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Factors affecting the permeability of urea and water through nude mouse skin in vitro. I. Temperature and time of hydration

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Summary

Temperature studies on nude mouse skin in vitro indicated that a complete deterioration of the barrier to urea and water permeation occurred after 48 h (irrespective of temperature), with the most pronounced effect at 50 °C. The deterioration of the permeation barrier with increasing time of hydration was a very significant factor in the increasing permeation pattern of urea and water. This study indicated that the closed diffusion cell system is unsuitable as a method for determining percutaneous absorption in vitro. Because of the changes in the permeability characteristics that accompany deterioration of skin structure, the permeability data obtained with the closed diffusion cell system may be unrepresentative of normal skin. However, it may still have limited applications, e.g. to determine the percutaneous absorption of highly toxic substances prior to in vivo tests, provided a few aspects are kept in mind, namely: experiments should not be run at temperatures above 37 °C or longer than 24 h.

Introduction

The in vitro diffusion of drugs and other substances through human or animal skin is a well-known technique in preliminary studies regarding the flux and mechanism of percutaneous absorption. The validation of the closed diffusion cell system is the concern of this paper. It came to our attention in previous studies that the barrier in nude mouse skin changed continuously as a function of time during the permeation of hydrophilic compounds in a closed diffusion cell system (Ackermann and Flynn, 1987). Various possible causative factors were systematically studied (Van der Merwe, 1986). Temperature and time of hydration had pronounced effects on the permeability and physical deterioration of the skin (Van der Merwe and Ackermann, 1987). The permeability coefficients and activation energies for urea and water at various temperatures and times of hydration will be reported.

Materials and Methods

[^{14}C]Urea and [^{3}H]water were used as the permeating substances through nude mouse skin in a closed diffusion cell system as described by Van der Merwe and Ackermann (1987).

The permeability coefficients were calculated according to the procedure as described by Ackermann and Flynn (1987).

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Activation energies for urea and water permeation were calculated using the logarithmic form of the Arrhenius equation.

$$\log P = \log A - E/2.303RT$$

where P = the permeability coefficient of urea or water (cm/h); A = a constant called the frequency factor; E = the activation energy (cal/mol); R = the gas constant (1.987 cal/kmol); T = the absolute temperature (K). Log P was plotted against $1/T$ and the activation energies for urea and water permeation were calculated from the slope of the statistically fitted line which was $-E/2.303R$ (Blank et al., 1967).

Results and Discussion

Variance analysis of the permeability data by means of the SAS5 computer programme (SAS Institute, 1982) on a 5% level of significance was done on each variable and all possible combinations of the variables. The temperature at which the experiments were performed and the time of hydration had a significant effect on the permeability coefficients of urea and water. When evaluated in combination with temperature and time of hydration, the urea concentration and stirring of the donor and receiver cell phases in the diffusion cell system did not affect the permeability coefficients significantly. These two factors were therefore not considered during the determination of mean cumulative receiver cell concentrations and permeability coefficients of urea and water.

The permeability coefficients of urea and water at different temperatures as a function of time are given in Tables 1 and 2, respectively, and the activation energies for urea and water permeation as a function of time are listed in Table 3.

The same pattern of increased permeation with time for the simultaneous permeation of urea and water observed by Ackermann and Flynn (1987) is evident (Tables 1 and 2). It seems as if there is no significant difference in the mechanism of, or the barrier to the permeation of urea and water.

The temperature coefficient for the permeation of compounds is expressed as the Q_{10}-value, the increase in permeation rate due to a 10 °C rise in temperature (Scheuplein and Blank, 1971). The Q_{10}-value for water permeation through human skin in vitro is given as 2.0–2.4 in the temperature range 20–50 °C (Mali, 1956; Scheuplein, 1965). The calculated Q_{10}-value for water permeation through nude mouse skin in this study was 0.9–82 in the temperature range 10–50 °C. The value of 82 was obtained after 48 h of permeation and a temperature increase from 37 to 50 °C. This very high Q_{10}-value for water permeation indicates a total deterioration of the barrier to water permeation under these conditions.

Even though using Arrhenius-like activation energies to characterize the temperature dependency of diffusion has many limitations (Blank et al., 1967; Smith, 1982), valuable information may be gained relative to specific mechanisms of transport and diffusion pathways. The similarity in the

TABLE 1

Permeability coefficients of [^{14}C]urea at different temperatures for 48 h (irrespective of urea concentration and stirring)

Time (h)	*Permeability coefficient × 10 (cm/h)*			
	10 °C	25 °C	37 °C	50 °C
6 (16) [a]	0.01 ± 0.01 [b]	0.01 ± 0.01	0.02 ± 0.01	0.3 ± 0.1
12 (12)	0.2 ± 0.1	0.3 ± 0.2	0.5 ± 0.4	19 ± 7
24 (8)	1.7 ± 0.5	2.8 ± 1.6	4.3 ± 0.5	286 ± 54
48 (4)	5.5 ± 0.2 [c]	7.9 ± 2.3	11 ± 7 [d]	1573 ± 304 [c]

[a] Number of determinations in parentheses.
[b] ± S.D.
[c] Mean of 3 determinations.
[d] Mean of 2 determinations.

