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Partitioning of some 21-alkyl esters of hydrocortisone and cortisone

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Summary

The temperature dependency of the partitioning of some steroids has been determined between dimyristoyl phosphatidylcholine liposomes; isopropyl myristate and 0.9% saline (0.15 M). Partition coefficients increased as a function of temperature below the endothermic phase transition temperature (T_c) of the phospholipid, but decreased above this temperature. The IPM–saline partition coefficients of the esters of hydrocortisone and cortisone decreased as a function of temperature. Free energies ($\Delta G_{w\rightarrow l}$) for all steroids studied were negative. The transfer process was found to be entropy-dominated in both cases. Partitioning into liposomes occurs into areas slightly more hydrophilic than *n*-octanol and IPM. Ketones substituted on the 11-position of 21-OH steroids have greater hydrogen-bonding capability than 11-OH compounds.

Esterification of the 21-hydroxyl leads to an increase in partitioning, because of a positive entropic effect and in spite of a large positive enthalpic effect. This opposing enthalpic effect is due to the increased difficulty in inserting the steroid acetate into a bilayer, due to its increased dimensions relative to the parent steroid. The negative free energy effect of steroidal side-chain lengthening per $-CH_2-$ group is higher than those reported for methylene group contribution of other solutes in DMPC liposome partitioning systems.

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Introduction

Thermodynamic analysis of drug partitioning can lead to an insight into drug transport processes and relative biological activity. Membrane permeability to drug molecules is governed by the partition coefficient and the diffusivity of the drug both across the membrane interface and within the structured membrane interior. Model partitioning systems range from bulk organic solvents (e.g. octanol, chloroform, alkanes, olive oil) to more complex phases (e.g. liposomes, erythrocytes, toad bladder), the structure of which are close to that of biological membranes.

In this work we report on the thermodynamics of partitioning of cortisone and hydrocortisone, in addition to the 21-*n*-alkyl esters of these steroids, between normal saline and the lipophilic phases of isopropyl myristate (IPM) and liposomes formed from dimyristoyl phosphatidylcholine (DMPC).

Liposomes undergo a transition from the L-β-crystalline to the L-α-liquid crystalline phase at a temperature (T_c) which is characteristic of the phospholipid and its purity. Drug partitioning into phospholipid phases as a function of temperature can therefore be measured, and the change in free energy ($\Delta G_{w \to l}$), enthalpy ($\Delta H_{w \to l}$) and entropy ($\Delta S_{w \to l}$) of transfer from aqueous (w) to lipid (l) phases determined. Such determinations have been reported for alcohols (Katz and Diamond, 1974); phenothiazines (Ahmed et al., 1981); phenols (Davis and Rogers, 1980) and steroids (Arrowsmith et al., 1983). In addition other studies have reported partition coefficients of a number of solutes in liposomes (Heap et al., 1970; Hill, 1975; Tomkliewicz and Corker, 1975).

Materials and Methods

Ethanol (98%) was redistilled and the 78–79 °C boiling fraction collected. Chloroform was BDH reagent grade which was redistilled and the 61–62 °C boiling fraction collected. Sodium chloride (BDH analar) and double-distilled water were used to prepare the aqueous phase. Pyridine was BDH reagent grade. Phospholipid (L-α-dimyristoyl phosphatidylcholine (DMPC) 98% crystalline) was purchased from Sigma Chemicals, U.K. Thin-layer chromatography of DMPC produced a single spot using a solvent mixture of chloroform/methanol/water (65 : 25 : 4, v/v), $R_f = 0.68$. IPM was reagent grade (Fuka A.G.). Steroids: 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. Hydrocortisone 21-acetate, -propionate, -valerate, -hexanoate and -octanoate were prepared and synthesized from acid chlorides of chemical grade reagents and their purities were checked by TLC.

