ANALYSIS OF PERMEABILITY DATA FOR THE CASE OF PARALLEL DIFFUSION PATHWAYS

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ABSTRACT A method of analyzing permeability or diffusion temperature-flux data, for the case of two parallel, independent diffusion fluxes is presented. Separate activation energies and frequency factors corresponding to independent and concurrent Arrhenius mechanisms are obtained from this procedure when the fluxes are of comparable magnitude. The method is developed in terms of membrane permeability theory since spatially separate and distinct diffusion pathways are of frequent occurrence in biological membranes. It is illustrated in the specific case of water diffusion through a keratin membrane. The results implicate the presence of "bound" water as a crucial factor in passive diffusion of water and polar molecules through the membrane.

INTRODUCTION

Transport processes in biological systems are often more complex than their classical analogues in experimental physics or physical chemistry. This is true in particular of passive processes occurring in closed, in vitro systems where no internal energy sinks or sources exist. The structural complexity of the tissue itself can account for much apparently anomalous diffusion behavior; e.g., sorting effects (1), rectification of flows, and deviations from Fick's law (2). In addition to these effects, which can result from asymmetric outer membrane surfaces, other anomalies attributable to the variety of internal substructures and the chemical differences associated with these substructures may occur. One of these, viz. the occurrence of multiple diffusion pathways and the resulting temperature behavior of the permeability constant, is the subject of this communication.

Consider, as an example, the steady-state diffusion rate of a pure substance through a biological membrane and through a thin, homogeneous solid slab of similar dimensions. Diffusion through the slab will be governed, exclusive of surface

effects, by a single diffusion constant which quite generally exhibits Arrhenius' temperature behavior; i.e.,

$$D = D_0 e^{-E/RT} \tag{1}$$

and

$$\ln D = \ln D_0 - \frac{E}{RT}. \tag{2}$$

The slope and intercept of the linear plot, $\ln D$ vs. $1/T$ give respectively, the activation energy, E, and the frequency factor, D_0. The relationship of these parameters to the free energy, enthalpy, and entropy of activation governing the rate process may be determined from absolute rate theory by well known methods (3). This analysis is quite generally applied to passive rate processes and can be extremely useful in aiding the discovery and interpretation of the molecular mechanism of the diffusion process. In the common application of the method to experimental data, it is assumed that the rate-determining step in the mechanism is characterized by a single activation energy, E. That diffusion should occur by a single, unique, molecular mechanism, involving a specific activated complex, is clearly dependent on the homogeneous structure and composition of the pure material composing the solid. In contrast, the typical "biological membrane"[1] has a nonhomogeneous, differentiated, mosaic structure associated with the enormous variety of specialized cellular and subcellular structures. If a significant difference in chemistry is associated with, say, two types of continuous internal structures, then activation energies for diffusion along these pathways may be very different. For example, in a filament-matrix tissue such as keratin, diffusion between filaments or on their surfaces may be quite different and independent of diffusion occurring within the filament interior. Also, if a membrane has a low intrinsic bulk diffusivity exclusive of a small number of pores, diffusion through both the pores and the bulk of the tissue can occur simultaneously. If the systems of molecules diffusing along these distinct pathways largely maintain their independence from one another, the total diffusion is the sum of both fluxes. It is easily shown that when a diffusion process occurs through two or more independent pathways an Arrhenius plot of the data will not in general be linear. The temperature behavior of the observed diffusion constant analyzed in this manner will not yield a meaningful activation energy or frequency factor and consequently a physical interpretation of the data is difficult.

A method is described for obtaining unique values for the activation energies and entropy factors in the simplest case when diffusion occurs through two independent pathways.[2] As an example of the procedure, results are applied to the

[1] We refer to anatomical membranes generally, i.e. skin, bladders, basement membranes, cellular membranes etc., and not specifically to the bimolecular unit membrane model. For this discussion of diffusion "a slice of tissue" would perhaps be the better term.

[2] The author is indebted to his referees for pointing out a similar and more general method,

specific case of water diffusion through human stratum corneum, a macroscopic lipid protein membrane.

Membrane Permeability along Parallel Pathways. Consider the particular case of membrane permeability where it has been established that the total experimentally measurable flux is actually the sum of two independent fluxes. We consider diffusion only (hydrodynamic flow is excluded) and assume a steady state near enough to equilibrium so that a proportionality of flux to gradient of free energy exists (4). In this case we can use the results of irreversible thermodynamics for the relationship between solute flow per unit area, J, and concentration difference across the membrane, ΔC. Under conditions of zero volume flow we have the Fick's law approximation (5),

$$J = J_1 + J_2 = k_1 \Delta C_1 + k_2 \Delta C_2. \tag{3}$$

The permeability constant, k, is a phenomenological quantity and is here defined by the relation $J = k \Delta C$. It can be related (with assumptions) to the classical diffusion constant through Fick's first law $J = (D/\delta X)\, \delta c$ and has dimensions of lt^{-1}. J represents the total experimental flux and since the same ΔC will apply for each flux,

$$k = k_1 + k_2, \tag{4}$$

where k is the observed permeability constant and k_1 and k_2 are the specific permeability constants corresponding to distinct mechanisms along separate pathways in the membrane. Qualitatively this is analogous to the addition of parallel conductances in electrical circuits.

Derivation of Basic Equations. We assume that both k_1 and k_2 (but not k) obey the Arrhenius equation; i.e.,

$$k_1 = A_1 e^{-E_1/RT} \tag{5}$$

$$k_2 = A_2 e^{-E_2/RT}. \tag{6}$$

We have, therefore

$$k = A_1 e^{-E_1/RT} + A_2 e^{-E_2/RT}. \tag{7}$$

Without loss of generality we can assume $E_2 > E_1$.

A routine ln k vs. $1/T$ plot of the experimental data which conforms to this equation will clearly be nonlinear. Fig. 1 shows the characteristic, positively curved plot obtained. The low temperature end asymptotically approaches a straight line of slope E_1 the (lower activation energy) as required. Similarly the high temperature end becomes asymptotically linear with slope proportional to E_2, reflecting the increasing influence of the high activation energy process at higher temperatures.

