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Permeation of oestrone, oestradiol, oestriol and dexamethasone across cellulose acetate membrane

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The permeation across cellulose acetate of three oestrogens, differing only in the number of hydroxyl groups attached to the nucleus, and a 'standard' steroid, dexamethasone, was investigated using the lag-time method for calculating diffusion parameters, between 10 and 40°. Diffusion coefficients for the similarly-sized oestrogens were relatively insensitive to marked changes in polarity, but increased permeation was correlated with increased partition coefficients, decreased polarity and fewer hydroxyl groups on the nucleus. Permeation increased with temperature and energies of activation were calculated from Arrhenius-type plots. E_p values ranged from 4·84 k cal mol^{-1} (20 kJ mol^{-1}) for the least polar steroid (oestrone) to 6·91 k cal mol^{-1} (29 kJ mol^{-1}) for the most polar steroid (oestriol). The results implied that steroid diffusion occurred through aqueous membrane channels, but that it was impeded to various extents by both obstruction and polar interaction effects.

Molecular permeation across capillary endothelium has been described by restricted diffusion and molecular filtration theory (Pappenheimer, 1953). This theory was expanded in studies with cellulose membranes by Renkin (1954), who suggested that diffusion through these membranes was solely via waterfilled pores. Diffusion of four typical steroids, hydrocortisone, dexamethasone, testosterone and progesterone, across cellulose acetate membrane was examined by Barry & El Eini (1976).

Here, we have investigated the influence of steroid polarity on permeation across cellulose acetate membrane, by employing three oestrogens of similar molecular volumes, but of widely differing polarities. Dexamethasone was also included in diffusion experiments as a control 'standard' steroid.

MATERIALS AND METHODS

Tritiated steroids. [1(2)-^{3}H] Dexamethasone, [2,4,6,7(n)-^{3}H]oestrone, [2,4,6,7(n)-^{3}H]oestradiol and [2,4,6,9(n)-^{3}H]oestriol were obtained from The Radiochemical Centre (Amersham, England). Oestrogens were in benzene–ethanol and dexamethasone was in ethanol. Steroids were prepared for experiments by evaporating the solvent, drying the residues over silica gel and redissolving them in 100 ml water.

Membranes. Wet thickness micrometer measurement of cellulose acetate was $10{\cdot}7 \times 10^{-3} \pm 0{\cdot}041 \times 10^{-3}$ cm (n = 30); membrane obtained from Scientific Instrument Centre Ltd (London) was washed in warm water and stored in cold water.

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Liquid scintillator. NE250 scintillation fluid obtained from Nuclear Enterprises Ltd, Edinburgh.

Diffusion experiments and calculations. Methods and calculations were as described by Barry & El Eini (1976) except that sampling intervals were 100 s from 300 s to 900 s to reduce receptor volume changes. Compartment volumes in diffusion cells were 22·2 ml and exposed membrane areas were 15·9 cm^2. Cells and solutions were equilibrated in a water bath (± 0·1°).

RESULTS AND DISCUSSION

Permeation parameters for various applied dexamethasone concentrations (Table 1) confirm Fickian steroid diffusion, since values of diffusivity, D, derived from lag time, L, were essentially constant at

Table 1. *Effect of applied dexamethasone concentration on permeation parameters across cellulose acetate membrane at 25°.*

C_1 g ml^{-1} × 10^7	L s	D cm^2s^{-1} × 10^8	dM/dt g $cm^{-2}s^{-1}$ × 10^{11}	C_0 g ml^{-1} × 10^7	K	P cm^2s^{-1} × 10^8
25°C						
23·8	86·2	22·1	6·95	33·6	1·41	31·2
5·91	84·5	22·6	1·72	8·15	1·38	31·2
0·535	84·6	22·6	0·167	0·791	1·48	33·5
30°C						
5·61	75·9	25·1	1·92	8·19	1·46	36·7
1·74	72·8	26·2	0·663	2·71	1·56	40·9
0·693	74·7	25·6	0·282	1·18	1·70	43·5

each temperature. Scheuplein, Blank & others (1969) considered that even *saturated* aqueous solutions of steroids were only 'very dilute concentrations' in the context of Fick's Law. Typical steady-state diffusion plots (for dexamethasone at 25 and 30°) are in Fig. 1 and data from other diffusion experiments with the four tritiated steroids between 10 and 40° are in

