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Influence of non-ionic surfactants on permeation of hydrocortisone, dexamethasone, testosterone and progesterone across cellulose acetate membrane

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The lag-time method of diffusion has been used to investigate permeation of hydrocortisone, dexamethasone, testosterone and progesterone across cellulose acetate membranes between 10° and 40°. The process depended mainly on membrane-water partition coefficients of the steroids so that the least polar compound permeated the fastest. Permeation generally increased with increasing temperature and from the temperature dependance of the diffusion coefficient, energies of activation were derived. These varied from 2·4 kcal mol^{-1} for the least polar steroid, progesterone, to 7·4 kcal mol^{-1} for the most polar, hydrocortisone. n - C_{16} Polyoxyethylene surfactants when present below and above the cmc increased the steroids permeation rates. Varying the polyoxyethylene chain length (OE = 17–63) did not significantly affect permeation rates, suggesting that the enhancing effect of surfactants arises from their hydrophobic group.

That surfactants influence the rate and extent of absorption of certain drugs has been reviewed by Gibaldi & Feldman (1970). Often, drugs are absorbed from dosage forms by passive diffusion. Although *in vitro* studies do not substitute for *in vivo* studies, they are important since they contribute significantly to the development and evaluation of new dosage forms and aid in understanding underlying principles controlling absorption.

Here we report on the permeation of tritiated steroids across cellulose acetate using the lag-time method (Barrer, 1957) with rapid sampling times and "sink" conditions. The effect of long-chain polyoxyethylene non-ionic surfactants on the permeation process was also examined.

MATERIALS AND METHODS

Tritiated steroids. [1(2)-^{3}H]Dexamethasone, [1,2-^{3}H]hydrocortisone, [1α,2α-^{3}H]-progesterone and [1β, 2β-^{3}H]testosterone were obtained from The Radiochemical Centre (Amersham, England). Progesterone and testosterone were in benzene, dexamethasone in ethanol, and hydrocortisone in benzene–ethanol. Steroids were prepared for diffusion experiments by evaporating the solvent, drying the residue over silica gel and redissolving it in 100 ml water.

Non-ionic surfactants. Polyoxyethylated cetyl alcohol surfactants (Texofors, Glovers Ltd., Leeds) contained 17, 32, 44 and 63 mol of ethylene oxide (Barry & El Eini, 1976a). Cmcs at 25° were reported earlier (El Eini, Barry & Rhodes, 1973).

Membranes. Cellulose acetate, wet thickness micrometer measurement, $9{\cdot}01 \times 10^{-3} \pm 0{\cdot}042 \times 10^{-3}$ cm (n = 100), obtained from Scientific Instrument Centre Ltd. (London), was washed in warm water and stored in cold water.

Liquid scintillator. NE250 liquid scintillator obtained from Nuclear Enterprises Ltd., Edinburgh.

Diffusion experiments. Diffusion cells were as described by Barry & El Eini (1976b). The "recipient" compartment contained 24 ml water and the cell was temperature equilibrated in a water bath ($\pm$ 0·1°). 24 ml of equilibrated steroid solution was rapidly introduced into the "donor" compartment at time, t = 0. The contents of both compartments were stirred using Teflon coated magnetic bars rotated by immersible stirrers. 100 μl samples were removed from the recipient compartment, at set time intervals, using a microlitre-pipette (Eppendorf, Hamburg, West Germany). Each sample was mixed with 10 ml liquid scintillator and assayed using an Intertechnique ABAC SL40 Scintillation Spectrometer (Intertechnique, Plaisin, France).

