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The partitioning of some 21-alkyl steroid esters between human stratum corneum and water

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Summary

Partition coefficients of [4-^{14}C]hydrocortisone and [1,2,6,7-^{3}H]cortisone and their esters, between stratum corneum and 0.9% saline decreased with increasing temperature. Free energies of transfer ($G_{w \rightarrow l}$) were negative, and the process was entropy controlled for the longer chained esters. Esterification of 21-OH led to an increase in partitioning; the incremental free energy of transfer from normal saline to stratum corneum being about 75% of the (negative) free energy of transfer from water to octanol. The logarithms of the stratum corneum–normal saline partition coefficients were rectilinearly related to the logarithms of the octanol–water partition coefficients. Partition coefficients of the hydrocortisone series were lower than those of the corresponding cortisone compounds between stratum corneum and normal saline; with isopropyl myristate, octanol and DMPC liposomes as the oil phase the order is reversed. There was no significant difference between the stratum corneum–normal saline partition coefficients of hydrocortisone when skin from three different body sites were compared, the inclusion of stratum corneum from the finger resulting in a difference in partitioning. It was concluded that of the various lipophilic phases examined as potential models for the stratum corneum with respect to cortisone and hydrocortisone ester partitioning, the liquid crystalline liposome was most suitable. When a thermodynamic evaluation was undertaken using the methylene group contribution to the free energy of partitioning, cyclohexane gave values closest to those of stratum corneum. This suggests that the ester chain occupies a site in the stratum corneum of low dielectric constant.

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Introduction

Since Overton and Meyer's original concept that membranes take up lipophilic materials more readily than hydrophilic materials, it has been generally accepted that the rate of absorption of a drug increases with lipid solubility, and that lipid–water partition coefficients provide a convenient scale for expressing the relative polarities of molecules (Konigstein, 1923). The stratum corneum presents the principal resistance to diffusion of molecules through intact skin. Recently, electron microscope studies of the horny layer showed that the surface is composed of flattened polygonal cells and the cell membranes are not in contact with each other. The intercellular spaces are filled with multilaminated structures which are derived from membrane coating granules. The cells are densely packed with keratin fibrils and are separated from each other by narrow spaces (Kligman, 1983). With the hydrophilic protein-rich intercellular spaces within the corneocytes, the hydrophobic cell membrane and the extracellular lipid, the stratum corneum can be represented as a hydrophilic-lipophilic multilayered structure. Recently, a quantitative analysis of human stratum corneum lipids was reported together with data of the variability in lipid composition for skin taken from sites exhibiting known differences in solute permeability (Elias et al., 1983). The water content of the stratum corneum varies with both the humidity of the environment and the bodily location of the skin. If the surface is occluded with a water-impermeable barrier, the stratum corneum slowly imbibes water, and will ultimately absorb up to six times its dry weight (Scheuplein, 1965). A certain degree of water solubility is therefore an additional prerequisite for solute uptake. These considerations are discussed in relation to stratum corneum permeability by Scheuplein et al. (1969).

Partitioning is a major contributing factor in the penetration of a wide variety of solutes through biological membranes. Knowledge of the factors involved in the partitioning of a solute can lead to a better understanding of the process of permeation and the nature of the barrier phase. From a thermodynamic analysis of the process by which solutes distribute between water and membranes Diamond and Wright (1969) have shown that the solute–water interactions tend to have a greater influence on the permeation of solutes through biological membranes than solute–membrane interactions.

In this work the partition coefficients of hydrocortisone and cortisone esters between stratum corneum and 0.9% saline were determined and compared with partitioning of the same solutes between some lipophilic fluids and normal saline. Such studies may lead to a better understanding of the lipophilic environment, the energetics of the process and the development of suitable models for the investigation of transdermal delivery of drugs.