Synthesis of hydrocortisone esters (acetate, propionate, hexanoate, octanoate)

The method of synthesis has been described by Arrowsmith et al. (1983). 1.4 mmol of steroid was dissolved in chilled pyridine (4 °C) and 2.8 mmol of acid

chloride added dropwise whilst stirring constantly. The reaction mixture was stirred at 4°C for 72 h. Acylation was confirmed by TLC analysis using a toluene/ethyl acetate (13 : 7) solvent. Pyridine was removed by rotary evaporation at 37°C under vacuum for 20 h. The resulting brown oil was dissolved in 20 ml chloroform, 5 g of Kieselgel 60 (Merck, F.R.G.) added, and the solvents removed. The silica gel was packed into a 1 cm glass column to produce a bed height of 12 cm. This was eluted with petroleum ether (60–80°C boiling fraction) with increasing concentrations of ethyl acetate, and 20 ml fractions were collected. The latter were analyzed by TLC and identification was by ultraviolet light and a 50% concentrated sulphuric acid spray, followed by charring at 150°C for 1 h. Appropriate fractions were pooled and recrystallized twice from hot aqueous ethanol (20% water) before air-drying. Yield: 66% hydrocortisone acetate; 61% hydrocortisone propionate; 50% hydrocortisone valerate; 39% hydrocortisone hexanoate; 35% hydrocortisone octanoate.

Radioactively labelled steroids: [4-^{14}C]hydrocortisone (1.85 MBq · ml^{-1}) and [1,2,6,7-^{3}H]cortisone (37 MBq · ml^{-1}) were purchased from Amersham International, U.K.

Synthesis of [4-^{14}C]hydrocortisone esters (acetate, propionate, valerate, hexanoate, octanoate)

0.185 MBq of [4-^{14}C]hydrocortisone was dissolved in dry pyridine (0.4 ml) at 21°C. 20 µl acid chloride was added and the reaction allowed to proceed at room temperature for 2 days (short esters) to 7 days (long esters). The pyridine was removed by rotary evaporation at 40°C and under vacuum, overnight. The residue was dissolved in chloroform/methanol and applied to preparative TLC plates (silica gel GF 254, 0.5 mm) and developed with toluene/ethyl acetate (7 : 3 v/v). The plates were viewed under UV light (254 nm) and the fluorescence quenching zone corresponding to the ester band was located. The silica gel in this zone was scraped into the scintillation fluid and counted using an LKB 1217 Rack Beta liquid scintillation counter.

Assay of radioactively labelled 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 90% for [^{14}C]samples and 40–45% for [^{3}H]samples.

The scintillation cocktail consisted of:

2,5-diphenyloxazole (PPO)	15 g
1,4-di-2(4-methyl-5-phenyl oxazol)benzene (dimethyl POPOP)	300 mg
Toluene	2 litres
Triton X-100	1 litre

All materials were of scintillation grade and obtained from BDH, U.K.

Liposome preparation

To form mixed films of DMPC and solutes, 5 ml quantities of a 5 mg · ml^{-1} stock

solution of DMPC in chloroform were delivered to 50 ml conical quickfit flasks. [4-^{14}C]steroids were added (0.1 mg of specific activity 0.37 MBq · mg^{-1}), and the chloroform removed by rotary evaporation at approximately 40 °C. Evaporation was continued for 1 h followed by overnight drying in a vacuum oven at 35 °C. This resulted in uniform dry lipid films being formed on the inside walls of the flasks.

Multilamellar liposomes were formed by transferring sufficient sterile 0.9% saline to the dried lipid, to produce an aqueous–lipid phase ratio of 300 : 1 for hydrocortisone, 500 : 1 for hydrocortisone acetate and 625 : 1 for hydrocortisone propionate. 4 or 5 small glass beads were added, and the mixture heated to 40 °C, swirling with the aid of a vortex mixer until all the lipid was dispersed. Subsequently, the flasks were placed in a water bath at the desired temperature ±0.1 °C to equilibrate. Addition of water to dry lecithin, at temperatures above the phase transition of the dry lipid, results in formation of liposomes, but liposomes are not formed if water is added below the transition temperature (Chapman and Fluck, 1966).

Determination of partition of labelled hydrocortisone and cortisone esters between IPM and 0.9% saline over the 22–42 °C temperature range

Partition coefficients were determined by the shake-flask method described by Leo et al. (1971).

The solutes were dissolved in IPM saturated with saline. The two phases were shaken for 3 h and then allowed to equilibrate at the required temperature for 48 h. The phases were separated by centrifugation at 18,000 rpm for 1 h, and samples were taken for scintillation counting. Partition coefficients were calculated from:

$$K = \frac{\text{fraction in oil}}{\text{fraction in aqueous}} \times R \qquad (1)$$

where R = ratio of phases (aqueous/oil); fraction in oil = weight fraction of steroid in IPM; and fraction in aqueous = weight fraction of steroid in aqueous phase.

