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A two phase series model for the transport of steroids across the fully hydrated stratum corneum

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A two phase series model for the permeability behaviour of the fully hydrated stratum corneum has been examined using Scheuplein's data on steroids, since these strongly encouraged the investigation of possible heterogeneous diffusion models that showed the dependence of the effective diffusion coefficient, D_e, upon the effective partition coefficient, K_e. The model described can be characterized by V_c and V_w, the volume fractions for the "cytoplasm" and the "cell wall" phases, K_c and K_w, the solute partition coefficients for the "cytoplasm" and the "cell wall" phases and D_c and D_w, the respective diffusion coefficient for the two phases. Reasonable correlations were found between the experimental P_e, effective permeability coefficient, values and the partition coefficients obtained with amyl caproate and those obtained with hexadecane. Also the magnitude of D_w was estimated and found to be about 10^{-13} and 10^{-11} cm^2/s when the lipoidal nature of the cell wall was equated to hexadecane or amyl caproate. In general, reasonable self-consistencies among the various experimental results and parameters of the model were found.

In a recent investigation on the transport of steroids through the hydrated stratum corneum, Scheuplein, Blank & others (1969) found that the permeability coefficients for many steroids were not proportional to the partition coefficients as might be expected for compounds of such relatively constant size diffusing through a single homogeneous barrier. The data strongly encouraged the examination of possible heterogeneous diffusion models showing dependence of the effective diffusion coefficient, D_e, upon the effective partition coefficient, K_e, since these might lead to better correlation with the experimental results.

The two phase model for the stratum corneum

The simplest heterogeneous model consistent with the known anatomical data is the standard two phase series barrier (Fig. 1). The simple two phase model ignores transport through the appendages such as hair follicles or sweat ducts and considers only the contributions to the membrane volume from the cytoplasm and the cell wall. Air spaces in the membrane, for example, are neglected. More refined models should include these additional considerations as well as allow for the heterogeneous nature of the cytoplasm and the cell wall. For the fully hydrated stratum corneum, the membrane consists of about twenty cells with a total thickness of around 40 μm (Zelickson, 1967), with the cell wall thickness being around 10 to 20 nm (Zelickson, 1967). Therefore, the model with an hydrated value of around 40 μm and a cell wall

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"phase" volume fraction of around 0·01 to 0·10 should be reasonable for those situations encountered in Scheuplein's studies.

This model can be characterized by V_c and V_w, the volume fractions for the cytoplasm and the cell wall "phases" respectively, K_c and K_w, the solute partition coefficients (referred to water) for the cytoplasm and the cell wall "phases" respectively, and D_c and D_w the respective diffusion coefficients for the two "phases".

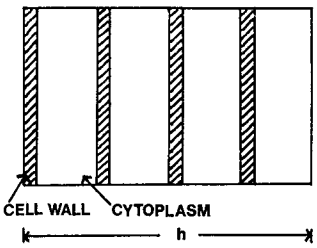

FIG. 1. Schematic two-phase model of the stratum corneum. Each phase has three parameters: a diffusion coefficient, a partition coefficient, and the volume fraction.

The effective permeability coefficient, P_e, for this system is given by

$$P_e = \frac{1}{\frac{V_w}{P_w} + \frac{V_c}{P_c}} \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where P_w and P_c are the permeability coefficients for the cell wall and the cytoplasm phases, respectively, and are given by

$$P_w = K_w D_w \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$\text{and } P_c = K_c D_c \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where the K's and D's have already been defined.