Materials and Methods

Δ^4-Pregnen-11,17,21-triol-3,20-dione (hydrocortisone) was donated by Boots, U.K. and Δ^4-pregnen-17,21-diol-3,11,20-trione (cortisone) was purchased from Sigma

Chemicals, U.K. Labelled steroids [4-^{14}C]hydrocortisone (1.85 MBq · mg^{-1}) and [1,2,6,7-^{3}H]cortisone (37 MBq · mg^{-1}) were purchased from Amersham International, U.K. Hydrocortisone and cortisone esters were prepared and synthesized from acid chlorides of chemical grade. Reagents and their purities were checked by TLC. The materials used and synthesis of hydrocortisone acetate, propionate, valerate, hexanoate and octanoate, and of cortisone acetate, butyrate, hexanoate and octanoate have been described previously (Saket et al., 1984). Sodium chloride (Analar) and double-distilled water were used to prepare the aqueous phase. Trypsin and cyclohexane were obtained from BDH, U.K. Isopropyl myristate (IPM) was reagent grade (Fluka AG).

Preparation of human stratum corneum

Skin was obtained from surgical specimens. In keeping with the method of Kligman and Christophers (1963), the fat was trimmed, and the skin immersed in a water bath at 60°C for 3 min at which time the intact epidermis could readily be teased off with forceps. The epidermal sheet was placed dermal side down on the surface of an aqueous solution of trypsin (0.0005%) and sodium bicarbonate (0.5%) in a petri dish and incubated at 37°C for 15 h. After incubation, the lower softened layers of the epidermis were removed by firmly rubbing the tissue, and the stratum corneum smoothed out on a glass plate, with moistened cotton wool. The tissue was then examined by phase-contrast microscopy for the presence of epidermal cells other than those of the stratum corneum.

Assay of radiolabelled steroids

All aqueous samples were adjusted to 1 ml and incorporated into 10 ml of scintillation cocktail prior to counting. Counting efficiencies were of the order of 89% for ^{14}C-samples and 35–40% for ^{3}H-samples.

The scintillation cocktail consisted of 2,5-diphenyloxazole (PPO) 15 g, 1,4-di(4-methyl-5-phenyl oxazol)-benzene (dimethyl POPOP) 300 mg, toluene 2 l and Triton X-100 1 litre. All materials were of scintillation grade and obtained from BDH, U.K.

Determination of partition coefficients

Weighed aliquots of stratum corneum were equilibrated with 0.9% saline containing the labelled ester. Partition coefficients were determined from the loss in concentration of the solution phase as described by Scheuplein (1965).

Results and Discussion

It has been commonly recognised, but poorly documented, that the thickness of the epidermis as a whole (Southwood, 1955; Rushmer et al., 1966) and of the stratum corneum portion of the epidermis specifically, vary in thickness and numbers of cell layers in different regions of the body.

Maibach et al. (1971) showed that the palm, of which the thick stratum corneum is allegedly almost impenetrable, allowed approximately the same penetration as the

TABLE 1

PARTITIONING OF HYDROCORTISONE AT 37°C BETWEEN HUMAN STRATUM CORNEUM AND 0.9% SALINE DETERMINED FOR DIFFERENT SITES

Site	Partition coefficient (K_m) ± S.D. (n = 4)
Axilla	7.02 ± 1.03
Breast	8.00 ± 1.70
Abdomen	8.38 ± 1.97
Finger	11.25 ± 1.37

forearm. The abdomen and dorsum of the hand had twice the penetration of the forearm, whereas follicle-rich sites, including the scalp, angle of the jaw, postauricular area, and forehead, had fourfold greater penetration. The intertriginious axilla had a 4-fold increase, whilst the scrotum allowed almost total absorption.

In this work stratum corneum samples from regions of the human body were selected for initial partitioning studies. The partition coefficients (K_m) of hydrocortisone at 37°C between stratum corneum from these regions and 0.9% saline are shown in Table 1.

Analysis of variance showed that the results for axilla, breast and abdomen were not significantly different. However, the partition coefficients from the horny layer of the finger's friction surface is significantly larger then the other sites at the $P' = 0.05$ level. The higher K_m value for finger stratum corneum, which is much thicker than from the other sites, probably results from differences in structure and chemistry of the tissue from this region. Subsequent work was carried out only on abdominal skin.