Determination of partitioning into liposomes over the 5–40 °C temperature range

Liposome suspensions were shaken for 48 h at temperatures of 5, 10, 15, 20, 25, 30, 35 and 40 °C ± 0.1 °C. Temperatures below 25 °C were obtained through the use of a circulating cooler (Grant Instruments, U.K.).

Following equilibration of partitioning, the suspensions were shaken briefly and vigorously, and duplicate 0.1 ml samples taken for scintillation counting, in order to determine the activity of the suspension.

Separation of the DMPC from the aqueous phase was accomplished by centrifugation. 5 ml of the liposome suspension was transferred into a centrifuge tube (polycarbonate 10 ml, M.S.E., U.K.). The use of polycarbonate tubes reduced steroidal loss through adsorption to an insignificant amount. The tubes were then placed in a fixed rotor precooled or prewarmed to the desired temperature (average transfer time 1.3 min). These were centrifuged (M.S.E. Prespin 50) at 50,000 *g* for 1 h. The centrifuge was thermostatically controlled to maintain the temperature within ±0.5 °C. Duplicate 0.1 ml samples of supernatant were carefully removed with a Finn pipette and were transferred as quickly as possible to scintillation vials.

Calculations

The weight fraction of steroid in the supernatant is calculated from the ratio division of supernatant dpm per 0.1 ml volume to suspension dpm per 0.1 ml volume. The weight fraction of steroid in the liposome pellet was found by difference, and the partition coefficient (K) calculated from Eqn. 1.

Thermodynamic analysis of partitioning

The thermodynamics of partitioning of a solute between an aqueous phase (w) and a lipid phase (1) are described by:

$$\Delta G_{w\to 1} = -RT \ln K = \Delta H_{w\to 1} - T\Delta S_{w\to 1} \quad (2)$$

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.

Partition coefficients derived from the linear regression at each temperature were used to obtain the calculated thermodynamic function for the steroids. These thermodynamic parameters, ΔH, ΔS and ΔG 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; Pimental, 1960; Hegna, 1977).

Results and Discussion

Many compounds possessing polar functional groups have been shown to undergo self-association in non-polar solvents, resulting in changes in thermodynamic activity (Anderson et al., 1979). In fact, self-association has been shown to be a dominant factor for alcohols (Fletcher et al., 1967). The changes in transport properties resulting from the self-association of phenol, have been studied by Mickelson et al. (1980), and described quantitatively. Testosterone has also been shown to self-associate (James and Ramgoolon, 1975).

To investigate the possibility of self-association of solute molecules in the solvent system, 4 different concentrations were taken (Table 1) and analysis of the data showed a constant log partition coefficient over the concentration ranges studied.

TABLE 1

LOG K AS A FUNCTION OF STEROID CONCENTRATION. EACH VALUE IS THE MEAN OF TWO DETERMINATIONS. T = 298° K

Drug	Log concentration			
	−0.52	−0.30	−0.15	0
Cortisone	1.444	1.445	1.436	1.439
Hydrocortisone	1.626	1.627	1.631	1.630
Hydrocortisone acetate	1.850	1.851	1.851	1.849

thus indicating that both lipid and aqueous phases were behaving ideally. The results illustrated that these compounds remain as monomers in IPM. The lack of association in this system can be accounted for by the relatively high tendency for the solvent to bond with the drug molecules. It is known that strong solute–solvent interactions can compete with the solute–solute interactions and thus prevent self-association of the solute molecules.

IPM / saline partitioning

[^{14}C]Labelled hydrocortisone 21-esters (acetate, propionate, valerate, hexanoate, octanoate) and [^{3}H]cortisone 21-esters (acetate, butyrate, hexanoate, octanoate) were used in the partitioning process.

