FINAL COMPREHENSIVE SUMMARY REPORT
July 1964 - July 1965

MOLECULAR STRUCTURE AND DIFFUSIONAL PROCESSES
ACROSS INTACT EPIDERMIS

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Penetration of Non-Electrolytes

INTRODUCTION

The specific aim of the past work has been to obtain meaningful quantitative permeability data on small molecular weight non-electrolytes particularly homologous series of aliphatic alcohols and acids. Included under this heading are both kinetic and equilibrium measurements. The former data are derived directly from membrane diffusion fluxes (permeability and diffusion constants, entropies and energies of activation etc.); the latter from solution membrane solubility equilibria (partition coefficients, desorption energies, etc.). Such measurements for several homologous series of molecules can provide the critical information necessary for understanding the molecular mechanism of skin penetration.

Previous work has shown that extended immersion in water can drastically interfere with the measurement of diffusion fluxes in epidermis! Epidermal membranes are not ideally stable in aqueous solution; the stratum corneum swells and pores are opened in the membrane. Because of the importance of accurate kinetic data the penetration rates and the temperature dependence of the penetration rates have been re-measured in accord with the scheme proposed in an earlier work¹. (See Experimental Technique). The major difficulties due to the alteration of the membrane during measurement appear to have been circumvented and
the data is probably of quantitative significance. This is discussed under Results (I).

Penetration through skin can obviously be increased by damaging the "barrier." This can be done by treating the skin with any number of chemical reagents which destroy or partially dissolve the stratum corneum. Of these substances, those which leave the membrane mechanically intact and merely increase the number and size of the pores are the most interesting for our purposes. The effect of such solvent treatment on the water permeability is discussed under Results (II).

EXPERIMENTAL

Samples, diffusion cells, techniques and the analysis of permeability data are covered in earlier reports, viz.- Final Comprehensive Summary Report July 1953-1964 ---- Molecular Structure and Diffusional Processes Across Intact Epidermis.

Temperature Measurements

The following scheme was adopted to circumvent the effects of water immersion on the membrane and the rate data

1. The diffusion cell with the membrane was conditioned for at least three days in water prior to accepting the observed fluxes.

2. Membranes with higher than normal fluxes were discarded.
3. The most dilute solutions were used to decrease the membrane damage due to the solvent action of the alcohols.

4. Data was measured first at the highest temperature and then at lower temperatures to insure a minimal amount of change during the measurements. Then the temperature was increased and repeat measurements were taken with the same membrane. Only when the fluxes were reversible with temperature was the data accepted.

5. Adjacent pieces of epidermis were used to minimize differences in thickness and chemistry of the membranes.

RESULTS (I)

Permeability of Homologous Series

Permeability constants at various temperatures from 0°C - 50°C are listed in Tables I and II. Some general discussion of our experimental procedure is appropriate here. Due to varying membrane thicknesses and probable differences in lipid chemistry of samples from different individuals, meaningful comparisons of permeabilities for molecules within a homologous series ought to be based on fluxes measured on adjacent sections of membrane from the same large piece of tissue. Many experiments of this kind were done, each involving a group of alcohols. The absolute values of the permeability constants differed by as much as 100 o/o for different individuals. This factor would be expected in comparing any two membranes one of which is exactly twice as thick as
Log $K_p$ is log (kg $\times 10^{-5}$ cm/hr). $K_p$ is in units of 10$^{-3}$ cm/hr.

$K_p = \frac{d_p \times 10^{-3}}{1}$

<table>
<thead>
<tr>
<th>$d_p$ (cm)</th>
<th>Log $K_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.17</td>
</tr>
<tr>
<td>2</td>
<td>3.07</td>
</tr>
<tr>
<td>3</td>
<td>3.09</td>
</tr>
<tr>
<td>4</td>
<td>3.11</td>
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<td>5</td>
<td>3.13</td>
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<td>3.15</td>
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<td>7</td>
<td>3.17</td>
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<td>8</td>
<td>3.19</td>
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<td>9</td>
<td>3.21</td>
</tr>
<tr>
<td>10</td>
<td>3.23</td>
</tr>
</tbody>
</table>