The nature of the organic phase and the choice of the reference solvent to be employed for partition studies has been a topic of active discussion. The two extremes that have been employed are inert hydrocarbon solvents, such as cyclohexane, and polar solvents such as octanol. Polar solvent systems have been advocated by many workers who have attempted to correlate biological activity with physicochemical properties of drug molecules (Burton et al., 1964; Flynn, 1971; Hansch and Dunn, 1972). In direct contrast, others (Beckett and Moffat, 1969; Bickel and Weder, 1969; Shibab, 1971) have found that the partition coefficients obtained with the system heptane or cyclohexane–water (buffer) correlate biological data better than those obtained using polar solvents.

Leo et al. (1971) have shown that the logarithms of binding constants to proteins and of lipid–water partition coefficients are often linearly related to the logarithms of the corresponding octanol–water partition coefficients. Since the stratum corneum contains mainly protein, lipid and bound water, similar types of relationships between log stratum corneum–water partition coefficients (K_m) of solutes and log octanol–water partition coefficients (K_0), may be anticipated, that is:

$$\log K_m = a \log K_0 + c \qquad (1)$$

TABLE 2

PARTITION COEFFICIENT VALUES OF HYDROCORTISONE AND CORTISONE ESTERS IN PARTITIONING BETWEEN STRATUM CORNEUM, OCTANOL AND NORMAL SALINE AT 25°C

Solute	log K_m (stratum corneum)	log K_0 (octanol)
Hydrocortisone	0.85	1.55
Hydrocortisone acetate	1.23	2.19 [a]
Hydrocortisone propionate	1.51	2.77 [a]
Hydrocortisone valerate	2.10	3.27 [a]
Hydrocortisone hexanoate	2.39	3.70
Hydrocortisone octanoate	2.91	4.37
Cortisone	0.91	1.42
Cortisone acetate	1.30	2.10 [a]
Cortisone butyrate	1.80	2.80 [a]
Cortisone hexanoate	2.43	3.30
Cortisone octanoate	2.93	4.22

[a] Calculated from data reported by Hansch and Leo (1979).

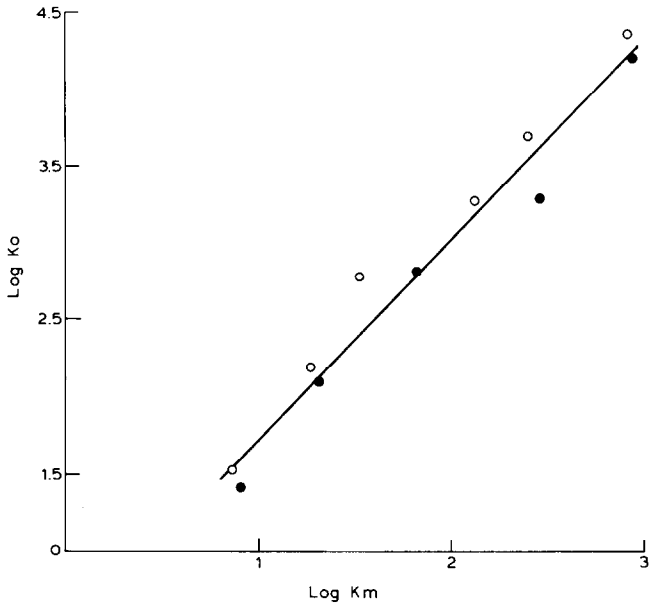

Fig. 1. The logarithmic relationship between the stratum corneum–water partition coefficient (K_m) and the octanol–water partition coefficient (K_0) for the various esters of cortisone and hydrocortisone.

$\log K_m = 1.313 \log K_0 + 0.45$

○, hydrocortisone esters; ●, cortisone esters.