Calculations. The time-lag method is based on the premise that a finite time is needed for a diffusant to traverse the thickness of the membrane before steady state is attained (Daynes, 1920). As soon as diffusant is introduced to one side of the membrane and before a steady state is established, both flow rates and the concentration at any point in the membrane vary with time. If the diffusion coefficient is constant, the membrane is initially completely free of diffusant and diffusant is continually removed from the recipient side of the membrane, i.e. a sink condition is maintained, then the cumulative mass of diffusant per unit area, M, which passes through the membrane in time, t, is (Jost, 1960)

$$M = \frac{DC_0 t}{h} - \frac{hC_0}{6} - \frac{2hC_0}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp \frac{-Dn^2\pi t}{h^2} \quad .. \quad .. \quad (1)$$

where h is thickness of membrane, n is number of displacements, C_0 is concentration at the membrane surface on the donor side and D is the diffusion coefficient.

As $t \to \infty$, steady-state is approached and exponential terms in equation (1) become negligibly small, so that

$$M = \frac{DC_0}{h}\left(t - \frac{h^2}{6D}\right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

A plot of M against t gives an intercept, L (lag-time,), given by

$$L = \frac{h^2}{6D} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The partition coefficient, K, of diffusant between membrane and phases adjacent is

$$K = C_0/C_1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where C_1 is applied phase concentration. Differentiating equation 2 gives the steady-state flux, dM/dt,

$$\frac{dM}{dt} = \frac{DC_0}{h} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5)$$

which when combined with equation 4 gives

$$\frac{dM}{dt} = \frac{DC_1K}{h} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

Knowledge of K allows calculation of the permeability coefficient, P, from

$$P = KD$$

Steady-state slopes, dM/dt, and lag-times, L, were calculated by least square treatment of data, extrapolating the steady-state portion of diffusional curves to the time axis. Essentially constant donor phase concentrations and sink recipient phase conditions were maintained throughout.

RESULTS AND DISCUSSION

The effect of applied phase concentration of steroid on diffusion was studied by varying concentrations of hydrocortisone in the donor compartment at 25° (Table 1). Results indicated that diffusion of hydrocortisone through cellulose acetate was Fickian since D remained essentially constant within concentration range studied. This agrees with Short (1971) who showed that a transport constant, k_t (directly related to diffusion coefficient) of hydrocortisone through cellulose acetate was independent of concentration below 0·1 mg ml^{-1}; above this, k_t increased rapidly. Crank & Park (1968) suggested that this dependence may be due to sudden swelling of the membrane above a certain concentration of diffusant. Until this concentration is reached, the membrane may possess long "relaxation times" and resist swelling. Another factor may be saturation of bonding sites in the membrane so that above a saturating concentration diffusion increases with concentration. Flynn & Roseman (1971) showed that permeation of *p*-aminoacetophenone and ethyl *p*-aminobenzoate through dimethylpolysiloxane membranes at 37° was concentration-dependent for high applied phase concentratons. This deviation from Fickian behaviour was attributed to significant solute-solute interactions, and thus a decrease in activity coefficients, as saturation was approached. Since at all times in our work steroid concentrations in the applied phase were of the order of 10^{-5} mg ml^{-1}, their diffusion across cellulose acetate will be considered as Fickian.

Diffusion parameters of steroids in water through cellulose acetate between 10° and 40° are in Table 2 (full data are shown to illustrate the method of calculation). Steady state portions of diffusional curves of hydrocortisone at various temperatures, typically representative of the steroids examined, are in Fig. 1.

At constant temperature, permeation of the steroids increased as their polarities, expressed at R_m values, decreased (Fig. 2). R_m was determined from R_F (Barry & El Eini, 1976b). Steroid permeation through relatively thick barriers, such as cellulose acetate, may be described by the following sequence: adsorption of the molecule on to the donor surface of the membrane, its diffusion through membrane, and its desorption from the recipient surface of the membrane. Adsorption and desorption processes

Table 1. *Effect of applied phase concentration of hydrocortisone on its permeation across cellulose acetate membranes at 25°.*

C_1 mg ml^{-1} × 10^5	L s	D cm^2 s^{-1} × 10^8	dM/dt mg cm^{-2} s^{-1} × 10^{10}	C_0 mg ml^{-1} × 10^5	K	P cm^2 s^{-1} × 10^8
7·553	54·05	25·0	24·85	8·944	1·18	29·5
6·518	50·54	26·6	21·40	7·246	1·11	29·5
5·179	55·86	24·2	17·29	6·430	1·24	30·0
3·949	52·20	26·0	12·85	4·465	1·13	29·4