Table II: Permeability Constants for the Alcohols at Temperatures from 5 to 50°C
### Table I

<table>
<thead>
<tr>
<th>Species</th>
<th>Octanol</th>
<th>HEP-TANOL</th>
<th>BUTANOL</th>
<th>HEPTANOL</th>
<th>PROPA-NOL</th>
<th>DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>---------</td>
<td>---------</td>
<td>-----------</td>
<td>---------</td>
<td>----------</td>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td>Test 1</td>
<td>3.59</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>5°C</td>
</tr>
<tr>
<td>Test 2</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>3.60</td>
<td>5°C</td>
</tr>
</tbody>
</table>

**Permeability Constants for the Alcohols at Temperatures from 5 - 50°C**
another. The thicker membrane would not change the observed activation energy as it would merely translate the $\ln K_p - 1/T$ plot 0.301 units vertically keeping the slope the same. The difference would appear in the pre-exponential factor $A$ which is calculated from the intercept. If the differences arise because of unique lipid chemistries or for reasons other than thickness, the error introduced into $E$ and $A$ would not be readily apparent. For these reasons a numerical average of all the data for different membranes would tend to obscure the real differences between the permeabilities of the alcohols and their temperature dependence. The results listed in the table are representative of stratum corneum only in the sense that they were obtained on two membranes of typical thickness ($\delta \approx 15\mu$) and chemistry. Penetration rates $J_5$ were obtained from the rate of increase of concentration in the receptor. Permeability constants $k_p$ were computed directly from the linear portion of the accumulation curve as previously described (see Appendix A). Each $k_p$ value is, therefore, an average over a dozen or more experimental points.

Permeability constants are plotted linearly vs. temperature in Figure I. This may be compared with Figure V in the Comprehensive Summary Report July 1963 - July 1964. The two sets of data appear quite similar, but significant differences are present and they become apparent when the data is more carefully analyzed. Corresponding Arrhenius plots are shown in Figures II, III and IV. Figure II shows
Figure I. Permeability constant $k_p$ vs. temperature °C for the homologous series of alcohols. Curves are drawn through initial data (filled circles) as explained in the text.
Figure II. Arrhenius plots for selected alcohols. The curve shows the entire permeability range included by the extreme numbers of the permeability series.
Figure III. Arrhenius plots for the polar alcohols. Curves are drawn through initial data (filled circles as explained in the text).
Figure 1D. Arrhenius plots for the non-polar esters. The curves show a decrease in activation energy reaching a constant value of about 10^0 K.

LOG (kp x 10^7 cal/hr^-1)

Temperatures: 25°C, 50°C, 75°C, and 100°C.

- Pentonal (2) •
- Hexonal (2) •
- Heptonal (2) ▼
- Octonal (2) ■

E = 1.70 K. cal/mole

E' = 10.0 K. cal/mole
the range in permeability for the homologous series of alcohols from
the most polar (ethanol) to the most non-polar (octanol). The alco-
hols separate rather surprisingly into two distinct groups rather than
being uniformly distributed over the permeability range. This was
also shown in our earlier report, Figures V and VI, ibid, but it was
thought to be an artifact and hence not emphasized. This corrobora-
tion makes it likely that a qualitative separation can indeed be
drawn between alcohols C_5^- and C_6^-.

The polar alcohols are, therefore, plotted separately on an ex-
panded scale in Figure III, the non-polar ones in Figure IV. Both
groups include pentanol for comparison. Two individual samples of
tissue were used for each group. The slightly different results ob-
tained for pentanol (1) and pentanol (2) are not in excess of what
one can expect for duplicate runs on the same sample. (The numbers
[1] and [2] after the names of the alcohol indicate the different
tissue samples).