Perl (32), for "unpeeling" exponentials, which can resolve the multicomponent exponential problem.

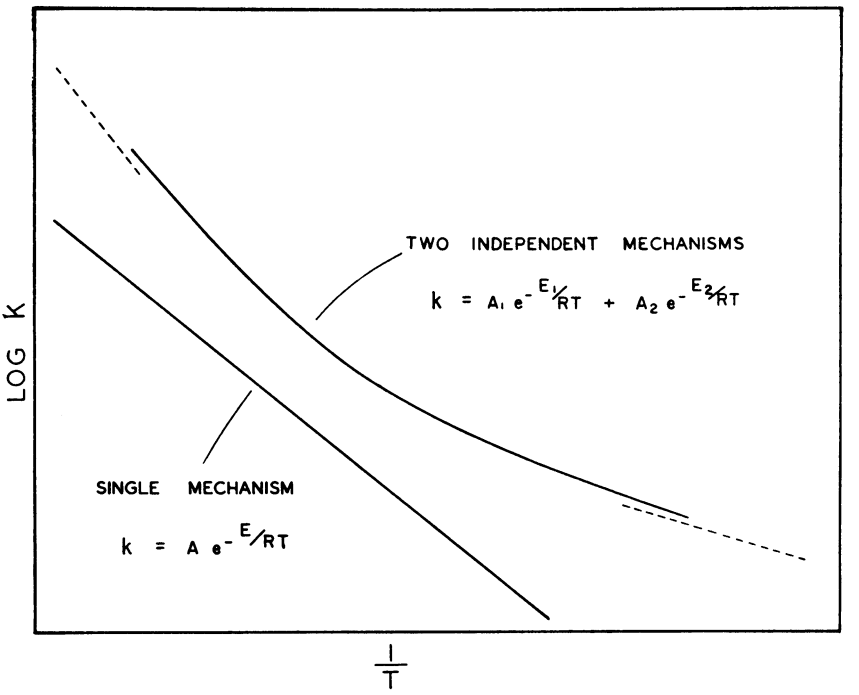

FIGURE 1 Arrhenius' plots, log k vs. $1/T$, for a single mechanism rate process compared with a rate process occurring by two concurrent and independent mechanisms.

Unfortunately experimental data on biological systems cannot always be obtained over a wide range of temperature to utilize this asymptotic behavior. It is obvious that Arrhenius' parameters computed from any chord approximation to the curve represent indefinite averages of the two processes and correspond exactly to neither.

By differentiation of equation (7) we have,[3]

$$RT^2 \frac{dk}{dT} = E_1k_1 + E_2k_2. \tag{8}$$

Equations (4) through (6) and (8) constitute a set of four independent equations, which in principle are solvable for the four unknowns E_1, E_2, A_1, and A_2. The experimental rate constant k can be obtained as usual from temperature rate measurements and dk/dT by graphical differentiation of the k vs. T curve. An analytical solution for this set of simultaneous equations cannot be obtained because the way k is split into k_1 and k_2 is registered only implicitly in the shape of the experimental curve; i.e., equation (7) and equations (5) and (6) cannot be used directly. A graphical solution is possible, however, by the following procedure. Multiplying

[3] We follow standard practice in assuming that the temperature dependence of A is small enough compared with that of $e^{-E/RT}$ to be safely ignored.

equation (4) first by E_1 and then by E_2 and subtracting each equation in turn from equation (8) we obtain,

$$\left(T^2 \frac{dk}{dT} - \frac{E_1 k}{R}\right) = \left(\frac{E_2 - E_1}{R}\right) k_2 \tag{9}$$

and

$$\left(\frac{E_2 k}{R} - \frac{T^2\, dk}{dT}\right) = \left(\frac{E_2 - E_1}{R}\right) k_1 \tag{10}$$

where $E_2 > E_1$.

Taking common logarithms of both sides of equation (9) we have:

$$\log\left(\frac{T^2\, dk}{dt} - \frac{E_1 k}{R}\right) = \log\left(\frac{A_2\, \Delta E}{R}\right) - \frac{E_2}{2.3RT}, \tag{11}$$

where equation (6) has been used for k_2 and $\Delta E = (E_2 - E_1)$. This is the equation of a straight line with slope, $= E_2/2.3\, R$, and intercept, $\log (A_2 \Delta E)/R$. Similarly with equations (5) and (10) we obtain,

$$\log\left(\frac{E_2 k}{R} - \frac{T^2\, dk}{dT}\right) = \log\left(\frac{A_1\, \Delta E}{R}\right) - \frac{E_1}{2.3RT}. \tag{12}$$

Method of Analysis of Experimental Data. If the left-hand sides of either equation (11) or (12) are plotted vs. $1/T$ a straight line will result if and only if the correct value of E_1, in equation (11) or E_2, in equation (12) is used. By varying E_1 or E_2 as parameters, a family of curves will be produced which converge to a straight line as the values assumed for either E_1 or E_2 become more nearly correct. The form of equations (11) and (12) suggests an interesting comparison with the standard Arrhenius equation. The argument of the logarithm on the left-hand side of equation (11) or (12) is precisely zero if $E_2 = E_1$, i.e. for the case of diffusion occurring by a single mechanism obeying $k = Ae^{-E/RT}$; i.e., the quantity $T^2\, dk/dt$ is identically equal to Ek/R.