TABLE 3

COLLANDER-TYPE RELATIONSHIPS FOR ESTERS OF CORTISONE AND HYDROCORTISONE DISTRIBUTING INTO STRATUM CORNEUM AND VARIOUS LIPOPHILIC PHASES

$\log K_m = m \log P + C$

Lipophilic phase	m	C	r	n
Octanol	1.313	0.449	0.984	11
Isopropyl myristate	1.382	0.125	0.993	11
Cyclohexane	0.730	−1.474	0.951	11
DMPC liposomes (fluid)	1.187	0.806	0.981	8

Stratum corneum–normal saline and octanol–water partition coefficients used to correlate log K_m with log K_0 are listed in Table 2. Octanol–water partition coefficients were obtained from published data (Leo et al., 1971) for cortisone and hydrocortisone acetates, while the other ester partition coefficients were determined experimentally.

A plot of log K_m against log K_0 containing data for both hydrocortisone and cortisone and their respective esters is linear (Fig. 1) and characterized by the following regression line:

$$\log K_m = 1.3 \log K_0 + 0.45 \quad (n = 11,\ r = 0.984) \tag{2}$$

where n is the number of data points and r the correlation coefficient.

Similar plots were obtained when cyclohexane, isopropyl myristate and liposomes were substituted for octanol. Regression data are given in Table 3. All correlations are good, and their slopes are close to unity, of which the liposome value is the nearest.

The partition coefficients of cortisone and hydrocortisone and their esters have been shown to increase logarithmically with carbon number when octanol, isopropyl myristate and liposomes were used as non-aqueous phases (Saket et al., 1984). Stratum corneum partitioning behaves in a like manner. Substitution of a keto group with hydroxyl, as in moving from cortisone to hydrocortisone decreases K_m from 8.5 to 7.0, and the permeability coefficient (k_p) from 10×10^{-6} cm · h^{-1} to 3×10^{-6}

TABLE 4

LOG PARTITION COEFFICIENT VALUES FOR CORTISONE AND HYDROCORTISONE DISTRIBUTING BETWEEN SALINE AND VARIOUS PHASES

	Log partition coefficient			
	IPM	DMPC	Octanol	Stratum corneum
Cortisone	1.419	1.793	1.418	0.930
Hydrocortisone	1.63	2.000	1.551	0.845

$cm \cdot h^{-1}$ (Schaeffer et al., 1982). Similarly in moving from cortexone to cortexolone, k_p decreases from 450×10^{-6} to 75×10^{-6} $cm \cdot h^{-1}$ and K_m from 37 to 23. Table 4 shows that, while the relative values of the cortisone and hydrocortisone series take this order with respect to stratum corneum partitioning, they take the reverse order with octanol, IPM and liposomal partitioning.

There is a strong hydrophobic interaction between the hydrated proteinaceous membrane and the rigid planar steroid molecules (Lien et al., 1971). In the absence of intramolecular bonding, inductive effects, and chain branching, each $-CH_2-$ group increases partitioning, whereas each polar group reduces partitioning depending on the ability of the group to form hydrogen bonds with water. The effect of the $-CH_2-$ group in the aqueous phase is explained in terms of hydrophobic interactions. On the other hand, the effect of the polar groups is explained by the greater amount of energy needed to break the hydrogen bonds and remove the solute from water.

The thermodynamics of partitioning of a solute between the aqueous phase (w) and a lipid phase (1) at a fixed temperature are described by:

$$\Delta G_{w \to 1} = -RT \ln K = \Delta H_{w \to 1} - T\Delta S_{w \to 1} \quad (3)$$

where $\Delta G_{w \to 1}$ is the free energy of transfer of a solute from an aqueous phase to a lipid phase. $\Delta H_{w \to 1}$ and $\Delta S_{w \to 1}$ are the corresponding enthalpy and entropy terms.

Linear regression of partition coefficients at a range of temperatures was used to calculate thermodynamic functions for the steroids. These thermodynamic parameters, $\Delta H_{w \to 1}$, $\Delta S_{w \to 1}$ and $\Delta G_{w \to 1}$ permit discussion of the role in the partition of drugs of such factors as solvation, magnitude of the energy of solvation, group transfer parameters and the effect of conformation upon solvation (Allawala and Riegelman, 1954; Pimentel, 1960; Hegna, 1977).