Polar Alcohols

Our earlier data were misleading in indicating that the activation
energies for methanol and ethanol were as high as 25 K.cal mole^{-1}. The
activation energies for conditioned membranes are uniformly around
16.5 \pm 2.0 K.cal mole^{-1}. Moreover there is no evidence to suggest any
real difference in the activation energy among the five polar alcohols
(C_1^- - C_5^-) as previously reported. Both the anomalously high apparent
activation energies and apparent differences in activation energies among the polar alcohols were artifacts introduced by the slow alterations in the physical properties of the swelling membrane. The previous calculations unwittingly included these changes in addition to the true temperature effect. As the tissue softens and swells in solution more regions become accessible to diffusion. This process occurs slowly (3 days or so at room temperature) and in our previous experiments occurred simultaneously along with the taking of temperature-rate data. Owing to the fact that the area accessible to diffusion is registered unavoidably in the entropy factor A, ln k thus also increases with time as well as with temperature. It is clear that these data fitted to an equation of the form \(\ln k = \ln A - \frac{E}{RT}\) (with A constant) would give a larger activation energy than normal. Even if the membrane changed in a more complicated manner than the way described, it is clear that any change which increased K would result in a higher apparent activation energy.

This knowledge is quite important when it comes to drawing the best straight line through a set of experimental points as may be seen in Figure II. The open symbols represent return measurements on the same membrane after initially reducing the temperature (filled symbols). They are all in cases close to the original points but always slightly higher. We now can interpret these points as being due to a slightly damaged membrane and draw the line through the original points with assurance. If the repeat measurements had been far off, we would have
to conclude the membrane had been severely damaged early in the run and throw out the data.

The thermodynamic quantities associated with the diffusion of the polar alcohols are given in the first part of Table III. These again should be compared with the values given on page 12 of the earlier report cited above. The method of computation for $\Delta S^\ddagger$ and $\Delta F^\ddagger$ is slightly different in that a correction for the partition coefficient $K_m$ is made, (See appendix B). It may be observed that the diffusion of polar molecules is characterized by a high activation energy, e.g., $16.5 \text{ K.cal mole}^{-1}$ and also a high entropy of activation $21-24 \text{ K.cal mole}^{-1} \text{ deg}^{-1}$. The significance of these quantities to the molecular mechanism of diffusion is discussed below. See Discussion of Results (I).

**Non-Polar Alcohols**

In contrast to the polar alcohols, the non-polar alcohols do not penetrate with a constant activation energy (Figure III). At low temperatures the activation energy is very roughly $20 \text{ K.cal mole}^{-1}$ or more. (More accurate values are presently being obtained). As the temperature is raised the activation energy decreases becoming constant at approximately $9 \pm 2 \text{ K.cal mole}^{-1}$. This can not be due to an irreversible change on the membrane as the change itself is reversible. These differences between the polar and the non-polar alcohols support our earlier thinking that two different mechanisms of transport are
<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>E</th>
<th>$\Delta H^\ddagger$</th>
<th>$\Delta S^\ddagger$</th>
<th>$\Delta F^\ddagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>119</td>
<td>16.4</td>
<td>15.8</td>
<td>18.7</td>
<td>10.2</td>
</tr>
<tr>
<td>Propanol</td>
<td>502</td>
<td>16.5</td>
<td>15.9</td>
<td>19.2</td>
<td>10.2</td>
</tr>
<tr>
<td>Butanol</td>
<td>1,760</td>
<td>16.7</td>
<td>16.1</td>
<td>21.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Pentanol</td>
<td>3,060</td>
<td>16.5</td>
<td>15.9</td>
<td>20.9</td>
<td>9.7</td>
</tr>
<tr>
<td>Hexanol</td>
<td>1.71</td>
<td>10.9</td>
<td>10.3</td>
<td>4.67</td>
<td>8.9</td>
</tr>
<tr>
<td>Heptanol</td>
<td>0.41</td>
<td>9.9</td>
<td>9.3</td>
<td>-0.36</td>
<td>9.4</td>
</tr>
<tr>
<td>Octanol</td>
<td>0.076</td>
<td>8.7</td>
<td>8.1</td>
<td>-4.17</td>
<td>9.4</td>
</tr>
</tbody>
</table>

A —— Experimental frequency factor (cm$^2$ sec$^{-1}$)

E —— Experimental activation energy (K. cal. mole$^{-1}$)

$\Delta H^\ddagger$ —— Enthalpy of activation (K. cal. mole$^{-1}$)

$\Delta F^\ddagger$ —— Free energy of activation (K. cal. mole$^{-1}$)

$\Delta S^\ddagger$ —— Entropy of activation (K. cal. mole$^{-1}$ deg$^{-1}$)

The values are not corrected for desorption energies and the resulting temperature variation in $K_m$.