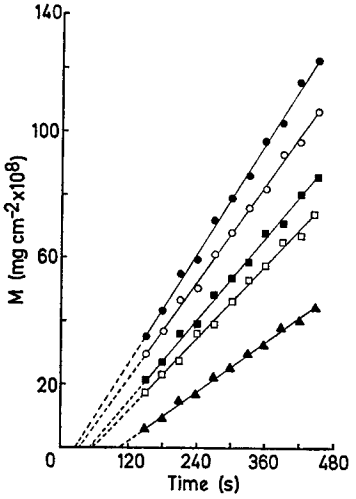

FIG. 1. Steady-state diffusional plots for hydrocortisone through cellulose acetate membranes at 10° (▲); 20° (□); 25° (■); 30° (○); 40° (●).

are affected by the relative affinity of permeant for aqueous phase and membrane, i.e. the partition coefficient. The diffusion process is related to the effective size of permeant, the extent of its interaction with the membrane, and the structural characteristics of the membrane.

Partition coefficients, K, of steroids between cellulose acetate and water were essentially independent of temperature (Table 2). They were directly related to R_m values. As expected for a membrane which although hydrophilic is slightly hydrophobic relative to water, K increases with decreasing steroid polarity because of greater tendency for a non-polar molecule to transfer from the aqueous phase to such a membrane. Since the amount of permeant diffusing through the membrane depends on the concentration gradient, the larger K, the faster is the permeation rate.

Molecular sizes of steroids, with molecular weights ranging from 288 for testos-

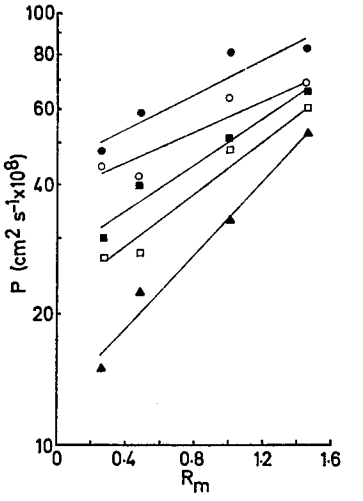

FIG. 2. Permeation of steroids, P, as a function of their R_m values at 10° (▲); 20° (□); 25° (■); 30° (○); 40° (●).

Table 2. *Permeation parameters for steroids across cellulose acetate membranes between 10°–40°.*

Steroid	°C	D $cm^2 s^{-1} \times 10^8$	K	P $cm^2 s^{-1} \times 10^8$
Hydrocortisone	10	13·4	1·13	15·1
	20	21·8	1·25	27·2
	25	26·4	1·14	30·1
	30	37·8	1·16	43·8
	40	45·2	1·06	47·9
Dexamethasone	10	14·8	1·52	22·5
	20	20·0	1·39	27·8
	25	25·9	1·55	40·2
	30	26·7	1·54	41·2
	40	39·9	1·47	58·7
Testosterone	10	17·9	1·85	33·2
	20	22·9	2·13	48·7
	25	24·1	2·13	51·4
	30	31·5	2·02	63·6
	40	33·4	2·41	80·6
Progesterone	10	17·0	3·11	53·0
	20	21·1	2·87	60·6
	25	21·7	3·03	65·9
	30	22·0	3·13	68·9
	40	28·5	2·92	83·1

terone to 393 for dexamethasone, are similar. Since the diffusion process is related to the permeant's molecular size, it would be expected that D for steroids would be similar at a given temperature. This is so (Table 2) at lower temperatures, 10–25°, but at 30° and 40° diffusion coefficients of more polar steroids, hydrocortisone and dexamethasone, increase more than those of testosterone and progesterone. This may be explained by considering energy of activation of steroids, E_D, for diffusion process, derived from Arrhenius equation

$$\ln D = \ln D_0 - E_D/RT \quad .. \quad .. \quad .. \quad (8)$$

where D_0 is the hypothetical diffusivity at infinite temperature and R is the gas constant. Plots of log D versus 1/T yielded straight lines with slopes of $E_D/2{\cdot}303$ R. Values of E_D and least-square analysis of log D versus 1/T plots are in Table 3.