Figs. 2 and 3 give the curves obtained for the case where

$$k = \exp -(5\ \text{kcal}/RT) + 10^7 \exp -(14\ \text{kcal}/RT). \tag{13}$$

The preexponential factors, 1 and 10^7, have been chosen in accord with the different activation energies so that the contributions from each mechanism are comparable. Obviously, if one term is overwhelmingly dominant the normal Arrhenius plot will be linear and no information about the lesser process can be obtained by the extended method. (These particular values are appropriate to water diffusion across dense biological membranes with a small fractional pore area. This is discussed below where the physical model consistent with the magnitude of these factors and the activation energies is discussed.) The dotted curve is the aberrant Arrhenius plot. Positive curvature is evident in this curve particularly at low temperatures where the small activation energy process becomes significant. The heavy line is the

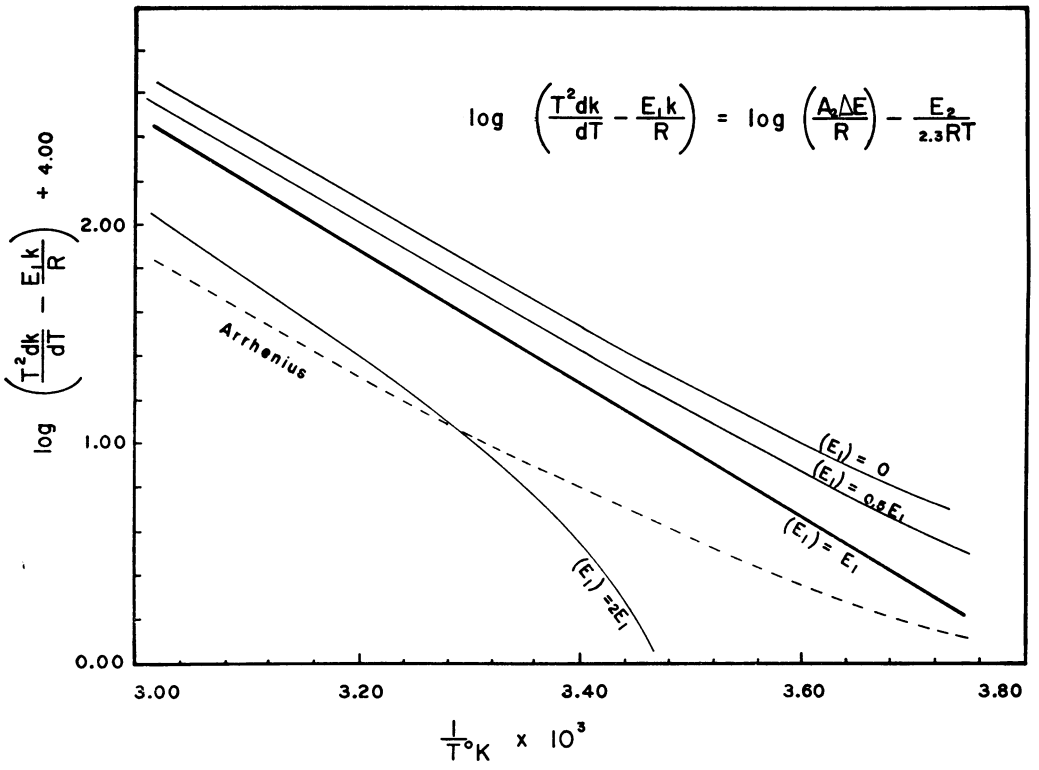

FIGURE 2 Flux-temperature plots for parallel mechanisms. Various estimates for the lower activation energy E_1 are used as parameters. The curves are computed from equations (11) and (13). Only the correct value, $E_1 = 5$ kcal mole^{-1}, yields a straight line with slope equal to $E_2/2.3\ RT$.

plot of equation (11) with the true value of E_1; i.e., 5 kcal. The negative slope is equal to $(-14$ kcal mole$^{-1})/2.3\ R$ as required. The intercept likewise yields the preexponential factor 10^7 from the term, log $(A_2\Delta E/R)$. Additional curves are shown, obtained by assuming other values for E_1 as indicated. Fig. 3 shows a similar family of curves for equation (13) where estimates for the higher activation energy are used as parameters. Both relations give regions where the slope is relatively insensitive to the particular value chosen for E_1 or E_2. In Fig. 2 the slopes converge to $E_2/2.3\ R$ at high temperatures where the k_1 process is comparatively negligible. In Fig. 3 the slopes converge to $E_1/2.3\ R$ at low temperatures where the k_2 process is negligible. For both relations the deviation from linearity is more extreme when the value chosen for either E_1 or E_2 makes the argument of the logarithm small. The correct values can be best obtained by plotting a family of curves and observing the convergence in this sensitive region. In the particular example shown, the lower activation energy; i.e., 5 kcal is small enough compared with 15 kcal that a rea-