The frequency factors A contain the partition coefficient $K_m$. This has been removed from $\Delta S^\ddagger$ and $\Delta F^\ddagger$ following the procedure outlined in Appendix B.
involve. The formation of pores by the solvent action of the water which measurably increases the flux of polar molecules does not seem to influence the flux of non-polar molecules. This is also consistent with the presence of independent pathways. The membrane-water partition coefficient of the higher alcohols \( K_m > 10 \) would decrease the relative importance of diffusion through water-filled pores as the concentration of solute within them would be quite low.

The frequency factors corresponding to the high temperature, linear portions of the plots in Figure III are quite low and similar to values obtained for liquid diffusion. Entropies of activation computed from these are small, Table III. Perhaps not too much significance should be given to the negative values shown in the table owing to the estimate for \( \lambda \), and the uncertainty in the intercepts. These entropies are, however, much lower than those for the polar alcohols and implicate a different kind of activated state in the diffusion process.
DISCUSSION OF RESULTS (I)

Before discussing the details of the diffusion mechanism it is well to summarize some earlier conclusions.

1. The stratum corneum behaves like a homogeneous slab of material in so far as diffusion is concerned. The complex anatomy of the membrane does not appear to provide many short-circuit pathways along which diffusion can occur with a lower heat of activation than through the bulk of the tissue. Morphologically this means that diffusion occurs transcellularly rather than between cells or within special appendages. The evidence for this is the small membrane diffusion constant $D_m$, the large heats of activation and the large and positive entropies of activation. The presence of a substantial number of active "short-circuit" pathways would imply a much lower and unrealistically low value of $D$ for the cellular walls (see Appendix A). If most of the flux were carried by diffusion shunts comprising a minor fraction of the tissue volume, the data would yield negative values for the entropy of activation. We might also expect $E$ and $\Delta H^\dagger$ to be small, consistent with our hypothesis of "short-circuit" diffusion. We, therefore, conclude that the low permeability of stratum corneum is due to a uniformly low intrinsic diffusivity which is measured by $D_m$.

2. Both polar and non-polar nonelectrolytes are sorbed by the membrane. Non-polar molecules are extensively soluble ($K_m \approx 50$), polar molecules much less so ($K_m \approx 1$). To a first approximation,
permeabilities increase in direct proportion to corresponding membrane-water partition coefficients. In this sense transport across the membrane is selective; the more soluble molecules are transferred more quickly. This is not caused by a higher mobility of the molecules within the membrane but is due to a. increased concentration gradient
\[
\frac{K_m}{\Delta C_s} \cdot \frac{\Delta c_s}{\delta}
\]

Temperature rate data give us additional information regarding the molecular details of diffusion. Figures I - III show the series of alcohols tend to separate into two groups between pentanol and hexanol. The separation is probably quantitative rather than qualitative in that a really distinct division between the diffusion mechanisms of adjacent molecules in a homologous series is unlikely, particularly in view of the complexity of the diffusing media. But it does support a loose classification of the molecules into a polar and a non-polar group according to diffusion evidence alone. Within each group the thermodynamic quantities associated with diffusion are similar, e.g., Table III. The observed partition coefficients and the physical properties of the alcohols also support such a grouping by implicating chemically different diffusion pathways. It seems likely that polar molecules would prefer to diffuse through other water molecules adsorbed on the extensively hydrated membrane. This assumes a strongly bound water layer adsorbed on the polar sites of the membranes which is essentially immobilized. Polar molecules would not tend to segregate
into the lipid rich regions of the cells. But these would be attractive sites for non-polar molecules. At present it is not possible to locate more precisely the lipid rich regions within the cells. It is true that when delipidizing solvents are used the fibrils themselves remain grossly unchanged even though 20-40 o/o of the dry mass of the membrane is removed. This suggests that the lipids are contained principally on the surface of the fibrils and between them. It is tempting to suggest that polar molecules diffuse within or near the surface of the fibrils and non-polar molecules between them.