It was found that the partition coefficients of hydrocortisone and its esters were higher than those of cortisone and its esters. Hydrocortisone has an 11-β-hydroxyl group, whereas cortisone has an 11-ketone group. Replacement of a ketone group with a hydroxyl group would lead to a decrease in the partitioning of a molecule, since a hydroxyl group has the greater capability of hydrogen-bonding with water. Solvation and entropy effects mainly control solution in aqueous phases due to the anomalous structure of water (Franks, 1973). However, the replacement of the 11-ketone with an 11-β-hydroxyl group results in an increase in partitioning, represented by a negative change in $\Delta G_{w\rightarrow 1}$ (Table 2). This is probably because hydrocortisone has one more hydroxyl group (11-hydroxyl) than cortisone, and therefore has an additional hydrogen atom available for hydrogen-bonding with the carbonyl oxygen of isopropyl myristate (IPM), therefore enhancing its solubility over that of cortisone. Hydrocortisone has 3–OH groups, each of which is capable of hydrogen-bonding with IPM; cortisone has only two. Acylation of the 21-hydroxyls therefore reduces this activity by 50% in cortisone, but only 33% in hydrocortisone. This is reflected by the greater difference (Table 2) between the free energies of partitioning between cortisone and cortisone acetate in comparison with the corresponding hydrocortisone compounds. The effect of addition of a hydroxyl group to progesterone, to give 17α-hydroxyprogesterone, has been shown by Lundberg (1979) to involve a free energy change of -1 kJ $\cdot$ mol^{-1} in the free energy of transfer from

TABLE 2

FREE ENERGY CHANGES ($\Delta G_{w\rightarrow 1}$) IN PARTITIONING OF STEROIDS IN IPM/SALINE SYSTEM

Steroid Hydrocortisone esters	$\Delta G_{w\rightarrow 1}$(kJ·mol^{-1})	Steroid Cortisone esters	$\Delta G_{w\rightarrow 1}$(kJ·mol^{-1})
Hydrocortisone	−9.15	Cortisone	−8.10
Hydrocortisone acetate	−10.421	Cortisone acetate	−10.060
Hydrocortisone propionate	−13.733	Cortisone butyrate	−14.471
Hydrocortisone valerate	−17.596	Cortisone hexanoate	−19.100
Hydrocortisone hexanoate	−20.200	Cortisone octanoate	−23.400
Hydrocortisone octanoate	−24.350		

water to octanol. This compares favourably with the difference between cortisone and hydrocortisone shown in Table 2.

Numerous investigators have studied the effects of the methylene group on the aqueous solubilities of homologous series (Davis et al., 1974). The group contribution method has been used to predict various thermodynamic parameters for the methylene group related to the solution properties of certain solutes. These parameters include the free energy, enthalpy and entropy of solution, hydration, vaporisation, etc. Application of this approach to free energy-related equilibrium such as solubility and partitioning has been successful.

Log partition coefficients are of an additive nature (Isawa et al., 1965) with the overall coefficient determined by modification of the partitioning behaviour of the parent compound through the effects of substituents, and now generally evaluated using Hansch's π-values. Flynn (1974) has demonstrated the applicability of such principles to the partitioning of a large group of steroids in the diethyl-ether/water system.

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 exlained by the greater amount of energy needed to break the hydrogen bonds and remove the solute from water.

Plots of $\Delta G_{w \rightarrow 1}$ versus total number of carbon atoms (Fig. 1) for hydrocortisone

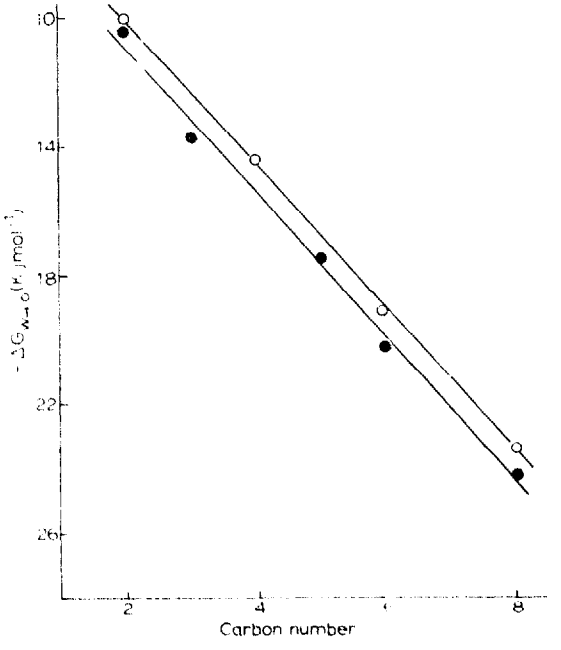

Fig. 1. RT·ln K versus total number of carbon atoms in the *n*-alkyl ester chain of hydrocortisone (●) and cortisone (○). Mean incremental free energies per methyl group of 2.27 kJ·mol^{-1} were determined for hydrocortisone esters and 2.23 kJ·mol^{-1} for cortisone esters.

and cortisone esters, resulted in linear relationships yielding a mean incremental free energy of 2.23 $KJ \cdot mol^{-1}$ for cortisone esters, and 2.27 $kJ \cdot mol^{-1}$ for hydrocortisone esters. [$-CH_2-$ in *n*-alkanes (transfer to cyclohexane) $\Delta G = -3.95$ $kJ \cdot mol^{-1}$, $-CH_2-$ in methyl phenols (transfer to octanol) $\Delta G = -2.4$ to -3.4 $kJ \cdot mol^{-1}$ (Nicholas et al., 1981)].