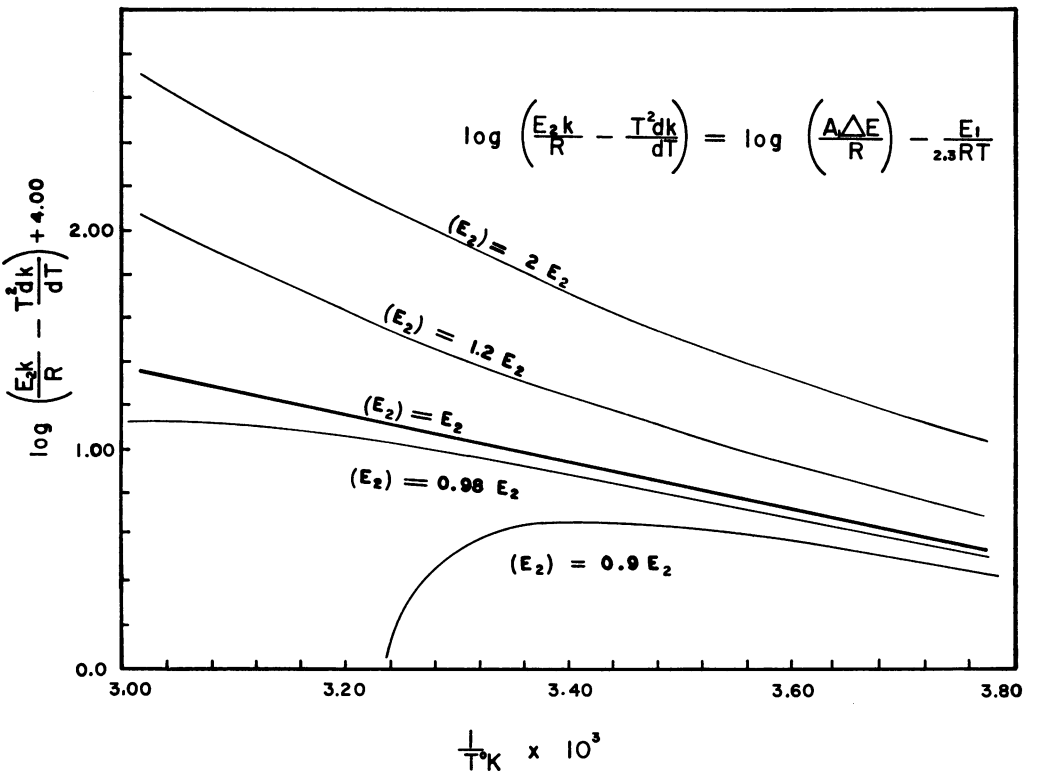

FIGURE 3 Flux-temperature plots for parallel mechanisms. Various estimates for the higher activation energy, E_2, are used as parameters. The curves are computed from equations (12) and (13). Only the correct value, $E_2 = 14$ kcal mole^{-1}, yields a straight line with slope equal to $E_1/2.3\ RT$.

sonable approximation to E_2 may be obtained from the Arrhenius plot itself. This is not true for the lower activation energy, E_1. The extent to which the Arrhenius slope gives a reasonable value for one of the processes depends, of course, on the magnitude of the ratio E_2/E_1 and the temperature range covered.

DISCUSSION

Scope and Precautions of the Method. Because of the necessity of differentiating the experimental k vs. T curve and the form of equations (11) and (12), large random errors in the values of k are ruinous. In the example discussed below, values of dk/dT were computed from a smoothed curve fitted to the k vs. T data. This avoids the difficulty, but introduces a certain arbitrariness to the analysis. Another shortcoming is the tediousness of the procedure which is more lengthy than that required for the routine Arrhenius plot. The desire for more ac-

curate information in those cases where parallel fluxes are suspected, may be for some, ample justification for doing the extra work.

Some evidence that independent parallel fluxes can indeed occur should be obtained before the analysis is accepted. In some cases the structural differentiation of the membrane makes such a situation likely; e.g., a filament-matrix or pore-matrix structure. It may also be possible to test the computed activation energies for physical consistency with the diffusion mechanism proposed. For example, diffusion of polar nonelectrolytes through liquid-filled pores should occur with an activation energy close to the corresponding solution diffusion values; i.e., 5 to 7 kcal mole^{-1}.

A necessary, if insufficient, criterion for the occurrence of parallel fluxes is a positive curvature of the normal Arrhenius plot. A change in the mechanism of diffusion with temperature such as occurs in ionic diffusion in solids cannot explain such a deviation in a biological membrane since the specialized mechanisms responsible for solid-state diffusion cannot apply to noncrystalline materials (6). A change in the diffusion mechanism might also occur with the structural breakdown of the biological membrane, but this implies a lower activation energy at high temperatures and negative curvature of the Arrhenius plot. This is, in fact, observed in the experimental example discussed below.

As stated above, the physical requirement implied by assuming independent pathways is that the systems of molecules associated with each pathway do not intermingle. This requires that most molecules do not jump back and forth between pathways but diffuse along the same pathway having a single activation energy throughout. If extensive crossing does occur most molecules will pass over both potential barriers in successive jumps to equilibrium positions. Temperature-rate data in this case will appear normal but can give information only about higher activation energy. This problem is best treated as a special case of multibarrier kinetics (7).

Application to Water Diffusion through Stratum Corneum.[4] Extensive permeability measurements have shown that stratum corneum membranes are not ideally stable in aqueous solution (8–10). Pores may slowly develop in these membranes after extended immersion in aqueous solution. Diffusion through the pores contributes an independent flux to the bulk diffusion occurring simultaneously throughout the undisturbed, intact regions of the membrane. The "pore" flux is comparable in magnitude and an accurate determination of the activation energy for the bulk diffusion process is impossible by the standard Arrhenius procedure. The extended method is applied below to a typical set of data, which is representative of the diffusion of water and polar aliphatic alcohols through stratum corneum.

Fig. 4 is an Arrhenius plot of the permeability constant as zero volume flow and

[4] The outermost (dead) layer and principal diffusion barrier of human skin. A multicellular lipid protein membrane composed of keratinized, hydrated epidermal cells.