Free energies of transfer of cortisone and hydrocortisone esters from water to isopropyl myristate are negative (Table 5), and decrease rectilinearly with increasing carbon number, giving free energy increments per methylene group of -2.25 and -2.27 $kJ \cdot mol^{-1}$, respectively. Enthalpies of transfer are also negative, and decrease as the homologous series are ascended, reflecting the greater solubility in isopropyl myristate than in water, the difference increasing with increasing carbon number. Entropies of transfer are positive, and increase rectilinearly as the homologous series are ascended. This is attributed to hydrophobic interactions (Ben Naim, 1980), in which solvation spheres of water molecules surround the solute molecules, giving a more ordered state than obtains in isopropyl myristate.

The free energies of transfer of hydrocortisone esters between normal saline and stratum corneum follow a similar pattern to the isopropyl myristate system. The enthalpies are negative but for the stratum corneum, the enthalpy increases with increasing carbon number. Entropies of transfer again increase with carbon number, but the values for the lower homologues are negative. This behaviour can be explained if the non-aqueous phase is regarded as hydrated stratum corneum, in which both water and lipid act as solvents. Solution in structured lipids will result in more random distribution, and hence an increase in entropy, while hydrophobic

TABLE 5

DERIVED THERMODYNAMIC PARAMETERS FOR THE TRANSFER OF HYDROCORTISONE AND CORTISONE ESTERS FROM AQUEOUS TO LIPOPHILIC PHASES AT 303°K

Drug	Liposome (DMPC)			Stratum corneum			Isopropyl myristate		
	ΔG	ΔH	ΔS	ΔG	ΔH	ΔS	ΔG	ΔH	ΔS
Hydrocortisone	−11.60 [a]	20.24 [a]	105 [a]	−5.44	−8.85	−11.25	−8.75	−2.74	19.83
Hydrocortisone acetate	−13.24 [a]	43.23 [a]	186 [a]	−7.3	−12.83	−18.25	−10.5	−7.03	11.40
Hydrocortisone propionate	−16.0 [a]	33.0 [a]	162 [a]	−8.6	−11.75	−10.29	−13.83	−8.60	17.26
Hydrocortisone valerate	–	–	–	−11.9	−10.6	4.31	−17.60	−11.41	20.43
Hydrocortisone hexanoate	–	–	–	−13.74	−8.85	16.14	−20.33	−12.82	24.78
Hydrocortisone octanoate	–	–	–	−16.81	−6.9	32.68	−24.44	−14.58	32.55
Cortisone	−10.4 [b]	22.7 [b]	109 [b]	–	–	–	−8.10	−19.21	−36.66
Cortisone acetate	−12.8 [b]	50.0 [b]	107 [b]	–	–	–	−10.09	−8.60	4.92
Cortisone butyrate	−18.5 [b]	40.40 [b]	196 [b]	–	–	–	−14.53	−11.43	10.23
Cortisone hexanoate	–	–	–	–	–	–	−19.39	−15.10	14.16
Cortisone octanoate	–	–	–	–	–	–	−23.5	−18.32	17.09

[a] Data reported by Saket et al. (1984).

[b] Data reported by Arrowsmith et al. (1980).

TABLE 6

FREE ENERGY VALUES $\Delta(\Delta G)_{-CH_2-}$ FOR THE TRANSFER OF DIFFERENT SOLUTES FROM AQUEOUS TO DIFFERENT LIPOPHILIC SYSTEMS AT 298°K

Solute	Stratum corneum [a]	Octanol [b]	IPM	(DMPC) liposomes [c]	Cyclohexane [d]
Phenolic [1]	−1.71	−2.4	–	1.55 *	−2.4
		−3.40			−3.1
Hydrocortisone esters [2]	−1.57	−1.99	−2.27	−2.9	−1.11
				−3.4 **	
Cortisone esters [3]	−1.57	−1.96	−2.23	−2.84 **	−1.29
Phenothiazine [4]	–	–	–	−1.90	–
Alcohols [5]	−1.64	−2.86	–	−1.9	–
				−2.70	
n-alkanes [b]	–	–	–	–	−3.95