The effective (or the average) partition coefficient for the membrane, K_e, is given by

$$K_e = K_w V_w + K_c V_c \quad .. \quad .. \quad .. \quad .. \quad (4)$$

The effective diffusion coefficient, D_e, may then be defined by

$$D_e = \frac{P_e}{K_e} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

If P_e is determined by a steady-state transport experiment and if K_e is determined by the conventional binding or partition experiment, the D_e may be directly calculated (Scheuplein & others, 1969) from the experimental data by means of equation 5. Expressions for P_e and D_e result when equations 1 to 5 are solved, i.e.

$$P_e = \frac{K_w K_c D_w D_c}{(V_c D_w K_w + V_w K_c D_c)} \quad .. \quad .. \quad .. \quad .. \quad (6)$$

$$D_e = \frac{K_w K_c D_w D_c}{(V_c D_w K_w + V_w K_c D_c)(K_c V_c + K_w V_w)} \quad .. \quad .. \quad (7)$$

Equations 6 and 7 then provide a description of P_e and D_e according to the two phase model when the parameters characterizing each of the two phases are known. Also, if independent estimates of V_c, V_w, K_c, K_w, D_c and D_w can be made, direct comparisons of the theoretical P_e, D_e, and K_e with the experimentally determined values would be possible.

Analysis of experimental data with model

From the experiments of Scheuplein & others, we have the experimental values for P_e, K_e and therefore D_e (see Table 1). In addition Scheuplein determined the o/w partition coefficients of the steroids using amyl caproate and hexadecane as the oil phases (see column 4 and 5 of Table 1).

According to equations 4, 6 and 7, we have three equations in nine "unknowns". Since P_e, K_e and therefore D_e may be determined independently by appropriate experiments and since

$$V_c + V_w = 1 \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (8)$$

there are actually five unknowns in three equations. These are K_w, K_c, D_w, D_c and V_w which are still too many to perform a usual comparison of experiment with theory. Thus a rigorous test of the model with the available data is impossible.

It was therefore decided to examine the model by means of Scheuplein's data in the following manner. First reasonable values for D_w, D_c, K_w, K_c and V_w were selected. Then equations 4, 6 and 7 were tested to see if they showed reasonable self-consistencies among the various experimental results and parameters of the model.

Let us first consider simplifying equation 6. If, in the fully hydrated state, the cytoplasm portion of the stratum corneum is regarded as a porous bed of (protein and lipid) material containing aqueous pathways through which the solute diffuses, the quantity, K_cD_c, might be expected to be the order of 10^{-6} cm s^{-1}. This should be true as long as the external (or the continuous) phase of the cytoplasm in the hydrated state is substantial and primarily aqueous. If this is a reasonable supposition, then the assumption that

$$V_cD_wK_w << V_wK_cD_c \qquad \ldots \quad \ldots \quad \ldots \quad (9)$$

is reasonable since all of the permeability coefficients, P_e, listed in Table 1 corresponds to much smaller values than 10^{-6} cm s^{-1}. Equation 9 essentially states that for the experiments considered in Table 1, the rate limiting step is transport through the cell wall. Employing equation 9 we may therefore simplify equation 6 to

$$P_e = \frac{K_wD_w}{V_w} \qquad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (10)$$

This is much easier to examine than equation 6.

Fig. 2 shows reasonable correlations between the experimental P_e values and the partition coefficients obtained with either amyl caproate and those obtained with hexadecane. The broken line is merely given to show the unit slope, i.e. the trends of the data are consistent with a unit slope on this plot. The numberings indicate respective steroids which are listed in Table 1. These correlations would be expected if D_w remains relatively constant for the steroids in Table 1 and if the partition coefficients obtained with amyl caproate and with hexadecane are proportional to K_w. The latter supposition is not an unreasonable one, although it is apparent that some hydrogen bonding would probably occur in amyl caproate with OH group containing steroids but not in hexadecane. The constancy of D_w also appears to be reasonable although not enough is really known about this point. Certainly if the cell wall is viewed as a highly viscous liquid phase and the Stokes-Einstein relation is approximately obeyed, then the small molecular weight range considered is consistent with a relatively constant D_w.

