skin permeability. From the overall data available, it appears that neonatal rat stratum corneum responds to heat in a manner that is essentially similar to that of full-thickness human and hairless mouse skin. The present work also agrees generally with the results of Polano et al [5], Vinson et al [6], and Cooper [11], although differing in some details.

Figure 1 shows that heat treatment at 80, 85, and 90°C produced a large, approximately linear, increase in skin permeability over the first 2 h exposure and little further change when heated for up to 12 h. This suggests that the permeability barrier was damaged within 2 h at these temperatures. In contrast, heating at 75°C produced an approximately linear increase in skin permeability throughout 12-h exposure. The permeability coefficient (Kp)-time of exposure profiles for both 75 and 90°C heating, being linear (first 2 h only at 90°C) suggest that this impairment of the barrier is following zero-order kinetics. The slope at 90°C is 8 times that at 75°C, showing that the rate of destruction of the barrier depends on both time and temperature of exposure. However, such a relationship is only observed at temperatures in excess of 70°C.

Figures 2 and 3 present results of a study in which we compared the permeability of stratum corneum to both propanol and hexanol after a standard 6-h heating. With preheating between 40°C and 70°C, only small changes in the Kp of both propanol and hexanol occurred. For propanol the value was constant, while for hexanol there was a small increase. This difference between the polar and nonpolar alkanols is not understood, although Behl and coworkers [2–4] have reported a similar effect with stratum corneum heated at 60°C and concluded that it was due to a hydration effect. In the present work the stratum corneum was desiccator-dry on heating, thus, as far as possible eliminating the effect of water as a cause and, perhaps, indicating that the differences arose from a direct effect of heat on the stratum corneum.

With both penetrants there was a dramatic increase in skin permeability after heating between 70 and 80°C, above which temperature it appears that a plateau was reached. This latter suggests that the maximum effect of heat on the permeability of stratum corneum could be achieved by heating at 80°C for 6 h. Comparing permeability coefficients in unheated and heat-treated stratum corneum shows that there was a 16-fold increase for propanol and a 14-fold increase for hexanol (using the average of Kp's at 80 and 85°C). It follows from the similarity of these

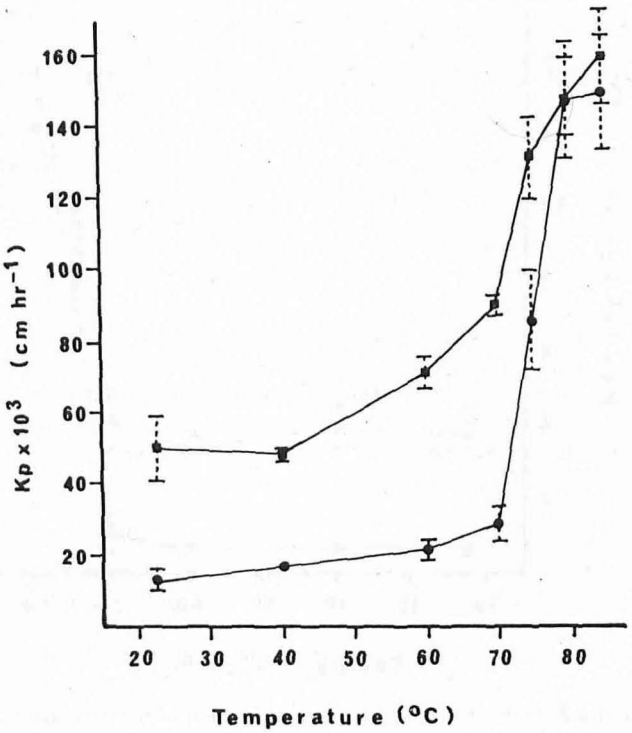

Figure 3. Plots of Kp of hexanol as a function of the temperature of exposure of neonatal rat stratum corneum to heat for 6 h. *solid circle*, heat alone; *solid square*, heat plus exposure to DMSO vapor.

changes in Kp that, irrespective of the polarity of the alkanol, the thermally damaged skin displays a markedly increased permeability. However, the difference between the actual Kp's of the penetrants indicates that, despite any damage to the skin barrier, partition coefficient remains an important factor in determining the overall rate of penetration. These results do not agree with the observations of Behl and coworkers [8–10], who reported a greater effect on the penetration of polar than nonpolar alkanols in skin heated above 80°C. However, direct comparison with their work is probably not valid because of species differences and their use of full skin, which, on heating, would produce severe heat injuries, protein denaturation, and cellular necrosis [13] in the viable layers and to which they attributed their results. From the observations reported here, one may conclude that there was a dramatic change in the stratum corneum barrier function between 70 and 80°C.

From DTA studies [1], it appears that the increase in skin permeability after exposure of stratum corneum to temperatures in excess of 70°C was due to changes in the normal lipid structures of the stratum corneum. A disordering of the lipid structure, perhaps by a slow melting, diffusion, and solution process, may increase the diffusivity of materials through the stratum corneum. Such a mechanism would also indicate that the normal lipid structure forms a significant part of the skin barrier.