TABLE 2

Permeability coefficients of [^{3}H]water at different temperatures for 48 h (irrespective of urea concentration and stirring)

Time (h)	Permeability coefficient × 10 (cm/h)			
q	10 °C	25 °C	37 °C	50 °C
6 (16) [a]	0.02 ± 0.01 [b]	0.05 ± 0.02	0.10 ± 0.04	1.0 ± 0.5
12 (12)	0.4 ± 0.3	0.8 ± 0.4	1.8 ± 0.9	51 ± 17
24 (8)	4.7 ± 1.9	10 ± 3	17 ± 6	539 ± 142
48 (4)	16 ± 4 [c]	21 ± 5	31 ± 17 [d]	3313 ± 574 [c]

[a] Number of determinations in parentheses.
[b] ± S.D.
[c] Mean of 3 determinations.
[d] Mean of 2 determinations.

activation energy values for urea and water permeation (Table 3) again signifies the common diffusional pathway for these two compounds. The near constant activation energies for the first 24 h of urea and water permeation in the temperature range 10–37 °C (Table 3) indicate that no change occurred in the mechanism of transport or the diffusion pathway during the first 24 h of permeation. However, at 48 h there is a significant decrease in the activation energy for the permeation of both urea and water, indicating a definite change in the diffusional pathway for these compounds. The activation energy for urea diffusion in water at 19 °C is 4.5 kcal/mol (Longsworth, 1954) and that for water at 25 °C is 4.6 kcal/mol (Wang et al., 1953). These values correspond well with those for urea (4.5 kcal/mol) and water (4.4 kcal/mol) permeation at 48 h in the temperature range 10–37 °C obtained in this study. It seems that the resistance of the barrier to urea and water permeation at 48 h (irrespective of temperature) is the same as that offered by water. The skin offers thus no more resistance than a layer of water.

TABLE 3

Activation energies (E_a) of [^{14}C]urea and [^{3}H]water as a function of time for temperatures ranging from 10 °C–37 °C (irrespective of urea concentration and stirring)

Time (h)	Activation energy (kcal/mol)	
	Urea	Water
6	6.7 ± 0.6 [a]	10.1 ± 0.7
12	6.6 ± 1.1	9.3 ± 1.4
24	6.2 ± 0.3	8.5 ± 0.5
48	4.5 ± 0.3	4.4 ± 0.2

[a] ± S.D.

Because of the deviation of the permeability coefficients at 50 °C from the straight line relationship found for temperatures 10–37 °C, it could not be included in the calculation of the activation energies. It seems likely that the change in the diffusional pathway occurs more quickly at 50 °C. It is possible that there may be a complete change in the mechanism of transport of urea and water at this high temperature, but not enough data points at high temperatures are available to further investigate this possibility. The change in the mechanism of urea and water permeation at higher temperatures would involve a study on its own and did not fall within the scope of this investigation.

To conclude, it seems that the increasing permeation of urea and water is independent of the concentration of urea used and the stirring of the donor and receiver cell phases. Temperature studies indicated that a complete deterioration of the barrier to urea and water permeation occurred after 48 h (irrespective of temperature), with the most pronounced effect at 50 °C. The deterioration of the permeation barrier with increasing time of hydration is therefore a very significant factor in the increasing permeation pattern of urea and water.

Conclusion

This study indicated that the closed diffusion cell system is unsuitable as a method of determining percutaneous absorption in vitro. Because of the changes in the permeability characteristics that accompany deterioration of skin structure, the permeability data obtained with the closed diffusion cell system may be unrepresentative of normal skin. However, it may still have limited applications, e.g. to determine the percutaneous absorption of highly toxic substances prior to in vivo tests, provided a few aspects are kept in mind, namely the following.

- Not to measure absorption rates after a period of 24 h, since structural deterioration of the skin permeation barrier would lead to erroneous conclusions regarding absorption rates obtained. Deterioration of the skin permeation barrier occurs upon immersing the skin in the diffusion medium, but activation energies indicated that this deterioration became very prominent after a period of 24 h.
- To avoid temperatures higher than 37 °C due to the rapid deterioration of the skin at higher temperatures.

It should therefore be stressed that in vitro permeability studies with diffusion cells should include the validation of all methods used. Experimental artifacts may occur, for example, when skin is left in the closed cells to equilibrate for hours or overnight. If the stratum corneum is exposed to a hydrating diffusion medium, the barrier starts changing from the instant that it comes into contact with the medium.

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