Table 1. *Transport constants and partition coefficients for the steroids.*

	Steroid	P_e(cm²/s) $\times 10^{12}$	D_e(cm²/s) $\times 10^{13}$	K_e	K_{ac}	K_{hex}
1.	Progesterone	1668	160	104	56	17·0
2.	Pregnenolone	1668	220	50	52	4·2
3.	Hydroxypregnenolone	668	155	43	49	1·6
4.	Hydroxyprogesterone	668	166	40	46	2·5
5.	Cortexone	500	135	37	30	3·0
6.	Testosterone	444	195	23	16	2·6
7.	Cortexolone	83·2	36·1	23	11·2	0·1
8.	Corticosterone	66·8	39·2	17	6·8	0·024
9.	Cortisone	11·1	13·1	8·5	1·52	0·28
10.	Hydrocortisone	3·34	4·8	7	1·30	0·009
11.	Aldosterone	3·34	4·9	6·8	—	—
12.	Esterone	4000	870	46	80	3·0
13.	Oestradiol	334	72·4	46	66	0·63
14.	Oestriol	44·4	19·3	23	1·64	0·23

K_e = Stratum corneum/water partition coefficient.
K_{ac} = Amyl caproate/water partition coefficient.
K_{hex} = Hexadecane/water partition coefficient.
P_e were calculated using Scheuplein's K_p values and the thickness of 40 μm.

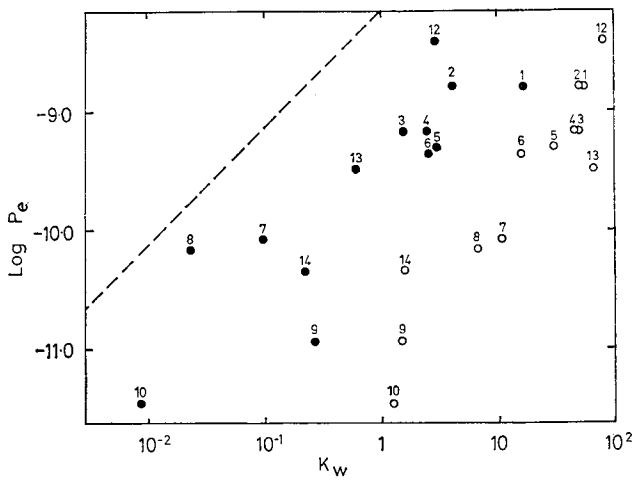

FIG. 2. Correlations between the experimental P_e values and partition coefficients obtained with amyl caproate (○) and those obtained with hexadecane (●). Numbers refer to Table 1.

By means of equation 10, an estimate of the magnitude of D_w may be obtained. If K_w is approximated by the partition-coefficients obtained with hexadecane, then $D_w \simeq 3 \times 10^{-12}$ and 3×10^{-11} cm² s^{-1} for $V_w = 0{\cdot}01$ and $0{\cdot}10$ respectively. If K_w is approximated with the amyl caproate data, then $D_w = 1{\cdot}0 \times 10^{-13}$ and $1{\cdot}0 \times 10^{-12}$ cm² s^{-1} for $V_w = 0{\cdot}01$ and $0{\cdot}10$ respectively. Thus, if the lipoidal nature of the cell wall can be equated to hexadecane or amyl caproate, a reasonable D_w value for steroids would be somewhere in the neighbourhood of 10^{-13} and 10^{-11} cm² s^{-1}.

Such low values for D_w are interesting for two reasons. First they imply that the stratum corneum cell wall membrane is extremely viscous or semi-solid like, as described by Scheuplein (1965). It is about a million times more resistant to diffusion than is liquid water. The second reason is that while values for D_w are extremely

small, they are of the same order of magnitude as those diffusivities found for other membranes, both biological and synthetic. For example, the transfer of sulpha drugs across the red blood cell membrane may be characterized by diffusivities of this order of magnitude (Holder & Hayes, 1965). Transport of many organic solutes across phospholipid bilayer membranes also encounters resistance of the same order of magnitude (Bean, Shepherd & Chan, 1968). Analyses (Suzuki, Higuchi & Ho, 1970) of data on rat intestinal absorption of sulpha drugs and of barbiturates have been found to be consistent with diffusivities of this order of magnitude. Thus the intrinsic barrier nature of the stratum corneum appears to be similar to that of other biological cell membranes and synthetic membranes. Of course, the stratum corneum, *in vitro* and *in vivo*, can be a more effective barrier than the single plasma membrane because it consists of many such cell membranes in series and because under dehydrated conditions it appears to exhibit enhanced barrier properties.