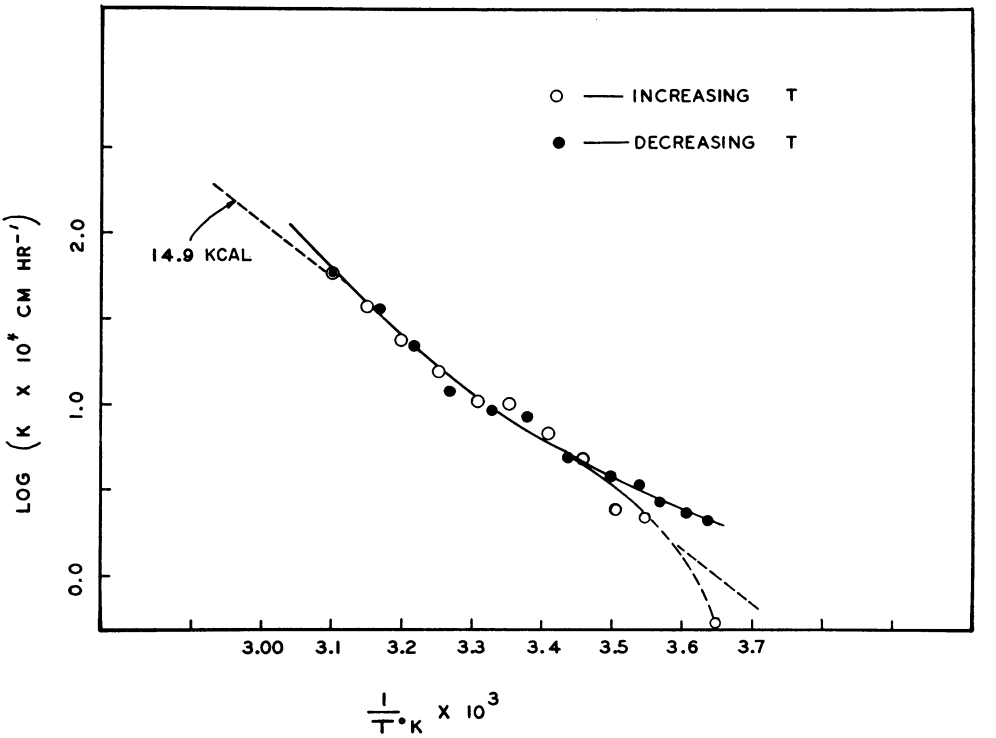

FIGURE 4 Arrhenius' plot of water permeability through stratum corneum. Open circles correspond to steady-state fluxes at various temperatures as temperature was increased from 0° to 50°C. Filled circles correspond to similar measurements on the same membrane as temperature was decreased.

zero hydrostatic pressure difference for water diffusion (HTO tracer) through a stratum corneum membrane.[5] The open circles represent the permeability constants at various temperatures as the temperature was increased from 0° to 50°C. The slope has initially, at low temperature, a high value which then decreases and appears to become constant at 14.9 kcal mole^{-1} (see dotted lines). Fig. 5 shows this change in slope with greater resolution in another experiment (and another membrane) with the temperature values taken one degree apart. The slope changes from an apparent value of 47.8 to 14.0 kcal mole^{-1} (least square lines are shown).

It would be incorrect to regard this steep slope as determining a correspondingly high activation energy as this part of the curve is not reproduced in the descending curve (filled circles in Fig. 4). This latter curve exhibits a definite positive curvature, is reproducible, and corresponds to the diffusion behavior of the fully hydrated

[5] The central purpose of this communication is to present the method of analysis and illustrate its use in a particular case. For additional experimental details and data on the permeability of water and polar nonelectrolytes through stratum corneum, see references (8–10).

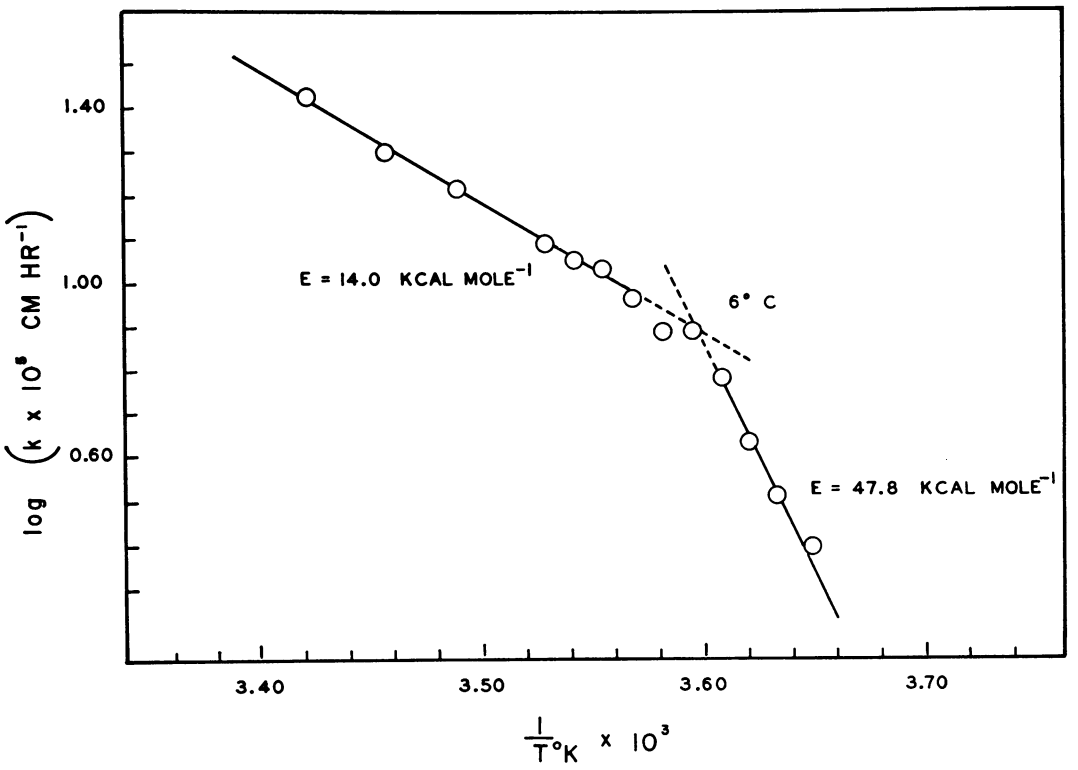

FIGURE 5 Low temperature range (0° to 20°C) Arrhenius' plot of water permeability through stratum corneum. Measured while temperature was increasing (lines fitted by least squares).

membrane after equilibrium with the bathing solution is attained. (This illustrates the importance of insuring that biological membrane permeability constants are reproducible throughout the temperature range before attempting to extract Arrhenius' parameters. Absolute rate theory assumes that the rate constant depends reversibly on the temperature and not on the previous history of the system. This is an important point which is often neglected in biological applications where it is most necessary.)

The low temperature diffusion through the now sensibly stable membrane is much greater than previously and is inconsistent with an activation energy which broadly fits the high temperature data; i.e., the dotted straight line in Fig. 4. The change in slope observed initially at low temperatures invites the suspicion of a more complex situation than that provided for by a single mechanism, Arrhenius' interpretation.

Supporting evidence indicates that as the keratin filaments slowly swell and soften in solution, the accessibility of the diffusing solute and solvent to the internal regions of the membrane increases (11). It appears that this is accompanied by the

opening of pores in local regions through which easier diffusion can occur. An additional increase in flux is registered unavoidably in the data during this period since ln k increases with time as well as with temperature. This accounts for the anomalously steep slope in the Arrhenius plot. After equilibrium diffusion in the membrane can occur through the two available pathways, through the bulk of the intact membrane, and through the newly formed pores. The physical distinction between these two diffusion pathways is made clearer below.

