Ether–water partitioning and permeability through nude mouse skin in vitro. II. Hydrocortisone 21-n-alkyl esters, alkanols and hydrophilic compounds

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Summary

The permeability coefficients obtained by means of an infinite dose diffusion cell system with nude mouse skin in vitro were compared to the ether–water partition coefficients of the following compounds: glucose, urea, glycerol, thiourea, alkanols (methanol to octanol), hydrocortisone and hydrocortisone 21-n-alkyl esters (-acetate to -heptanoate). The permeability coefficients ranged from 1.0×10^{-6} cm/h (glucose) to 0.34 cm/h (hydrocortisone-21-heptanoate). The ether–water partition coefficients ranged between 9.0×10^{-6} (glucose) and 1.4×10^{4} (hydrocortisone-21-heptanoate). No general pattern emerged when the permeability coefficient is taken as a function of the ether–water partition coefficient for these compounds. Therefore the skin can not be regarded as a simple lipoidal barrier.

Introduction

The skin has been regarded as a simple lipoidal barrier by Treherne (1956), Scheuplein and Blank (1973) and Dürrheim (1977) to various compounds. It can therefore be expected that the ether–water partition coefficients could be used to model the partitioning of compounds into the lipid phase of the skin. The objective when comparing the permeability coefficients of compounds ranging widely in hydrophobicity to their ether–water partition coefficients is to see whether a pattern emerges which can be used as a guide to

the possible mechanisms by which these compounds traverse the skin and particularly the stratum corneum. If that could be done, the ether–water partition coefficient of a compound can be used as a parameter to estimate its permeability coefficient through the skin.

The compounds used in this study, their permeability coefficients and ether–water partition coefficients are listed in Table 1.

The ether–water partition coefficients of hydrocortisone were reported by Katz and Shaikh (1965), Nathansohn et al. (1969) and Flynn (1971), those of hydrocortisone-21-acetate by Katz and Shaikh (1965), Mantica et al. (1970) and Flynn (1971) and those of hydrocortisone-21-butyrate and hydrocortisone-21-hexanoate only by Flynn (1971).

The values found by the different workers dif-

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fer substantially and except for hydrocortisone and hydrocortisone-21-acetate the data on the ether–water partition coefficients of the other hydrocortisone 21-*n*-alkyl esters were only assessed by Flynn (1971).

The ether–water partition coefficients were determined to supplement the information available in the literature and to verify the values found by previous workers. The ether–water partition coefficients of hydrocortisone and a series of 21-*n*-alkyl esters were determined by means of a HPLC method. This method obviated concerns of impurity of the compounds as it is molecularly specific.

Materials and Methods

The materials and methods for the determination of the permeability coefficients and ether–water partition coefficients of all the compounds mentioned in this study, had been described previously as indicated in Table 1. All the permeability coefficients were obtained by means of an infinite dose diffusion cell system with nude mouse skin (SKH-hr-1 strain) in vitro. Only the determination of the ether–water partition coefficients of hydrocortisone and the hydrocortisone-21-*n*-alkyl esters will be described here.

Hydrocortisone was obtained from the Upjohn Company and was used without further purification. The hydrocortisone 21-*n*-alkyl esters (acetate through to heptanoate) were synthesised as outlined by Hagen (1979) and Smith (1982) by reacting an excess of the anhydride of a specific acid with 5 g of hydrocortisone in 100 ml of pyridine.

Double-distilled water and analytical reagent ether were used to prepare the cosaturated phases. Water and ether were added to a 2-litre separator and shaken to obtain cosaturation of the phases. The separator was set aside for 24 h to allow for temperature and further phase equilibrium. All remaining steps were carried out at room temperature $(23 \pm 1^{\circ}C)$. An appropriate amount of steroid (1–10 mg, depending on the steroid) was accurately weighed and put into a 50 ml conical flask. This was dissolved in the ether phase, which was maintained at approximately 25–50 ml. An ap-

TABLE 1

Permeability coefficients and ether–water partition coefficients of glucose, urea, glycerol and thiourea, a homologous series of alkanols and hydrocortisone and its 21-n-alkyl esters