It is noteworthy that plotting P_e against K_e gives a slope that is much greater than unity, while an homologous series of alcohols gives a slope unity between K_p and K_e (Scheuplein, 1965), (Fig. 3). This lack of proportionality between P_e and K_e for the

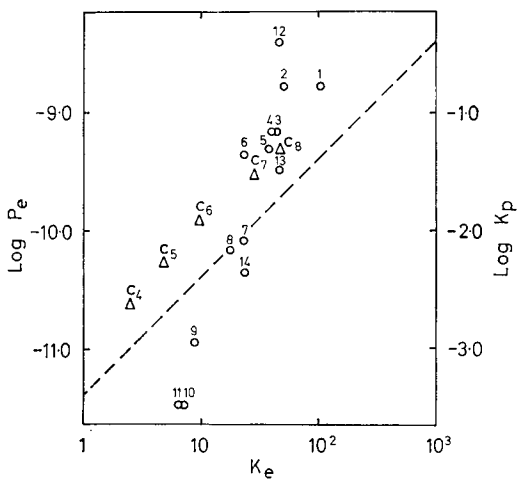

FIG. 3. The relation between the experimental P_e and the experimental K_e for steroids (○) and the relation between the experimental K_p and the experimental K_e for alcohols (△). The data are provided by Scheuplein. The broken line is given to show the unit slope.

steroids has been essentially the basis for the present two phase model. The steep slope of the P_e *vs* K_e curve corresponds to an effective diffusion coefficient, D_e, that is strongly dependent upon the polarity of the solute as can be seen in column 2 of Table 1. This relation from the two phase model's viewpoint is discussed next.

Equation 7 for D_e may also be simplified by the condition expressed by equation 9. We may therefore write

$$D_e = \frac{K_w D_w}{V_w(K_c V_c + K_w V_w)} \quad .. \quad .. \quad .. \quad .. \quad (11)$$

Fig. 4A and B present the results of theoretical calculations with equation 11 for the two situations $V_w = 0{\cdot}10$ and $0{\cdot}01$ respectively. D_e values as a function of K_w/K_c are plotted for the different D_w cases. It is noteworthy that all curves in both figures approach the same slope of unity as K_w/K_c decreases. This follows from equation 11, i.e. when

$$K_c V_c >> K_w V_w \quad .. \quad .. \quad .. \quad .. \quad (12)$$

$$D_e \simeq \frac{D_w K_w}{V_w V_c K_c} \quad .. \quad .. \quad .. \quad .. \quad (13)$$

Therefore D_e should become proportional to K_w/K_c under these conditions if, again, D_w remains relatively constant in the range considered.

To attempt a comparison of the experimental D_e with the theoretical results given in Fig. 4A and B, the plots given in Figs 5 and 6 were constructed. Fig. 5 gives the plots in which K_c was set equal to unity, and Fig. 6 gives those in which K_c was set equal to the experimental K_e. In both situations the amyl caproate and the hexadecane partition coefficients were used for K_w.

For comparison purposes an analysis was also made with the data on the homologous series of alcohols whose transport behaviour may be shown to follow the one phase limit of the two phase model. The relation between D_e and K_e, the partition coefficient in the stratum corneum, is shown in Fig. 5. In contrast to the steroid