Application of the theory is shown in Figs. 6 and 7. Activation energies of 19.7 ± 0.5 and 6.0 ± 0.5 kcal mole^{-1} corresponding to bulk and pore diffusion are obtained.

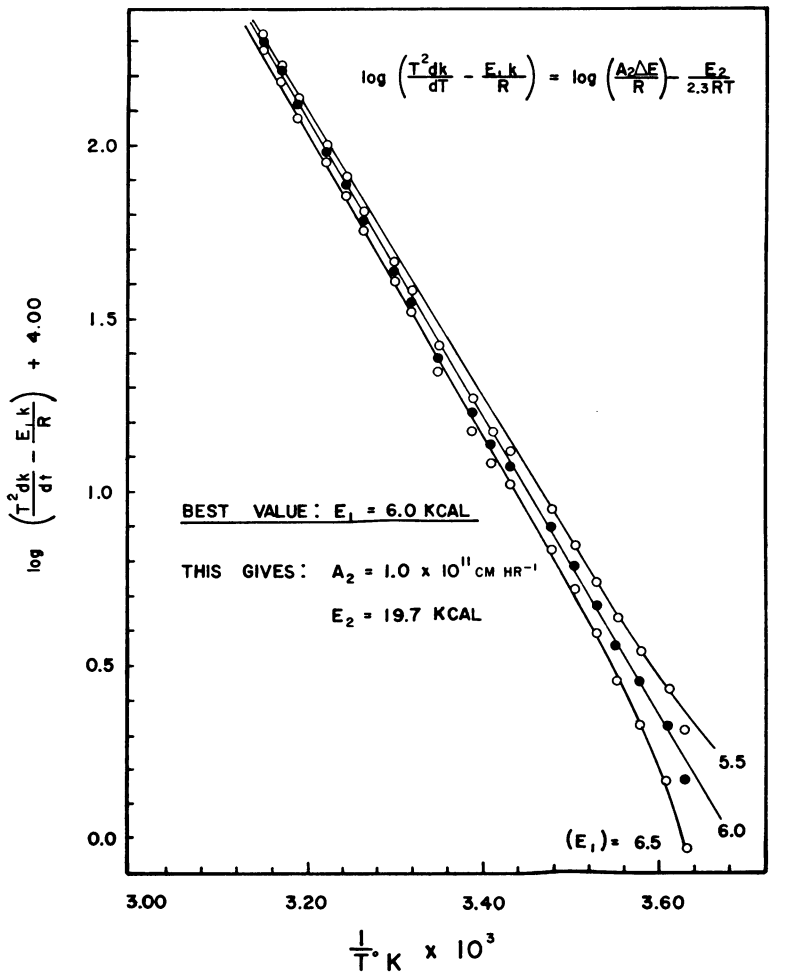

FIGURE 6 Flux-temperature plots for water permeability data of Fig. 4. This corresponds to Fig. 2; i.e., the low activation energy value E_1 is selected to give the best straight line. In this case E_1 is near 6.0 kcal mole^{-1}.

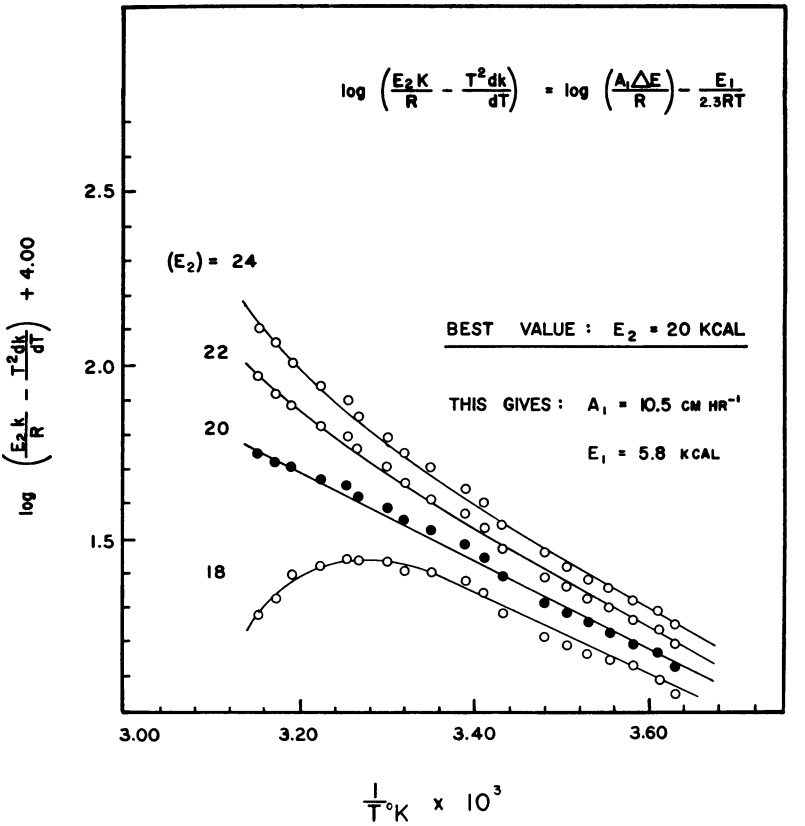

FIGURE 7 Flux-temperature plots from water permeability data of Fig. 4. This corresponds to Fig. 3; i.e., the high activation energy value E_2 is selected to give best straight line. In this case E_1 is near 20 kcal mole^{-1}.