Danielli (1943) described E_D as a measure of ease with which a molecule diffuses. In solution when diffusing molecules are large compared to molecules of the medium, E_D is theoretically about one-third the energy of vaporization (Benson, 1960) and is in the range 3–5 kcal mol^{-1} in many liquids (Chemburkar, 1967). However, molecules diffusing through polymeric membranes are small compared to polymer

Table 3. *Energies of activation, E_D, for steroids across cellulose acetate membrane from equation 8.*

Steroid	E_D k cal mol^{-1}	Slope	Intercept	Correlation Coefficient (5 points)
Hydrocortisone	7·43	1·626	−1·116	0·984
Dexamethasone	5·74	1·255	−2·407	0·989
Testosterone	3·85	0·841	−3·765	0·969
Progesterone	2·76	0·604	−4·631	0·969

molecules which are also fixed in position. Thus, unlike diffusion in liquids, where solvent molecules are mobile and move with diffusant thus offering less resistance, diffusing species move on their own. This demands higher activation energies than postulated for diffusion in liquids.

Activation energies of steroids ranged from 2·8 to 7·4 kcal mol^{-1}, in the region of values postulated for diffusion of molecules in a liquid (Benson, 1960). This suggests that steroids diffuse in water hydrating the membrane. Relatively low membrane/water partition values also support this view. The membrane absorbs water which forms clusters around hydrophilic groups of cellulose acetate polymer chains. The water content in a fully wetted membrane is about 40–50%. It would therefore be expected that diffusivity of steroids in water, D_w, would be similar in magnitude to that in cellulose acetate. Flynn, Yalkowsky & Roseman (1974) showed that for solutes whose molar volume, v, is greater than or equal to the molar volume of solvent, diffusivity, D, can be expected to range from

$$D = \frac{kT}{4\pi\eta}\left(\frac{4\pi N}{3v}\right)^{1/3} \quad .. \quad .. \quad .. \quad (9)$$

for small particles to

$$D = \frac{kT}{6\pi\eta F}\left(\frac{4\pi N}{3v}\right)^{1/3} \quad .. \quad .. \quad .. \quad (10)$$

for large particles, where η is the solvent viscosity, k is the Boltzman constant, N is Avogadro's number, and F is the frictional ratio. For water at 25° equations 9 and 10 become

$$D = \frac{4{\cdot}95 \times 10^{-5}}{v^{1/3}} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

and

$$D = \frac{3{\cdot}3 \times 10^{-5}}{v^{1/3}F} \quad .. \quad .. \quad .. \quad .. \quad (12)$$

Inclusion of F amounts to less than a 10% correction for all but the most elongated structures and can therefore be ignored to a first approximation of D_w. Values of v for steroids can be estimated with reasonable accuracy from their chemical formulae since molar volume is an additive property of constituent atoms and functional groups (Yalkowsky & Zografi, 1972).

Values of D_w calculated using equations 11 and 12 (F assumed to be unity) are in Table 4 together with calculated values of v. Flynn & others (1974) showed that for a wide range of diffusants, experimentally determined aqueous diffusivities always lay between diffusivities calculated from equation 11 and 12. It is therefore reasonable to assume that the aqueous diffusivities of steroids lie within values defined by these equations. These values are on average 25 times larger than the membrane diffusivities of the steroids. Slower membrane diffusion rates are caused by "hindrance" of diffusing molecules by cellulose acetate polymer chains.