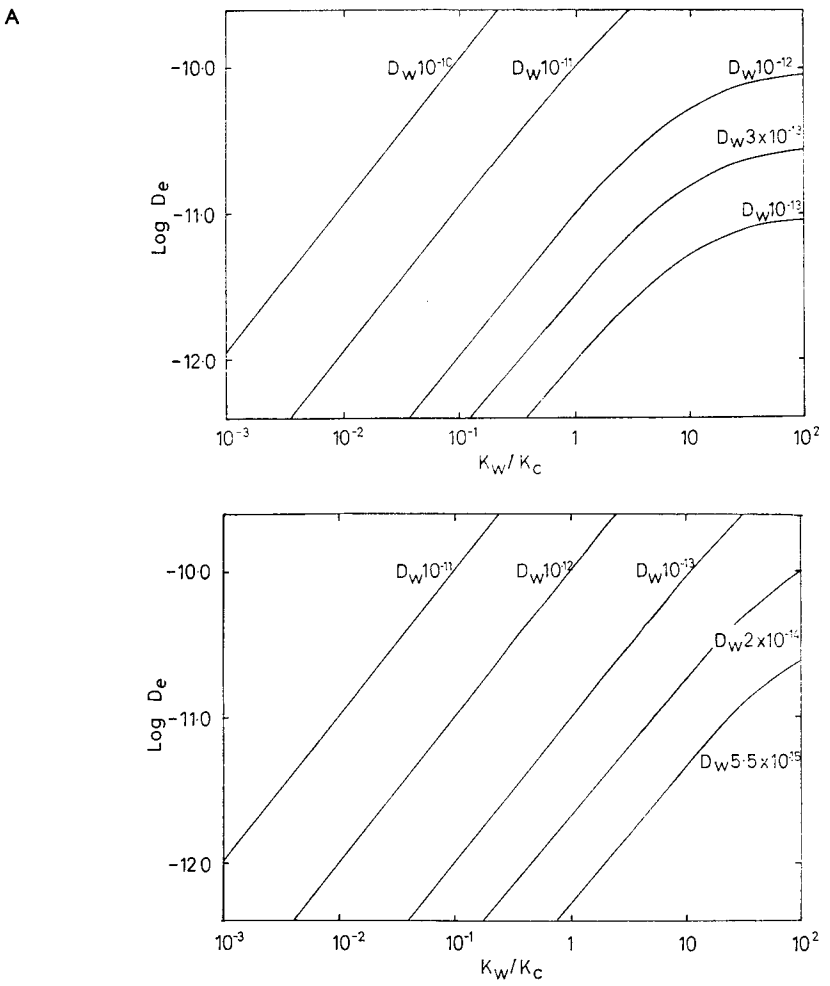

FIG. 4. Theoretical D_e values *vs* K_w/K_c under the various D_ws. $V_c = 0{\cdot}90$, $V_w = 0{\cdot}10$ (A) and $V_w = 0{\cdot}01$ (B).

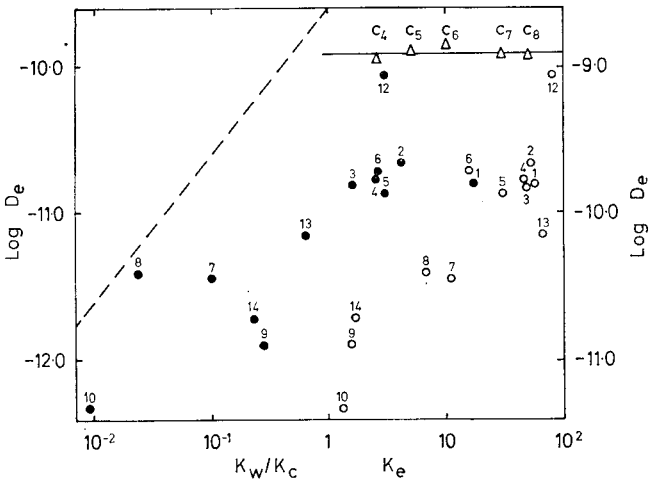

FIG. 5. Plots of experimental D_e values of steroids (left hand side) *vs* K_w/K_c and plots of experimental D_e values of alcohols (right hand side) *vs* K_e. The amyl caproate partition coefficient (○) and the hexadecane partition (●) taken from Table 1 were equated to K_w. $K_c = 1{\cdot}0$. △ indicates a homologous series of alcohols from butanol (C_4) to octanol (C_8). The broken line is given to show the unit slope. The solid line is given to show a distinct difference of the tendency.