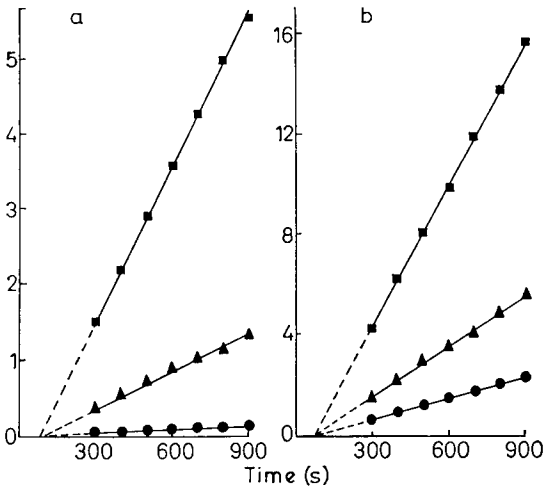

FIG. 1. Effect of applied phase concentration of dexamethasone on steady-state diffusion plots, through cellulose acetate membrane, (a) at 25°: (■) $2{\cdot}38 \times 10^{-6}$ g ml^{-1}, (▲) $5{\cdot}91 \times 10^{-7}$ g ml^{-1}. (●) $5{\cdot}35 \times 10^{-8}$ g ml^{-1}; and (b) at 30°: (■) $5{\cdot}61 \times 10^{-7}$ g ml^{-1}, (▲) $1{\cdot}74 \times 10^{-7}$ g ml^{-1}, (●) $6{\cdot}93 \times 10^{-8}$ g ml^{-1}. Ordinates a—M (g cm^{-2} × 10^{8}), b—M (g cm^{-2} × 10^{9}).

Table 2. Results confirmed the findings of Barry & El Eini (1976), in that permeability, P, was related to membrane/water partition coefficient, K, so that the least polar steroid permeated the fastest at any given temperature. The steroids all diffused to a similar extent so it was concluded that D was fairly insensitive to changes in polarity rather it was more dependent on molecular size.

The partition coefficient, K, was derived from the steady state flux dM/dt, since

$$\frac{dM}{dt} = \frac{D\,C_0}{h} \quad .. \quad .. \quad (1)$$

where h is membrane thickness, and C_0 is membrane surface concentration; K for diffusant between membrane and adjacent phases is then given by

$$K = C_0/C_1 \quad .. \quad .. \quad (2)$$

where C_1 is applied phase concentration. Knowledge of K permits calculation of P from

$$P = KD \quad .. \quad .. \quad (3)$$

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

Steroid	°C	D cm^{2}s^{-1} × 10^6	K	P cm^{2}s^{-1} × 10^6
Oestrone	10	13·9	2·67	37·1
	20	18·6	2·67	49·7
	25	21·0	2·70	56·7
	30	24·5	2·92	71·5
	40	32·0	2·55	81·6
Oestradiol	10	10·2	2·49	25·4
	20	14·8	2·50	37·0
	25	16·6	2·72	45·2
	30	19·6	2·75	53·9
	40	24·1	2·49	60·0
Oestriol	10	12·4	1·20	14·9
	20	17·2	1·44	24·8
	25	20·1	1·34	26·9
	30	23·3	1·45	33·8
	40	31·7	1·58	50·1
Dexamethasone	10	14·2	1·53	21·7
	20	19·3	1·55	29·9
	25	22·6	1·48	33·5
	30	26·2	1·62	42·4
	40	33·8	1·78	60·2

Steroid polarities were represented by R_m values from reverse-phase t.l.c. using 60% aqueous acetone mobile phase and light liquid paraffin stationary phase. This gave particularly good separation of the three oestrogens (Table 3), where decreasing R_m values correlated with addition of hydroxyl groups to the nucleus. Both log P and log K were linearly correlated with R_m (Fig. 2). Regression equations were:

at 10°: $\log P = 0{\cdot}523\ R_m + 1{\cdot}39$ ($r = 0{\cdot}937$; $P < 0{\cdot}1$)

at 20°: $\log P = 0{\cdot}416\ R_m + 1{\cdot}55$ ($r = 0{\cdot}946$; $P < 0{\cdot}1$)

at 25°: $\log P = 0{\cdot}476\ R_m + 1{\cdot}61$ ($r = 0{\cdot}978$; $P < 0{\cdot}05$)

at 30°: $\log P = 0{\cdot}457\ R_m + 1{\cdot}70$ ($r = 0{\cdot}958$; $P < 0{\cdot}05$)

at 40°: $\log P = 0{\cdot}245\ R_m + 1{\cdot}80$ ($r = 0{\cdot}815$; $P < 0{\cdot}2$)

Table 3. *Reverse-phase t.l.c. data of steroids using 60% aqueous acetone mobile phase and light liquid paraffin stationary phase, at room temperature (20°).*

Steroid	R_F	R_m*
Oestrone	0·368	0·220
Oestradiol	0·401	0·176
Dexamethasone	0·589	−0·156
Oestriol	0·714	−0·397

* $R_m = \log\left(\frac{1}{R} - 1\right)$ (Bates-Smith & Westall, 1950).