Liposome partitioning

It has been postulated that steroids partition into membranes with the molecule lying parallel to the alkyl chains of the phospholipid molecules comprising the bilayer. The criteria for steroid orientation were that the most lipophilic part of the molecule would seek the alkyl chain region of the bilayer, while the most hydrophilic moiety would orientate towards the aqueous phase. Such a model is readily compatible with the incorporation of a monopolar steroid, such as cholesterol. However, a perpendicular orientation of polypolar steroids, including hydrocortisone and cortisone, would involve the close association of hydrophilic groups with the most lipophilic parts of the bilayer.

The temperature dependency of partitioning of hydrocortisone, hydrocortisone acetate and hydrocortisone propionate between DMPC liposomes and 0.9% saline is shown in Fig. 2. The amount of steroid used is within the maximum 1% recommended (Diamond and Katz, 1974) for such experiments, and also within the aqueous solubility of these steroids (Tonida et al., 1978; Batra, 1975). Snart and Wilson (1967) demonstrated that partition coefficients of several steroid solutes in egg yolk lecithin liposomes are concentration-independent, up to the solubility limit in water.

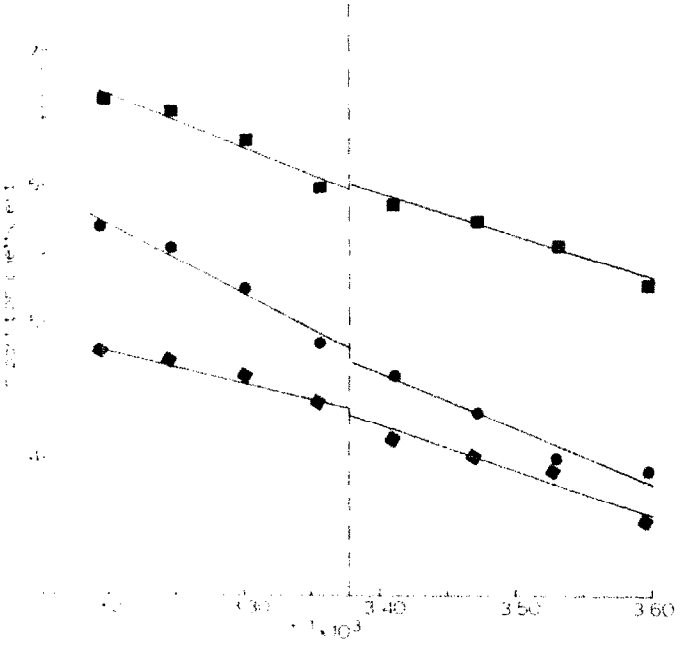

Fig. 2. Temperature-dependent partitioning of hydrocortisone and 21-derivatives between DMPC liposomes and 0.9% saline. Key: ◆, hydrocortisone; ●, hydrocortisone acetate; ■, hydrocortisone propionate.

The reported phase-transition temperature (T_c), above which the fluidity of the phospholipid acyl chains greatly increases, is 23°C for DMPC (Chapman et al., 1967), a temperature convenient to study the effect of T_c on partitioning.