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**Diagram:**

- **Keratin Fibril**
- **Lipid molecule**
- **Water molecule**

---

-11-
But it is also possible for the molecules to select separate pathways from a complicated, and confused arrangement of lipid and non-lipid elements within an amorphous mixture between the fibrils. We really don't know enough about the chemistry of this region to draw a more definite picture.

There seems to be adequate evidence to indicate both polar and non-polar diffusion pathways regardless of whether these actually correspond to prominent ultrastructural features.

Polar non-electrolytes - we suppose the polar pathways to consist of layers of partially immobilized water molecules anchored through other water molecules to the polar side chains of the protein. A low diffusivity and high activation energy seem to support this postulated immobilization of water molecules and the resulting high viscosity. The apparent bulk viscosity of the diffusing media is 10^6 times that for liquid water (Table IV). More direct evidence is given by the very high entropies of activation. Generally, large positive entropies imply a disordered activated state compared to the initial state. This normally arises in the self-diffusion of associated liquids as intermolecular bonds have to be broken in attaining the activated complex. Liquid water gains approximately 2.5 K.cal. deg\(^{-1}\) mole\(^{-1}\) (2.5 E.U.) in self diffusion\(^3,\ 4\). This may be profitably compared with the entropy gained by melting ice, i.e., \(\Delta H_f/T = 5.26\) EU. When a polar alcohol is dissolved in water it is well known that the water molecules become
TABLE IV

Apparent Membrane Viscosity from Diffusion Data

<table>
<thead>
<tr>
<th>Alcohol</th>
<th>(D_{25^\circ C})</th>
<th>(K_m)</th>
<th>(D_m)</th>
<th>(\eta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.11</td>
<td>0.6</td>
<td>2.0</td>
<td>(1.5 \times 10^4)</td>
</tr>
<tr>
<td>Propanol</td>
<td>0.42</td>
<td>2.0</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Butanol</td>
<td>0.99</td>
<td>2.5</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Pentanol</td>
<td>2.54</td>
<td>5.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Hexanol</td>
<td>15.9</td>
<td>15</td>
<td>11.0</td>
<td>(1.4 \times 10^3)</td>
</tr>
<tr>
<td>Heptanol</td>
<td>22.9</td>
<td>30</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>Octanol</td>
<td>30.0</td>
<td>50</td>
<td>6.0</td>
<td></td>
</tr>
</tbody>
</table>

\(D_{25^\circ C}\) — Diffusion constant \(\times 10^9\) \(\text{cm}^2\) \(\text{sec}^{-1}\)

\(D_m = \frac{D_{25^\circ C}}{K_m}\) — Intrinsic membrane diffusion constant \(\times 10^{10}\) \(\text{cm}^2\) \(\text{sec}^{-1}\) when \(K_m\) is membrane-water partition coefficient

Apparent bulk viscosity from \(\eta = \frac{kt}{\lambda D}\) poise.

\[
\frac{kt}{\lambda} = 1.37 \times 10^{-6} \text{ dynes}
\]

\[
\lambda = 3.1 \times 10^{-8} \text{ cm}
\]

\(25^\circ C\) (liquid water) = \(8.95 \times 10^{-3}\) poise
oriented and immobilized round the hydrocarbon chains\textsuperscript{5}. From measurements of hydrocarbon chain monolayers on water surfaces, one can calculate that about 5 E.U. are lost by the water molecules per-CH\textsubscript{2}-immersed\textsuperscript{6}. Assuming this figure is also reasonable for bulk processes as well as in monolayers, we might easily obtain an activation entropy of around 15 E.U.