Compound	Permeability coefficient (cm/h)	Ether–water partition coefficient
Glucose	9.5×10^{-5} a	8.7×10^{-6} a
Urea	1.2×10^{-4} a	3.2×10^{-4} a
Glycerol	1.4×10^{-4} a	5.1×10^{-4} a
Thiourea	9.6×10^{-5} a	7.3×10^{-3} a
Methanol	2.6×10^{-3} b	9.6×10^{-2} c
Ethanol	4.8×10^{-3} b	2.8×10^{-1} c
Propanol	5.4×10^{-3} b	9.4×10^{-1} c
Butanol	1.5×10^{-2} b	5.9 c
Pentanol	2.2×10^{-2} b	1.6×10^{1} c
Hexanol	4.8×10^{-2} b	6.3×10^{1} c
Heptanol	9.3×10^{-2} b	2.4×10^{2} d
Octanol	9.7×10^{-2} b	8.9×10^{2} d
Hydrocortisone	1.6×10^{-4} e	1.4 f
Hydrocortisone-21-acetate	1.7×10^{-3} e	2.7×10^{1} f
Hydrocortisone-21-propionate	9.8×10^{-3} e	9.5×10^{1} f
Hydrocortisone-21-butyrate	4.3×10^{-2} e	3.0×10^{2} f
Hydrocortisone-21-pentanoate	7.1×10^{-2} e	9.1×10^{2} f
Hydrocortisone-21-hexanoate	2.0×10^{-1} e	4.1×10^{3} f
Hydrocortisone-21-heptanoate	3.4×10^{-1} e	1.4×10^{4} f

Also see Fig. 2. a, Taken from Ackermann (1983); b, taken from Dürrheim (1977); c, taken from Hansch and Leo (1979); d, calculated from the literature values mentioned under c; e, taken from Smith (1982) – values obtained without the enzyme inhibitor; f, this study.

propriate amount of water phase (25–500 ml depending on the solubility of steroid) was placed in an appropriately sized separator (60–1000 ml). The steroid solution in the ether phase was added with no attempt to rinse any residual steroid from the flask or funnel used. The phases were shaken intermittently, allowing sufficient time between agitations for the phases to reform.

The system was allowed to stand for 20 to 30 minutes to assure complete phase separation. Then the aqueous phase was transferred to a clean, appropriately sized separator with great care taken to assure that no ether phase contaminated it at this point. The ether phase was immediately transferred to a 100-ml round bottom flask, the phase weight was recorded and the phase was evaporated to dryness on a Buchi evaporator. The aqueous phase was extracted with four 25-ml portions of
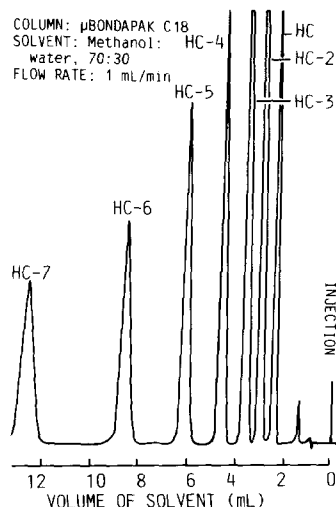
Fig. 1. Separation of hydrocortisone (HC) and its 21-*n*-alkyl esters (HC-2 to HC-7) on an HPLC chromatogram under the specified conditions (unequal concentrations).

TABLE 2

Logs of ether – water partition coefficients (log k_p) of hydrocortisone and its 21-n-alkyl esters obtained in this study and those of other authors

Compound	Log k_p
Hydrocortisone	0.15 a
	0.21 b
	0.18 c
	0.11 d
Hydrocortisone-21-acetate	1.43 a
	1.42 b
	1.09 e
	1.11 d
Hydrocortisone-21-proprionate	1.98 a
Hydrocortisone-21-butyrate	2.48 a
	2.37 b
Hydrocortisone-21-pentanoate	2.96 a
Hydrocortisone-21-hexanoate	3.61 a
	3.56 b
Hydrocortisone-21-heptanoate	4.14 a

a, This study; b, Flynn (1971); c, Nathansohn et al. (1969); d, Katz and Shaikh (1965); e, Mantica et al. (1970).

methylene chloride and the extracts were collected in a 250-ml round bottom flask. The combined extracts were then evaporated to dryness on the evaporator. The dried-phase residues were reconstituted with reagent grade methanol (usually 5–25 ml of methanol for the aqueous phase residue and 25–50 ml of methanol for the ether phase residue) and assayed by the following HPLC procedure (Hagen, 1979).

A Waters Associates solvent delivery system and absorbance detector were used with a reverse-phase high-efficiency liquid chromatography column. The mobile phase was a 70 : 30 mixture of reagent grade methanol and double-grade distilled water. The flow rate of the mobile phase was 1.0–1.5 ml/min. The attenuation reference used was 0.1, the injection volume was 50 μl and the chart speed 0.25 cm/min. An Omniscribe recorder was used. The peak heights of the samples were compared to a concentration standard curve. The separation for hydrocortisone and its 21-*n*-alkyl esters on an HPLC chromatogram is shown in Fig. 1.

Results and Discussion

The logs of the obtained ether–water partition coefficients for hydrocortisone and the hydrocorti-sone 21-*n*-alkyl esters were listed together with those of other workers in Table 2.

All the ether–water partition coefficients obtained in this study are approximately the same as those obtained by other researchers.