Thermodynamic parameters were derived from the van't Hoff (Eqn. 2) and Gibbs (Eqn. 2) equations for hydrocortisone, hydrocortisone acetate and hydrocortisone propionate, values of which are presented in Table 3. Calculation of ΔG, ΔH and ΔS from the temperature dependence of partition coefficients involves an interdependence of the parameters. Although microcalorimetry may be employed to determine ΔH directly, such techniques are not applicable to structured phases such as liposomal membranes. The approach used in this work provides information which can be used for comparative purposes. These are shown at only one temperature below and one temperature above T_c of DMPC. The assumption is made by fitting lines through the experimental points that, over a small temperature range (15°C) either side of T_c, $\Delta H_{w\rightarrow l}$ is independent of temperature (Katz and Diamond, 1974). For esters of hydrocortisone the energetics of partitioning become more favourable as the series is ascended. Partitioning increases with temperature both below and above T_c but this effect is reduced in the latter temperature range. Steroid partitioning above T_c is higher than below, indicative of a less ordered structure above the phase transition. As values of $\Delta G_{w\rightarrow l}$ were all < 0, and $\Delta H_{w\rightarrow l}$ and $\Delta S_{w\rightarrow l}$ values are positive, the transference process is entropy driven both below and above T_c. These observations are a result of a balance between two entropy effects. The observed positive entropy changes arise from the loss in water structure surrounding steroid molecules on transference to the liposome with the subsequent disordering of the phospholipid bilayers. Also the $-CH_2-$ group contribution to steroid partitioning into DMPC liposomes both above and below T_c is dominated by entropy effects, i.e. an increase in entropy of the aqueous phase pushes the group out of water, and the increase in entropy of the membrane lipid pulls the group into the membrane. For cortisone esters, the longer the acyl chain, the greater the structural change in the membrane, as measured by the enthalpy of transition from the solid to the liquid crystalline state (Arrowsmith et al., 1983). In the hydrocortisone ester series, the

TABLE 3

THE ENTHALPY ($\Delta H_{w\rightarrow l}$ kJ·mol^{-1}), ENTROPY ($\Delta S_{w\rightarrow l}$ J·mol^{-1}K^{-1}) AND FREE ENERGY ($\Delta G_{w\rightarrow l}$ kJ·mol^{-1}) CHANGES OF PARTITIONING FOR A SERIES OF STEROIDS IN DMPC LIPOSOMES BOTH BELOW AND ABOVE THE PHASE-TRANSITION TEMPERATURE

Steroids	Below T_c (288°C) gel			Above T_c (303°K) fluid		
	$\Delta G_{w\rightarrow l}$	$\Delta H_{w\rightarrow l}$	$\Delta S_{w\rightarrow l}$	$\Delta G_{w\rightarrow l}$	$\Delta H_{w\rightarrow l}$	$\Delta S_{w\rightarrow l}$
Hydrocortisone	−9.7	28.0	131	−11.6	20.2	105
Hydrocortisone acetate	−10.4	33.9	154	−13.24	43.2	186
Hydrocortisone propionate	−13.8	26.9	141	−16.04	33.0	162
Cortisone	−9.16	35.8	156	−10.4	22.7	109
Cortisone acetate	−9.7	49.7	206	−12.8	50.0	107
Cortisone butyrate	−16.5	20.8	129	−18.5	40.4	196

extent of membrane disordering follows the ester chain length and presumably outweighs the loss of translation and rotational entropy upon transfer of solute from a water to a lipid phase. This compensation effect gives rise to the observed positive change in entropy of the cortisol esters, which increases as a function of chain length.

The orientation of steroid 21-esters within a phospholipid bilayer is probably different for the unesterified molecules (Arrowsmith et al., 1983). All 21-OH steroids have an enthalpy of partitioning of a higher value below T_c than above. This reflects the extra energy required to insert a steroid molecule bearing a non-stereospecific -OH into the gel state bilayer compared with the liquid crystalline state.

Comparison of partition data of the same series of solutes in different solvents enables estimation of the relative hydrophobicities of solvents (Collander, 1950). Solutes with a hydrogen bonding capability will have a higher partition coefficient into a lipid solvent with a greater hydrogen-bonding capacity than with a less polar solvent. Comparison of $\Delta G_{w \rightarrow l}$ data obtained for DMPC liposomes with free energies calculated from partition data for IPM, ethyl ether (Flynn, 1971) and octanol (Hansch and Leo, 1979) may allow conclusions as to the environment of a liposomally incorporated steroid (Table 4). By using different solvents for the determination of partition coefficients and by plotting $\Delta G_{w \rightarrow l}$ of liposomes both below and above T_c versus $\Delta G_{w \rightarrow l}$ of IPM (a), octanol (b) and ether (c), (Fig. 3), the extent to which the partition data in the different solvent systems are suitable models for the phosphatidylcholine membrane can be evaluated.