Non-polar nonelectrolytes-lipid soluble molecules - The data with hexanol, heptanol and octanol indicate that non-polar molecules dissolve in the lipid rich portions of the membrane. The changing activation energy could arise from a "melting phenomenon" wherein lipid parts of the membrane become less rigid as hydrophobic bonds begin to break. Viscosity would decrease and diffusion would be enhanced. One would not expect such a change in the activation energy for polar molecules except at much higher temperatures where the stronger hydrogen bonding could be affected. Activation energies for diffusion appear to reach a constant value near 9 \pm 2 K.cal mole\textsuperscript{-1}. Since large molecules do not diffuse as a whole but in segments, this behavior is not surprising. The activation energy for much larger molecules containing long paraffin chains might be expected to increase since the medium is not a liquid and increased binding to fixed sites in the membrane would tend to occur.
DISCUSSION OF RESULTS (II)

Preliminary data on the effect of various solvents on the permeability of stratum corneum is shown in the series of Figures V - VII. The data in all cases represents the permeability of the stratum corneum to water (HTO) after various treatments and hence generally corresponds more with the permeability of polar rather than non-polar molecules. All membranes were equilibrated with water for three days prior to measurement. In Figure VII, corresponding D.C. resistance values, taken simultaneously are given; these may be ignored for the present.

Figure V shows the increased water-flux during treatment of six membranes with six solvents. The limiting $k_p$'s are given below:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Water Permeability ($k_p \times 10^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Chloroform-Methanol (2-1)</td>
<td>120</td>
</tr>
<tr>
<td>2. Acetic Acid (100 o/o)</td>
<td>20.3</td>
</tr>
<tr>
<td>3. Ether (100 o/o)</td>
<td>18.2</td>
</tr>
<tr>
<td>4. Dimethyl Sulfoxide (100 o/o)</td>
<td>6.3</td>
</tr>
<tr>
<td>5. Acetone (100 o/o)</td>
<td>4.8</td>
</tr>
<tr>
<td>6. Ethanol (100 o/o)</td>
<td>1.7</td>
</tr>
</tbody>
</table>
During this experiment the membranes are continuously held in contact with the solvent which bathed the receptor half of the cell (about ten hours). Chloroform methanol had the largest effect; ethanol the least. To test the conclusion that the principal effect was the opening of holes in the membrane, the permeability of several chloroform-methanol treated membranes was measured at 27°, 37° and 47°C. The results are shown in Figure VI. The activation energies are all close to 6.0 K.cal.mole⁻¹ confirming the hypothesis of liquid like diffusion through newly opened pores in the membrane.

Figure VII, the effect of scalding the membrane successively in water is shown. Permeability decreases as expected but the membrane is still largely intact.
Figure V. Receptor activity vs. time. Shows rate of increase in water permeability (HTO) during continuous contact with various delipidizing solvents and acetic acid.
Immersed fluid diffusion through water filled pores.

Ignores hydrophobic-membrane (S-1), low activation energy.

Arrhenius plot for water permeability (H2O) through membrane de-

$E = 6.54 \text{ kcal/mole}$

$E = 6.08 \text{ kcal/mole}$

$E = 6.14 \text{ kcal/mole}$
WORK IN PROGRESS AND FUTURE PLANS

The following work is in progress --------

1. Accurate measurement of membrane-water partition coefficients and desorption energies.

   The accuracy of the gas chromatographic method of measurement of $K_m$ was limited by the sensitivity and precision of the analytical method itself, the necessity of taking intermittent rather than continuous data and the loss due to volatilization of the alcohols from the chamber. These errors are avoided in a method involving continuous analysis of aliphatic acids by polarograph during their absorption by stratum corneum.

   The penetration of large molecules, ions and gases through stratum corneum presents specific and formidable difficulties which we believed could more easily be understood once a beginning had been made in the simpler area of non-electrolyte transport. Our experience in this area and our knowledge of the problems are now reasonably adequate and we believe experiments can profitably be done on some of these more complex problems.
2. Penetration of large water soluble molecules.