TABLE I

DERIVED QUANTITIES FROM ABSOLUTE RATE THEORY (3)

	f	E	$\Delta H\ddagger$	$\Delta S\ddagger$	$\Delta F\ddagger$	D 25°C
Bulk	1.0	19.7	19.6	28.5	10.6	4.2×10^{-10}
Pore	10^{-5}	6.0	5.4	[2.5	3.9]‡	—

‡ Other values listed in the table were calculated according to the usual expression:

$$D = (ekT\lambda^2/h\delta x)e^{\Delta S\ddagger/R}e^{-\Delta H\ddagger/RT} \text{ cm}^2 \text{ sec}^{-1}.$$

These can be considered only approximate owing to the estimates for $\lambda = 3.0$ A and $\delta = 1.5 \times 10^{-8}$ cm, the molecular jump distance and the membrane thickness. Thermodynamic quantities are expressed in kcal mole^{-1} or kcal mole^{-1} degree^{-1}.

§ The bracketed values were deduced from the self-diffusion data of water: Longsworth (12) and Wang et al. (13).

The 19.7 kcal value is significantly higher than the previous 14.9 kcal obtained by the standard Arrhenius procedure which does not correct for simultaneously occurring pore diffusion. Fig. 8 shows the increase of the permeability constant with temperature. The dotted lines represent the component permeabilities according to the derived equation:

$$k = 10.5e^{-6.0/RT} + 10^{11}e^{-19.7/RT} \text{ cm hr}^{-1}. \quad (14)$$

Pore diffusion is dominant at low temperatures but increases slowly with temperature owing to the small activation energy. At room temperatures the flux from both mechanisms is approximately equal. At higher temperatures diffusion through the bulk of the membrane rapidly becomes dominant owing to the larger temperature coefficient. This particular decomposition of fluxes is the physical consequence of fitting the temperature rate data with equation (14) (the heavy line of Fig. 8, and Fig. 4). It is shown below that the magnitudes of these quantities are physically plausible as well.

Discussion of the Physical Model

Pore diffusion. The derived activation energy for the pore diffusion

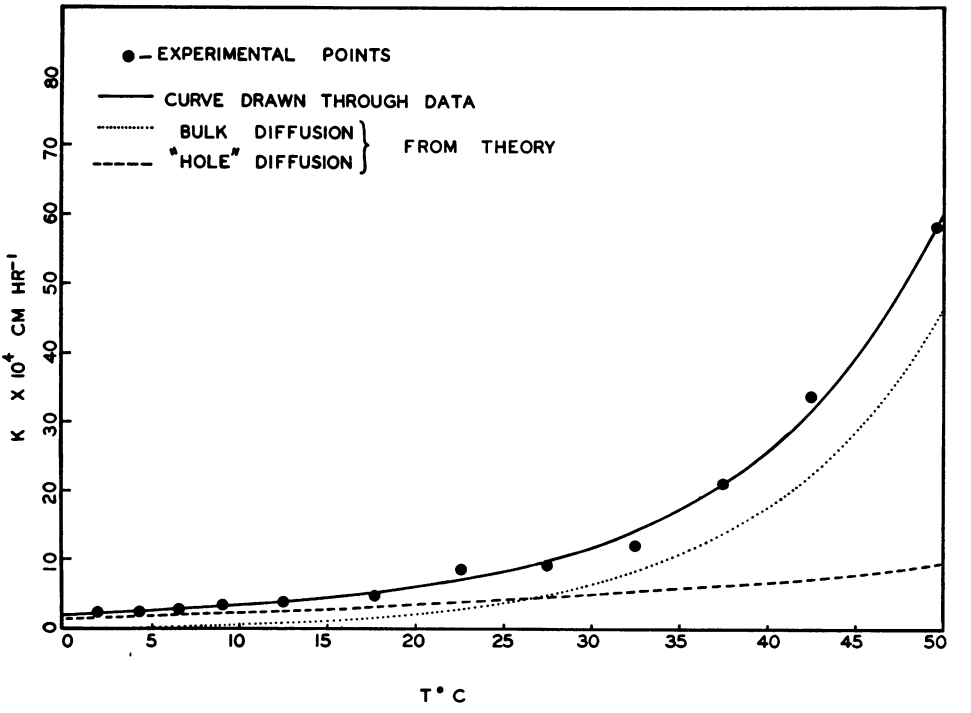

FIGURE 8 Permeability constant vs. temperature for water permeability through stratum corneum. The observed permeability (solid line) is decomposed into its two component permeabilities according to the theory.

process is reasonably close to the established value for the self-diffusion of liquid water, 4.7 kcal $mole^{-1}$ (12, 13). The observed value, 5.4 kcal $mole^{-1}$, is the same within the accuracy of the data. This agreement is a posteriori evidence of the validity of the pore diffusion hypothesis. Similar activation energies have been obtained for synthetic membranes where diffusion through water-filled pores is known to occur (14, 15). An estimate of the effective fraction of the membrane accessible to pore diffusion, $f = 10^{-5}$, is obtained from the ratio of the calculated pore diffusion constant (1.8×10^{-10} cm^2 sec^{-1} at 25°C) and the self-diffusion constant for water (2.3×10^{-5} cm^2 sec^{-1} at 25°C). Partial opening of intercellular spaces and appendages could account for this limited pore diffusion which occurs over a very small fraction of the stratum corneum membrane.

Bulk diffusion. The high heat of activation obtained for the bulk diffusion process; i.e., 19 kcal $mole^{-1}$ is approximately the same as that observed for the diffusion of water and polar nonelectrolytes through a host of cellular (17–19) and some multicellular membranes (16). As observed by Zwolinski et al. (17) these large activation energies " . . . bespeak a semi-solid structure for natural membranes. . . ." It is significant that in this tissue the "semi-solid structure" appears to be provided by "immobilized water" contained within the membrane itself. After extended immersion in aqueous solution the membrane swells as the keratin filaments become extensively hydrated (11). More or less continuous regions of water molecules are very likely present, adsorbed in irregular multilayers of the scaffolding provided initially by the polar side chains of the fibrous protein (20, 21). It is physically likely that the principal diffusion of water molecules occurs through these aqueous regions between the molecular or macromolecular components of the membrane rather than through the protein matrix per se. This would likewise hold true for other polar, nonlipid soluble molecules and perhaps other biological membranes as well. The concept of "structured water" within membranes is not new, Hays and Leaf (16) and Fernández-Morán (22) among others have discussed its potential implications. However, compelling evidence for the presence and influence of such structured water on membrane permeability is lacking. The observed passive diffusion of water through stratum corneum provides an experimental case in which active transport processes cannot interfere and in which the resistance to diffusion cannot be provided by a dense hydrophobic bimolecular leaflet (23, 24).