There is a close correlation (Fig. 3) between IPM and liposomes above and below T_c, and the regression slope is close to 1. The hydrophobicity of this solvent

TABLE 4

FREE ENERGY OF PARTITIONING (kJ·mol^{-1}) OF STEROIDS IN FIVE DIFFERENT LIPID/WATER SYSTEMS

Steroid	Liposomes (DMPC)		n-Octanol 298° K [b]	Diethyl-ether 296° K [c]	IPM 298° K
	Gel (288° K)	Fluid (303° K)			
Hydrocortisone	−9.70	−11.60	−8.85	−1.15	−9.15
Hydrocortisone acetate	−10.40	−13.24	−12.47	−8.01	−10.42
Hydrocortisone propionate	−13.80	−16.03	−15.80	-	−13.73
Cortisone	−9.16	−10.40	−8.09	−0.82	−8.10
Cortisone acetate	−9.70	−12.80	−11.98	−7.93	−10.06
Cortisone butyrate	−16.50	−18.50	−19.00	-	−14.47
Progesterone	−16.60 [a]	−18.60 [a]	−18.40	−15.75	-
Deoxycorticosterone	−12.80 [a]	−16.60 [a]	−16.40	−9.72	-
Corticosterone	−11.60 [a]	−14.50 [a]	−13.50	−3.71	-
17-α-Hydroxydeoxycorticosterone	−12.40 [a]	−14.90 [a]	−14.05	−6.52	-

[a] Data reported by Arrowsmith et al. (1983).

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

[c] Calculated from data reported by Flynn (1971).

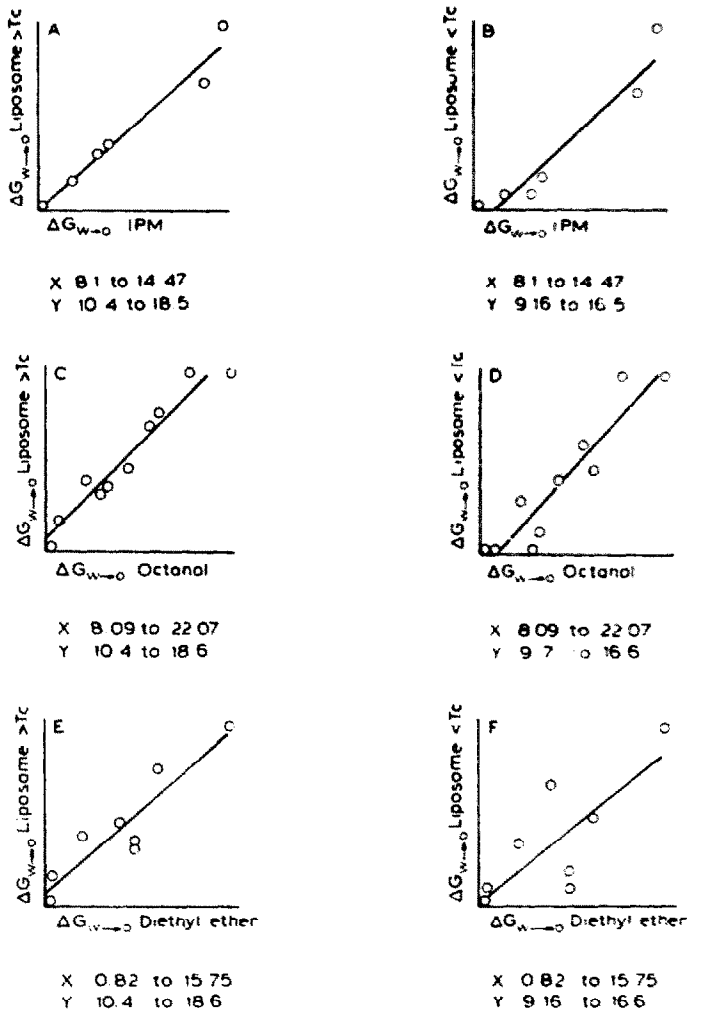

Fig. 3. Free energy change of partitioning in liposomes versus free energy change of partitioning in IPM, octanol diethyl ether. A, C, E above T_c; B, D, F below T_c. A: $r = 0.984$; slope = 1.15. B: $r = 0.959$; slope = 1.11. C: $r = 0.974$; slope = 0.616. D: $r = 0.935$; slope = 0.559. E: $r = 0.915$; slope = 0.492. F: $r = 0.780$; slope = 0.412.

therefore, is close to that encountered at the partition site of the steroids within the membrane. In all cases, the correlation was better for liposomal partitioning at temperatures above than below T_c. This may reflect the relative ease of experimental conditions both in terms of temperature control and the attainment of equilibrium in the two liposomal phases.

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