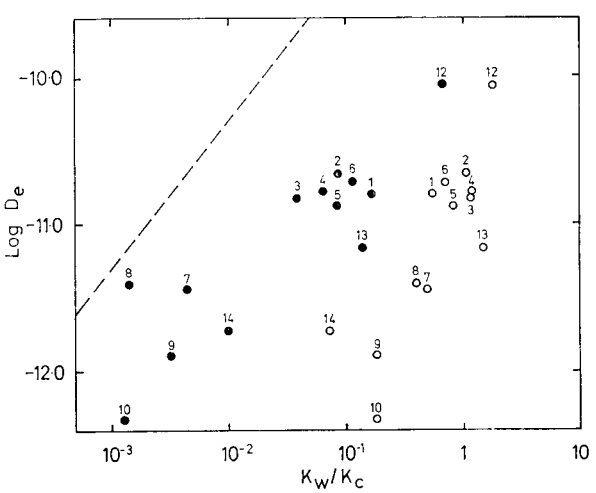

FIG. 6. Plots of experimental D_e values *vs* K_w/K_c. The K_w values are same as in Fig. 5. $K_c = K_e$, values taken from Table 1. The broken line is given to show the unit slope.

situation, it can be seen that D_e remains almost constant from C_4 (butanol) to C_8 (octanol). This would follow mathematically from equation 11, namely when

$$K_w V_w >> K_c V_c \quad .. \quad .. \quad .. \quad .. \quad (14)$$

accordingly

$$D_e \simeq \frac{D_w}{V_w^2} = \text{const.} \quad .. \quad .. \quad .. \quad .. \quad (15)$$

Thus there is a significantly different tendency with respect to the relation of D_e against K_e for the steroids and the alcohols. Also, it is seen from Figs 5 and 6 that there is reasonably good correlation of the experimental steroid data (slope $\approx$ unity)

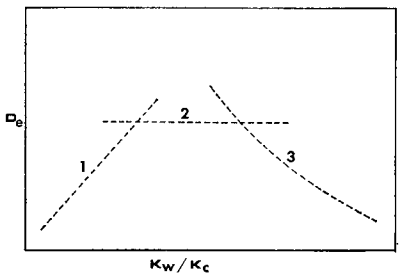

FIG. 7. A schematical relation between D_e, the effective diffusion coefficient and K_w/K_c, the ratio of partition coefficients. The numbered portions are characterized by the appropriate conditions, respectively.

with two phase theory, when either method of plotting the data is used, for both the hexadecane and the amyl caproate partition coefficients.

The general relation between D_e and K_w/K_c based upon equation 7 may be schematically classified according to the different conditions which are likely for a percutaneous transport system (Fig. 7). Under the condition expressed by equation 9, two different situations could be considered as already discussed. One is the situation which can be characterized by equations 12 and 13 for the steroids and corresponds to the linear portion #1 in Fig. 7. The other is what can be expressed by equation 14 and 15 for the alcohols and corresponds to the plateau portion (#2 in Fig. 7). The above analysis suggests that the steroids have a greater affinity for the phase other than that which is transport rate-determining. This might be interpreted as the steroids having a greater binding tendency for the protein-rich cytoplasm and the alcohols having a greater affinity for the more lipoidal cell wall phase. Finally, when $V_c \cdot D_w \cdot K_w$ is comparable to $V_w \cdot D_c \cdot K_c$ (e.g. if K_w is extremely large), the situation could be described by

$$D_e = \frac{D_w \cdot D_c}{V_c \cdot V_w \cdot D_w \cdot \frac{K_w}{K_c} + V_w^2 \cdot D_c} \quad .. \quad .. \quad .. \quad (16)$$

This case corresponds to the curve #3 in Fig. 7 and might apply to highly lipid soluble compounds. For this situation, D_e decreases as K_w increases. Although we do not have available data to illustrate this situation, highly lipid soluble, small molecular weight compounds are expected to fall into this category.

The present contribution represents a preliminary analysis of the two phase series model for the steroids. The absence of independent values for K_w and K_c preclude a rigorous test of the theory. However, recalling that one phase theory would predict no dependency of D_e upon K_w/K_c, the results presented in this report strongly encourage a further exploration of the two phase model and its extensions to more complex situations.

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