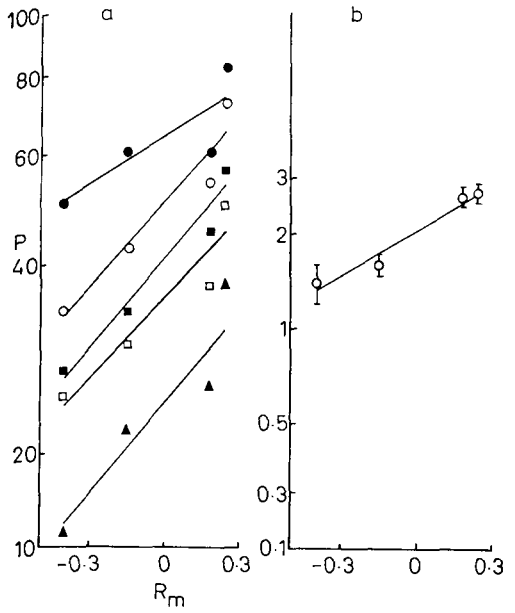

FIG. 2. a. Permeability of steroids, P ($cm^2 s^{-1} \times 10^8$), as a function of their R_m values at 10° (▲), 20° (□), 25° (■), 30° (○) and 40° (●); b. partition coefficient, K (ordinate), derived from equation 2, as a function of R_m.

and at 30° (a representative temperature)

$$\log K = 0{\cdot}483\, R_m + 0{\cdot}316 \quad (r = 0{\cdot}983;\ P < 0{\cdot}02).$$

During storage, the membrane thickness increased from 5×10^{-3} cm (dry) to $10{\cdot}7 \times 10^{-3}$ cm (wet). Measurements were made after 1 weeks soaking, at 4°, 17° and 30° and no difference in mean thickness was observed. Despite this apparent hydrophilicity, the polymer was slightly hydrophobic relative to water; i.e. the less polar the steroid the more it preferred the environment of the membrane.

Slopes of Arrhenius-type plots (see Flynn, Yalkowsky & Roseman, 1974b) of log D and log P versus reciprocal temperature showed that the more polar the steroid, the more rapidly did D and P increase with increasing temperature (Table 4). Diffusion energy of activation, E_D, for dexamethasone of 5·13 k cal mol^{-1} compared favourably with 5·74 k cal mol^{-1} (24 kJ mol^{-1}) found by Barry & El Eini (1976). Values of permeation energy of activation, E_p, were slightly higher than E_D for the more polar steroids, indicating that the overall permeation process involved a small positive heat of interaction. E_D and E_p values around 5 k cal mol^{-1} (21 kJ mol^{-1}) and low (near unity) membrane/water partition coefficients indicated steroid transport through aqueous

Table 4. *Energies of activation, E_D, for steroid diffusion and E_p, for steroid permeation across cellulose acetate membrane.*

Steroid	Ep kcal mol^{-1}	ED kcal mol^{-1}	Slope*	Intercept*	Corr Coeff.* (5 ps)
Oestrone	4·84	4·90	−1·07	−3·08	−0·999 ($P<0{\cdot}001$)
Oestradiol	5·22	5·05	−1·10	−3·08	−0·995 ($P<0{\cdot}001$)
Oestriol	6·91	5·51	−1·20	−2·66	−0·999 ($P<0{\cdot}001$)
Dexamethasone	6·01	5·13	−1·12	−2·88	−0·999 ($P<0{\cdot}001$)

* Refer to plot of D versus 1/T; as P = KD, values will be similarly ranked for P versus 1/T.

regions in the membrane, possibly around hydrophilic polymer groups (Kesting, 1971). The water content of fully hydrated membrane was estimated by weight difference to be approximately 44%.

The approximate approach of Flynn, Yalkowsky & Roseman (1974a) for calculation of theoretical, aqueous self-diffusivity, D_w, from estimated partial molal volumes (as used by Barry & El Eini, 1976) demonstrated the 'hindering' effect of the membrane (Table 5). Mean values of D_w were about 35 times larger than the observed values, D. Although the equations used related to gel solutions, it was felt that as the polymer chains are rigidly interlinked and the ratio of solute radius to pore radius is $< 0{\cdot}2$ the equations would hold adequately and any error introduced would be negligible. The results suggested that the polymer modified the observed diffusivity by either surface adsorption or interaction with the penetrant, as well as by a mechanical obstruction effect. The obstruction depended on diffusant molecular size and on volume fraction of polymer, Ø.