Further evidence that lipids were involved was obtained using chloroform/methanol extraction (C/M 2:1). After C/M 2:1 overnight extraction, the Kp for propanol was $258.4 \pm 36.0 \times 10^{-3}$ cm/h and for hexanol $404.6 \pm 13.6 \times 10^{-3}$ cm/h. A similar process carried out on thermally damaged skin (80°C for 6 h) gave Kp values of $326.0 \pm 13.0 \times 10^{-3}$ cm/h and $378.7 \pm 11.6 \times 10^{-3}$ cm/h for propanol and hexanol, respectively. Thus, lipid extraction increased the penetration of propanol 27 times over that of thermally damaged skin, but only 2.5 times for hexanol. Both penetrants had similar Kp's in the absence of lipid. It follows, therefore, that not only does thermally damaged skin still act as a strong barrier to penetration, but also that heat damage before lipid extraction does not produce an additive effect, further indicating that heat is altering the lipids. It is suggested, therefore, that both heat treatment and solvent extraction affected the same lipid structures in the stratum corneum, but to different extents.

Table I. Ratios of Propanol and Hexanol Permeability Coefficients for Stratum Corneum Preheated and Exposed to DMSO Vapor to That for Preheated Only Stratum Corneum

Heating temperature (°C)	Propanol	Hexanol
No heating	5.2	4.9
40	5.7	3.0
60	5.4	3.2
70	4.1	3.1
75	1.2	1.5
80	1.0	1.0
85	1.2	0.9

The exposure of unheated stratum corneum to DMSO vapor increased the Kp of both penetrants about five-fold (Figs 2 and 3). The same treatment produced lower lipid melting endotherm temperatures on DTA. This evidence shows that sufficient DMSO enters the stratum corneum to produce an effect and that this appears to involve the stratum corneum lipids. The standard deviations are similar to those without DMSO treatment, indicating that the method is reproducible.

Prolonged washing of stratum corneum exposed to DMSO vapor was used in an attempt to remove all traces of residual DMSO. The Kp for hexanol following the treatment was $40.2 \pm 7.4 \times 10^{-3}$ cm/h, which was not significantly different from stratum corneum exposed to the vapor and not washed ($50.3 \pm 8.8 \times 10^{-3}$ cm/h). It therefore seems probable that DMSO produced irreversible changes before the permeability determination. This is in contrast to samples exposed to DMSO vapor and then exposed to room conditions for 2 weeks before permeability measurement, which gave a Kp of $13.0 \pm 3.9 \times 10^{-3}$ cm/h for hexanol, the same as untreated stratum corneum ($10.3 \pm 2.8 \times 10^{-3}$ cm/h).

Evidence from thermal analysis suggests that the effect of DMSO vapor on lipid endotherms can be reversed either by rinsing with water or by prolonged evaporation. In contrast, the permeability studies suggest that the reversible effect of DMSO vapor was only obtained when removal of DMSO with water was avoided. This has not been examined further, but Scheuplein and Ross [14] reported a similar effect and Chandrasekaran et al [15] have suggested that a concentration gradient with DMSO produces high osmotic stress within the stratum corneum that may lead to swelling, distortion, and intercellular delamination. This may not show on thermal analysis, but could affect permeability. However, the values of Kp after exposure to DMSO vapor are less than those obtained after exposure to DMSO liquid [12], preheating in excess of 80°C for 2 h or longer, and lipid extraction. Thus, if the DMSO is having an effect on the stratum corneum lipids, it is small relative to the effect of these other treatments.

Figures 2 and 3 show that for both penetrants exposure of stratum corneum to DMSO vapor following preheating up to 70°C produced a constant increased permeability. However, over the temperature range 70 to 80°C, this difference was eliminated, so that above 80°C there was no significant difference between stratum corneum exposed and not exposed to DMSO vapor. These changes are also shown in Table I, where the ratio of Kp with DMSO treatment to that without DMSO treatment are given. This pattern of behavior strongly indicates that both heat treatment and DMSO vapor were acting on the same, or very similar, sites within the stratum corneum. The evidence from thermal analysis indicates that this site is the lipid structure. One cannot conclude that both heat and DMSO have the same mechanism of action, because thermal analysis shows that there is a dif-

ference in their effects. On thermal analysis, heat progressively eliminates the 80°C endothermic transition, while DMSO vapor causes a lowering of both the 71 and 80°C transition to 59 and 65°C, respectively. In addition, the effect of heat is irreversible, while that of DMSO vapor is reversible. It is possible that, because the concentration of DMSO within stratum corneum from the vapor is relatively low, it is sufficient to interact with lipids to increase penetrant diffusivity, but insufficient to cause extraction. Subsequent removal of DMSO would allow the lipids to return to their original structure and so restore the barrier effect. Heat treatment would produce melting and hence large changes to the lipid structures that could not reform on subsequent cooling. The addition of small amounts of DMSO to such a grossly distorted lipid structure would have no further effect on their function as a barrier. However, the lipids would still be within the stratum corneum and, therefore, still exert some barrier effect, which could only be reduced further by their extraction.

The technique of using preheating of stratum corneum followed by exposure to penetration enhancers may provide a method for studying their site of action within stratum corneum. This possibility is being further investigated.

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