Although the precise nature of bulk liquid-phase water is not yet understood, there is evidence indicating that water adjacent to surfaces (in an interface phase) exhibits remarkably different physical properties (24, 25). An increase in the density and viscosity and consequently in the degree of immobilization appears to result (25). Evidence suggests that both polar (26) and nonpolar groups (26–30) can enter cooperatively in the stabilization of this immobilized water which is also favored by the open structure of water itself (31). When the structure of a membrane can promote such stabilization it would seem that both free liquid diffusion

and restricted diffusion might occur together; the relative amounts depending essentially on the efficacy of the stabilization and the size of the intermolecular spaces.

A difficulty comes in deciding where the boundary between "bound" and "free" water should be drawn as far as the activation energy for diffusion is concerned. The primary adsorbed monolayer is certainly a more difficult path for diffusion for at least two reasons. First, the H_2O-protein bond is stronger than the H_2O-H_2O bond and secondly, the rotation of water molecules is severely restricted in the first bound layer. These factors would act to increase the time between successive jumps of a water molecule bound in the monolayer and thus decrease the rate of diffusion. In the next layer and possibly for several more, the freezing out of rotational degrees of freedom might still be a significant factor.

Assuming for argument that the bound water persists only for two molecular diameters, i.e. $\simeq$6 A, we might describe the physical picture as approximating a raggedly, continuous coat of water molecules $\simeq$6 A thick over the macromolecular elements of the membrane; e.g., Fig. 9.

We would expect a water molecule (or any small polar molecule) embedded within this region to diffuse with a high activation energy. Other water molecules located beyond $\simeq$6 A from the internal surfaces, we assume would diffuse with an

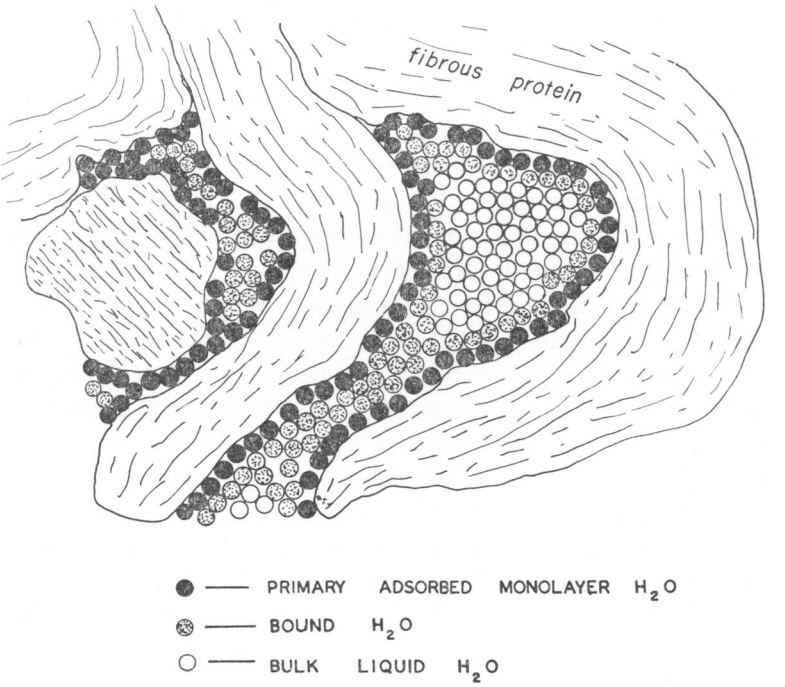

FIGURE 9 Idealized representation of bound and bulk water; i.e., liquid, such as water, adjacent to fibrous protein surfaces. The primary monolayer is probably strongly adsorbed to ionic side chains.

activation energy close to that of liquid water; viz., 4.7 kcal mole^{-1}. Regardless of the spectrum of sizes of the holes or pores actually present, there would be only two essentially different mechanisms for diffusion.

The observed low diffusion constant $\simeq 10^{-9}$ cm^2 sec^{-1} and high activation energy, 19 kcal, support the view that diffusion of water occurs through bound or immobilized water within the membrane. The high entropy of activation for the bulk diffusion process is also consistent with this idea. An appreciably greater entropy of activation would be involved if the diffusing molecules were oriented within a bound water matrix to begin with. The diffusion of liquid water involves approximately 2.5 e.u. or about half that released in the melting of ice. Comparison with the larger experimental value implies that bonds with several molecules must be broken in order for a single water molecule to diffuse.

Temperature-Flux Measurements on Biological Membranes. Studies of the effects of temperature on membrane permeability are more frequently found in the publications of early investigators than in the current literature. Danielli and Davson emphasized the importance of activation energies (i.e. Q_{10} values) to membrane transport theory and many cellular membranes were studied (19). Possibly there was an early overemphasis or tendency to expect too much too quickly from this kind of measurement. In any case the work appears to have led to few new developments and little work of this kind now seems to be done. This is equally unfortunate as such studies can give information which is related in a most direct way to transport mechanisms (3). A possible contributing reason for the indifferent results was perhaps a too facile employment of the method. Following the established procedures in chemistry, measurements were usually, and still are, made over wide temperature intervals, viz. every 10°C. This is suitable for in vitro chemical processes where activation energies are high and systems are stable over several hundred degrees, but rather poorly suited to aqueous biological systems. In liquid water, for example, several higher order phase transitions in the 0° to 60°C region have been reported (26). Similarly, conformational changes in the structure of lipid protein systems are produced in this temperature range. These phenomena ought to influence membrane permeability but could well be obliterated in poorly resolved data. Discriminating temperature-flux measurements on membranes with known structural differences might well show corresponding differences in permeability behavior which would be difficult to detect by other means.

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