Table 5. *Calculated aqueous diffusivities, D_w, and estimated partial molal volumes, pmv, of steroids at 25°.*

Steroid	pmv ml mol^{-1}	D_w^a $cm^2 s^{-1} \times 10^8$	D_w^b $cm^2 s^{-1} \times 10^8$	D $cm^2 s^{-1} \times 10^8$
Oestrone	193	857	571	21·0
Oestradiol	196	852	568	16·6
Oestriol	198	849	566	20·1
Dexamethasone	266[c]	770	513	22·6

[a] calculated from $D_w = 4{\cdot}95 \times 10^{-6}/pmv^{1/3}$.
[b] calculated from $D_w = 3{\cdot}30 \times 10^{-6}/pmv^{1/3}$.
[c] improved estimate of value from Barry & El Eini (1976).

Lauffer (1961) showed for diffusion of small solutes through aqueous gels, that

$$D = D_w/(1 + 0{\cdot}667\,\emptyset) \quad .. \quad (4)$$

But if the polymer also interacts with or adsorbs the penetrant, then

$$D = D_w/(1 + A\,\emptyset) \quad .. \quad .. \quad (5)$$

where A is the adsorption-obstruction constant of solute in unit volume of gel (Schantz & Lauffer, 1962). The adsorption effect differs from the obstruction effect, in that the chemical properties of solute, solvent and polymer affect diffusivity, rather than molecular size. As approximately 35 fold differences were found earlier (Table 5), the use of equation 4 describing the simple polymer obstruction effect was thus not applicable (if $\emptyset = 0{\cdot}44$ then $D_w/D = 1{\cdot}29$); substitution into equation 5 provided, for A, values of around 80 for the steroids examined.

Beck, Schultz & Jerome (1972) found that the equation

$$D_p/D_f = 1\text{-}(r_s/r_p)^4 \quad .. \quad .. \quad (6)$$

described the diffusion of certain solutes, with various molecular volumes, through right cylindrical pores. D_p and D_f were the pore- and free-diffusivities respectively, while r_s and r_p were solute radius and pore radius. Some authors (Pappenheimer, 1953; Renkin, 1954) have shown that solute flux through a small aqueous pore is dependent on r_s and r_p. Equation 6 is an approximation of the full expression (Flynn & others, 1974a,b) but holds adequately when $r_s/r_p < 0{\cdot}2$. Ratios of r_s/r_p and D_p/D_f for the oestrogens and dexamethasone are in Table 6, where

Table 6. *Relationship between free diffusivity (D_f), pore diffusivity (D_p), solute equivalent spherical radius (r_s) and pore radius (r_p), as calculated from equation 6.*

Steroid	Estimated partial molal volume (pmv) ml mol^{-1}	r_s cm	r_s/r_p*	D_p/D
Oestrone	193	$4{\cdot}25 \times 10^{-8}$	0·177	0·459
Oestradiol	196	$4{\cdot}28 \times 10^{-8}$	0·178	0·457
Oestriol	198	$4{\cdot}28 \times 10^{-8}$	0·178	0·456
Dexamethasone	266	$4{\cdot}72 \times 10^{-8}$	0·197	0·416

* r_p of cellulose acetate (Visking) membrane = 24×10^{-8} cm (manufacturer's information).

it may be seen that for the four steroids, if molecular size was the sole controlling influence in diffusion through cellulose acetate membrane, pore diffusivity would have been approximately half the free diffusivity. Thus the three oestrogens provided a series of diffusants of similar molecular dimensions, but of widely differing polarities. Diffusivity, D, did not readily correlate with polarity, but there was a correlation between permeability, P, and polarity (Table 2). This was because, in the system investigated, D was relatively insensitive to the marked change in molecular polarity, but P was positively influenced by decreasing steroid-membrane interactions.

REFERENCES

Barry, B. W. & El Eini, D. I. D. (1976). *J. Pharm. Pharmac.*, **28**, 219–227.

Bates-Smith, E. C. & Westall, R. G. (1950). *Biochim. biophys. Acta*, **4**, 427–443.

Beck, R. E., Schultz, J. S. & Jerome, S. (1972). *Ibid.*, **255**, 273–303.

Flynn, G. L., Yalkowsky, S. H. & Roseman, T. J. (1974a). *J. pharm. Sci.*, **60**, 1788–1796.

Flynn, G. L., Yalkowsky, S. H. & Roseman, T. J. (1974b). *Ibid.*, **63**, 479–510.

Kesting, R. E. (1971). *Synthetic Polymeric Membranes*, New York: McGraw-Hill.

Lauffer, M. (1961). *Biophys. J.*, **1**, 205–213.

Pappenheimer, J. R. (1953). *Physiol. Rev.*, **33**, 387–423.

Renkin, E. M. (1954). *J. gen. Physiol.*, **38**, 225–243.

Schantz, E. J. & Lauffer, M. A. (1962). *Biochemistry*, **1**, 658–663.

Scheuplein, R. J., Blank, I. H., Brauner, G. J. & MacFarlane, D. J. (1969). *J. invest. Derm.*, **52**, 63–70.