The degree of "difficulty" in diffusion of steroids, as indicated by E_D values (Table 3), may be explained by considering possible interactions that may occur with the membrane. Both water and cellulose acetate can hydrogen bond. Relative hydrogen bonding abilities of steroids are indicated by their aqueous solubilities which are, in descending order, hydrocortisone, dexamethasone, testosterone and progesterone.

Results suggested that permeation of steroids through cellulose acetate depended on the degree of interaction by hydrogen bonding, since E_D varies in the same direction as the steroids' ability to hydrogen bond.

Table 4. *Calculated aqueous diffusivities, D_W, and molar volumes, v, of steroids at 25°.*

Steroid	v ml mol^{-1}	D_W (Eq. 11) cm^2 s^{-1} × 10^6	D_W (Eq. 12) cm^2 s^{-1} × 10^6	D cm^2 s^{-1} × 10^6
Hydrocortisone	247	789	526	26·4
Dexamethasone	271	765	510	25·9
Testosterone	204	841	561	24·1
Progesterone	234	803	536	22·0

Table 5 shows permeation data at 25° for hydrocortisone through cellulose acetate when an equimolar, submicellar concentration (10^{-7} M) of each of four polyoxyethylene surfactants was added to the donor solution. Results indicate that although the permeation rate increases in the presence of each surfactant, no significant difference exists between effects due to varying their hydrophilic chain length. The mechanism by which this class of surfactants affects membrane permeation depends on the nature of the hydrophobic moiety, which is constant for all surfactants used. Since permeation of steroids is related to their membrane–water partition coefficients, it is possible that monomeric surfactant molecules may somehow enhance the adsorption of the permeating molecule on to the donor side of membrane and therefore increase the membrane surface concentration. This enhancement may arise from the lowering of interfacial tension between membrane and aqueous phase (Gibaldi & Feldman, 1970). Kesting, Subcasky & Paton (1968) showed that surfactant adsorption at the membrane/water interface allowed faster permeation rates for hydrogen bonding solutes than non-hydrogen bonding solutes.

Permeation rates of dexamethasone, testosterone and progesterone also increased in the presence of 10^{-7} M $C_{16}OE_{32}$. However, for supra-micellar concentrations of $C_{16}OE_{32}$, permeation of steroids, as indicated by steady-state flux rates, dM/dt, decreased (Table 6). Fig. 3 shows as an example steady-state portions of diffusional curves for hydrocortisone at 25° in the presence of sub-micellar (10^{-7} M) and supra-micellar (10^{-3} M) concentrations of $C_{16}OE_{32}$. The diffusion coefficients of steroids remained essentially the same as in water in the presence of both sub- and supra-micellar concentrations of surfactant. This indicated that observed changes in

Table 5. *Effect of submicellar concentration (10^{-7} M) of n-alkyl polyoxyethylene surfactants on the permeation of hydrocortisone through cellulose acetate membranes at 25°.*

Surfactant	D cm^2 s^{-1} × 10^6	K	P cm^2 s^{-1} × 10^6
—	26·4	1·14	30·1
$C_{16}OE_{17}$	29·3	1·09	31·9
$C_{16}OE_{32}$	31·1	1·21	37·6
$C_{16}OE_{44}$	30·0	1·19	35·7
$C_{16}OE_{63}$	31·5	1·10	34·6

Table 6. *Permeation parameters for steroids across cellulose acetate membranes at 25° in submicellar (10^{-7} M), a, and supramicellar (10^{-4} M for progesterone and 10^{-3} M for other steroids), b, concentrations of $C_{16}OE_{32}$.*