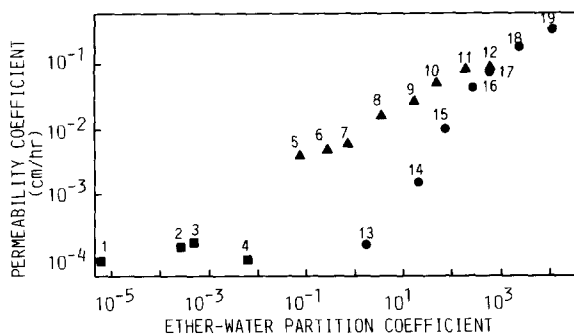When the logs of the ether–water partition coefficients were plotted as a function of alkyl

Fig. 2. The permeability coefficient (cm/h) for nude mouse skin as a function of the ether–water partition coefficient on a log–log scale for the following compounds: (1) glucose, (2) urea, (3) glycerol, (4) thiourea, (5–12) *n*-alkanols, methanol to *n*-octanol, (13) hydrocortisone, (14–19) hydrocortisone 21-*n*-alkyl esters, -acetate to -heptanoate.

chain length a linear line relationship is found and the slope of the plot is the π value as defined by Fujita et al. (1964). The π values in this study and for the data of Flynn (1971) are 0.542 and 0.536 with correlation coefficients of 0.9992 and 0.9985, respectively. The excellent correlation found in this study indicates that the HPLC is a reliable method to determine the partition coefficients.

The ether–water partition coefficients of urea, thiourea, glycerol and glucose, a series of alkanols and hydrocortisone and its 21-*n*-alkyl esters and their permeability coefficients through nude mouse skin in vitro are listed in Table 1. These values are plotted on a scale in Fig. 2.

The permeability coefficients of the hydrophilic compounds (glucose, urea, glycerol and thiourea) vary only from 1.0×10^{-4} cm/h while their ether–water partition coefficients vary from 9.0×10^{-6} to 7.2×10^{-3}. There is no correlation between the partition coefficients and permeability coefficients for these compounds, which might indicate that permeation of hydrophilic compounds, with ether–water partition coefficients very much less than unity, does not depend upon the lipophilicity of the compound.

The mechanism of permeation of such hydrophilic compounds through nude mouse skin might therefore be mainly through the water-filled intercellular channels in the stratum corneum.

For the alkanols, the permeability coefficients seem to level off on going below a partition coefficient of 1 on the scale in Fig. 2. This corresponds to a lower limit permeability coefficient for the alkanol series of about 5.4×10^{-3} cm/h.

It seems from Fig. 2 that there is a lower limit to permeability coefficients for nude mouse skin. This agrees with the suggestion made by Scheuplein and Blank (1973) that there is a finite lower limit of approximately 10^{-6} cm/h for human skin permeability coefficients. There seems to be an upper limit at approximately 0.5 cm/h, which corresponds roughly to the permeability coefficients found for different compounds through the dermis only of nude mouse skin (Dürrheim, 1977; Smith, 1982; Ackermann et al., 1985).

It is significant to note that with the compounds mentioned in Fig. 2 the ether–water parti-

tion coefficient changes with approximately 10 logarithmic orders while the permeability coefficient changes only 4 logarithmic orders. Thus π for partitioning into the lipids of the stratum corneum may be the same for both the alkanols and the hydrocortisone ester series but the π for permeation may deviate markedly from this value (Smith, 1982). This means that different diffusion pathways are not the only determinant of diffusivity and rate of permeation.

This discussion of the relation between ether–water partitioning and permeability was based on the permeability coefficients calculated from data obtained over the first 6 h of an experiment. But as mentioned earlier, the permeation of the hydrophilic compounds increased gradually as a function of time. This phenomenon was not mentioned in literature on the permeation of these compounds through oral mucosa (Siegel, 1981) or rabbit skin (Treherne, 1956); it is the topic of ongoing research in this laboratory.

Conclusion

The ether–water partition coefficients of hydrocortisone and its derivatives found in the literature were relatively reliable estimates easily and accurately complemented and verified in this work. When comparing the permeability coefficients to the ether–water partition coefficients of compounds ranging widely in hydrophobicity, no general pattern emerged. Thus the skin does not act as a simple lipoidal barrier with regard to compounds ranging much in hydrophobicity. The ether–water partition coefficients could therefore not be used to estimate the permeability coefficient of a compound through nude mouse skin in an infinite dose diffusion cell system.

Since a phenomenon of increasing permeability as a function of time was observed for urea, thiourea, glycerol, glucose, water and methanol by Ackermann (1983) and for hydrocortisone and hydrocortisone 21-*n*-alkyl esters (Smith, 1982 and Behl et al., 1984), one should be cautious about drawing extensive conclusions on the ether–water partitioning/percutaneous absorption relationships while there is uncertainty about the permeability coefficient that should be used.

Acknowledgements

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