[1] Calculated from data reported by: [a] Anderson et al. (1976); [b] Leo et al. (1971); [c] Rogers and Davis (1980); [d] Davis et al. (1976).
[2] and [3] Calculated from data reported by Saket et al. (1984).
[4] Calculated from data reported by Ahmed et al. (1981).
[5] Calculated from data reported by: (a) Scheuplein (1967); (b) Lien and Tong (1973); (c) Diamond and Katz (1974).
[6] Calculated from data reported by Wilhelm et al. (1977).
* 301°K.
** 288°K.

interaction will reduce the entropy of the hydrated component of the stratum corneum. As the homologous series is ascended, the balance between solubilities will increasingly favour the lipid component, so that the lower members give negative entropies and the higher members positive entropies.

There is not enough information to speculate on variations in transfer parameters between normal saline and the liposome system. However, the magnitudes and signs of the entropies and enthalpies of transfer indicate that on thermodynamic grounds, DMPC liposomes above the transition temperature are not likely to be a suitable model for skin.

The standard free energies of transfer for the methylene group from water to stratum corneum, octanol, IPM and cyclohexane for different solutes are given in Table 6. The nature of the lipophilic binding site(s) of the stratum corneum is likely to influence the incremental free energies of transfer (water to stratum corneum) for various hydrophobic groups. This site is probably less hydrophilic than octanol since the free energy of transfer per $-CH_2-$ group from water to stratum corneum is about 75% of the (negative) free energy of transfer of this group from water to octanol, and about 57% for phenolic compounds, 57% for alcohols, and 69% for hydrocortisone and cortisone esters from water to IPM (Table 5).

Davis et al. (1974) have suggested that since biological membranes are highly structured, there is a lower entropy contribution in the transfer as compared with an unstructured organic solvent. Leo et al. (1971), suggested that the solute molecules are partially desolvated when they are partitioned onto the surface of protein, whereas in the transfer to a lipid phase such as octanol complete desolvation occurs.

Steroids represent a class of substances exhibiting a complete spectrum of permeability from very high to very low (Scheuplein et al., 1969). Partition studies of hydrocortisone and cortisone esters using octanol and isopropyl myristate were reported by Saket et al. (1983). Cyclohexane was selected as a non-polar system as an extension to this work to see if partitioning of both solute esters in this system can be correlated to their stratum corneum–water partitioning. Partition coefficients of hydrocortisone esters are higher than those of cortisone esters in all systems examined (IPM, octanol, cyclohexane and DMPC liposomes) except in stratum corneum, where K_m values of cortisone are higher than those of hydrocortisone esters.

In conclusion, of the various lipophilic phases examined, none completely modelled the stratum corneum as assessed by the partitioning of esters of cortisone and hydrocortisone. Data analysis using the Collander relationship indicated that the 'fluid' or liquid crystalline liposome came closest to providing a partitioning environment for the test solutes comparable to that encountered with the hydrated stratum corneum. Although phospholipids form a minor component of the horny layer, it is unlikely that they are present as a smectic mesophase. Studies by Van Duzee (1975) indicated the presence of structured lipids following controlled rehydration of stratum corneum samples. Differential scanning calorimetry currently being undertaken in the authors' laboratories may provide information on the changes induced by partitioning solutes in the structural arrangements of lipids present in the stratum corneum. Thermodynamic assessment focussed on the 21-ester chain, and in particular the methylene group contribution to the free energy of partitioning. Cyclohexane, although lacking the required degree of polarity, was nevertheless a closer match with stratum corneum than the more polar solvents examined. The lipophilic phases which differentiated between the $\Delta(\Delta G)_{-CH_2-}$ for hydrocortisone and cortisone esters was the DMPC liposome, and cyclohexane where the constituent groups on the steroid nucleus were seen to influence the energetics of the C21-ester chain partitioning. A better definition of the morphological features of the stratum corneum and in particular the inter-relationship between protein and lipid components, should lead to a better understanding of solute interactions, and eventually the design of molecules with optimal skin permeability.

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