Steroid	C_1 mg ml^{-1} × 10^5		L s		D cm^2 s^{-1} × 10^8		dM/dt mg cm^{-2} s^{-1}		C_0 mg ml^{-1} × 10^5		K	P cm^2 s^{-1} × 10^8
	a	b	a	b	a	b	a	b	a	b	a	a
Hydrocortisone	8·049	7·975	43·49	47·32	31·1	28·6	33·64	23·75	9·743	7·483	1·21	37·6
Dexamethasone	11·94	13·38	43·82	45·21	30·9	29·9	68·76	42·68	20·06	12·85	1·68	57·9
Testosterone	5·437	5·932	46·22	53·89	29·3	25·1	41·04	9·873	12·63	3·543	2·32	67·9
Progesterone	5·699	5·841	52·68	55·31	25·7	24·5	51·04	2·710	17·91	2·713	3·14	80·7

permeation rates arose from changes in membrane–water partition coefficients due to the presence of surfactant. As argued earlier, it was likely that surfactant monomers increased the partition of steroids in favour of the membrane by acting at the interface so that the effective applied concentration increased and hence the flux rate increased. In the presence of surfactant micelles, steroids were involved in two partition equilibria simultaneously; between the aqueous phase and the micelles and between the aqueous phase and the membrane. Since steroid is continuously depleted from the donor compartment across membrane, the micellar phase acted as a reservoir of steroid, releasing molecules to maintain equilibrium. Micelles were too large to diffuse across the membrane so that solubilized steroid was effectively unavailable for permeation. So although the total steroid concentration in the donor compartment was essentially equal in the presence of sub- and supra-micellar surfactant concentrations, the effective applied concentration was different depending on the steroid's partition coefficient, K_m, between micellar and aqueous phases. K_m's for steroids were determined earlier (Barry & El Eini, 1974, 1976b). Using K_m at 25°, "true" effective applied steroid concentrations in presence of $C_{16}OE_{32}$ micelles, C_T, were estimated and K and P were calculated (Table 7).

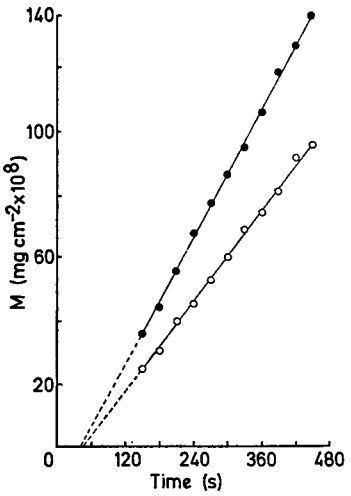

FIG. 3. Steady-state diffusional plots for hydrocortisone through cellulose acetate in the presence of submicellar (10^{-7} M), ●, and supramicellar (10^{-3} M), ○, concentrations of $C_{16}OE_{32}$.

Table 7. *"True" effective applied steroid concentrations, C_T, and their permeation parameters in the presence of $C_{16}OE_{32}$ micelles.*

Steroid	C_T mg ml^{-1} $\times 10^5$	C_0 mg ml^{-1} $\times 10^5$	K	P cm^2 s^{-1} $\times 10^8$
Hydrocortisone	5·648	7·483	1·33	38·0
Dexamethasone	6·443	12·85	1·99	59·6
Testosterone	1·641	3·543	2·16	54·2
Progesterone	0·738	2·753	3·73	91·2

Results showed that despite appreciable reductions in flux rates of the steroids in the presence of $C_{16}OE_{32}$ micelles (Table 6), permeation coefficients increased compared to those in water (Table 2). Short, Abbs & Rhodes (1970) showed that the diffusion coefficient for testosterone through cellulose acetate decreased linearly with surfactant concentrations above the cmc. However, the surfactant concentrations used by these workers were well above the cmc, so that the true effective concentration of testosterone in the donor compartment would have been changed appreciably from that in water. Diffusion coefficients in polymers are related to the diffusant concentration, C, by (Flynn & others, 1974),

$$D = D_{C=0}e^{AC} \quad .. \quad .. \quad .. \quad .. \quad (13)$$

where A is constant at a given temperature. Equation 13 shows that changes in diffusant concentration, C, could affect D appreciably. When effective concentrations of testosterone and hydrocortisone were maintained constant, diffusion constants decreased slightly for testosterone and increased for hydrocortisone (Short, 1971). The general effect of non-ionic surfactants on permeation of steroid across cellulose acetate seems to be an enhancement, provided that effective steroid concentration is constant.

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