Large molecular weight water soluble non electrolytes are being assembled and tested for their reducibility at the dropping Hg electrode. A diffusion chamber connected directly to a polarograph is now being used for penetrating studies of the homologous aliphatic acids. This system will also be used for the analysis "in situ" of large molecular weight molecules diffusing across epidermal membranes. Molecules which can exist also as ions will be used to compare permeabilities in both neutral and in ionic form.

3. Penetration of gases.

Gases may diffuse through dry skin by an entirely different mechanism than do molecules in solution through hydrated skin. The simultaneous diffusion of several gases through stratum corneum will be studied using infrared absorption as a method of gas analysis. The system will allow the humidity and the corresponding water content of the tissue to be controlled.
LIST OF REFERENCES

APPENDICES


B- Derivation of thermodynamic quantities from absolute rate theory.
APPENDIX A

MECHANISM OF PERCUTANEOUS ADSORPTION

I. ROUTES OF PENETRATION AND THE INFLUENCE OF SOLUBILITY

Robert J. Scheuplein, Ph.D.
Introduction

It is now generally accepted that the "barrier" function of the skin resides almost entirely in the stratum corneum. A host of work supports this conclusion (1-7) and it is in accord with the gross morphology of the epidermis. Roughly twice as much fibrous matter occupies almost one tenth as much space in the stratum corneum as it did in the viable epidermis. It is not unreasonable to expect that such a dense and compact structure ($\rho = 1.55$) would have a large influence on diffusion. No real evidence exists to further localize the functional "barrier" in an ultramicroscopic region near the lower layer or in any other local region of the stratum corneum. The division of the stratum corneum into a "disjunctum" and a "conjunctum" (6, 7) has split only the ranks of the investigators; the barrier has survived intact. Analysis of penetration data, evidence from controlled stripping experiments and the detailed picture of the stratum corneum gained from electronmicroscopy all support the idea that the entire anatomical stratum corneum serves as the "barrier" (3, 4, 8, 9).

Kligman has shown that human stratum corneum can be removed intact in large sections (100 cm$^2$) in the form of a thin (10-15$\mu$m) membrane and furthermore that this membrane is mechanically quite strong and resists chemical attack (5). The accessibility of stratum corneum and epidermal membranes makes possible a controlled physical chemical study of skin penetration in vitro. Unquestionably there still will be some differences between the
restricted experimental situation and the same process occurring in vivo and this must be kept in mind in extrapolating conclusions. But this narrowing of the problem is neither arbitrary nor unnecessary. In order to be able to draw fundamental conclusions and even to obtain accurate data it is essential to restrict the experimental system so that the many factors that influence penetration can be controlled and measured accurately and biological variation kept at a minimum.

An understanding of the diffusion mechanism through this membrane has possibly wider significance to the mechanism of membrane permeability in general. The stratum corneum is a collection of tightly adherent cells, densely packed with lipid enriched keratin, and presents a unique system for studying the chemical influence of naturally occurring protein-lipids on membrane transport. This is particularly significant when it is realized the many cell membranes are chemically quite similar to stratum corneum and transport through them is believed to be governed in large part by a lipid-penetrant interaction.

Blank (I) and Blank and Scheuplein (II)¹ have shown that penetration through epidermis varies inversely with thickness \( \delta \) and is directly

¹. This work was done in collaboration with Dr. I. Blank of these laboratories who has written a prior report of part of it, Ref. #10, to which frequent reference denoted by (I) is made. Another joint publication, Ref. #11 is denoted by (II).
proportional to the concentration difference $\Delta C_s$ in accord with the following expression:

$$J_s = k_p \Delta C_s = \frac{K D}{\delta} \Delta C_s$$

This communication presents some further work on the mechanism of penetration of water (HTO) and alcohols which shows that the expression above is also approximately true insofar as the third accessible factor, the partition coefficient $K$ is concerned. In permeability experiments the membrane is of necessity in contact with the pure compound being studied or with both the compound and its solvent. In either case it is possible that the tissue is altered by contact with the substance. The question naturally arises as to whether permeability constants so obtained are truly representative of the initial membrane and to what extent they are constant and reproducible. Strong reagents that destroy the membrane, e.g., alkaline solutions, and solvents that dissolve major constituents, e.g., chloroform-methanol, obviously cannot be used. The concentration of alcohol present in the aqueous solutions employed in our experiments can be kept small, but the water concentration is close to $55.5 \text{ M}$. The work discussed below shows that extended aqueous immersion does measurably alter the structure and permeability of the membrane. Fortunately this change appears to be relatively small and soon reaches an equilibrium value. Extended aqueous immersion does not appear to lessen the
influence of lipid solubility on penetration; these conclusions very probably apply without essential change to in vivo percutaneous adsorption.

**Experimental Techniques**

Samples -. Whole epidermal membranes were used in the diffusion experiments. These were removed from abdominal skin obtained at autopsy by immersion in water at 60°C for 30 sec. These membranes carry the stratum corneum intact and can be obtained in larger unruptured pieces than stratum corneum membranes per se. The permeability for both membranes is the same to the degree of accuracy reported. Stratum corneum pieces for partition coefficient measurements were obtained by excising cantharidin produced blisters.

Diffusion Cells -. Figure I A shows the pyrex cell which was used for the penetration experiments. The ends of each half of the cell in contact with the membrane were ground flat and then lapped on a flat glass plate with jewelers rouge. The inside of the receptor half of the

2. The author is indebted to his collaborator, Dr. I. Blank, for successfully separating epidermal membranes.

3. Stratum corneum membranes were obtained through the courtesy of Dr. Albert M. Kligman, Duhring Laboratories, Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania.

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cell was recessed to support a thin perforated metallic plate, e.g., Figure I B. A disk of porous filter paper was placed between the perforated plate and the membrane to insure the entire aperture was utilized for diffusion and to protect the membrane from damage. A thin film of silicone lubricant (stopcock grease) was spread on the lapped glass surface to insure a water-tight glass-to-membrane seal. The solution level on the donor side was kept a centimeter or two higher than the receptor side to fix the membrane firmly against the filter paper and prevent it from freezing out during the experiment. With this arrangement its thickness and area were as constant as possible. (The small hydrostatic pressure thus placed in the membrane has no perceptible effect on the penetration rate as the filtration coefficient for the membrane is extremely small [12]). The receptor side was stirred by a teflon-coated bar kept inside the cell during the experiment.

Micro-Solubility Cell -. For determining the membrane-water partition coefficients the cell shown in Figure II was used. It consists of two pieces of pyrex capillary tubing with an enlarged chamber (A in Figure II) for the tissue, fitted together with a ground glass joint. A 21 gauge syringe needle (B) was glued to one end of the capillary with epoxy cement. Approximately 5-10 mg of accurately weighed, dry stratum corneum was placed in the small chamber (A) and the cell was fitted together and weighed. Approximately 100 μl of 0.005 M aqueous alcohol solution was added through the capillary end (C) with a micro syringe and the cell
weighed. The solution was forced through the cell and mixed with the tissue by the piston action of a syringe attached at (B). Two spherical enlargements at D and E allowed complete mixing. The solution was forced from one chamber to the other periodically until the equilibrium was attained. The partition coefficients $K_m$ were computed from the loss in concentration of the original solution after equilibration with the tissue using the definition:

$$K_m = \frac{\text{moles alcohol absorbed per unit mass of dry tissue}}{\text{moles alcohol in solution per unit mass of water}} \quad (2)$$

Analysis of solutions - Alcohols were analyzed by gas-liquid chromatography using a glycerol-Chromasorb-W column and a hydrogen flame detector. Further details are given in (I).

Epidermal membranes, with the stratum corneum side next to the donor, were placed in the diffusion cells, and the cells filled with appropriate solutions. A constant temperature bath maintained the temperature to within $\pm \ 0.05^\circ C$. Penetration rates $J_s$ were obtained from the rate of increase of concentration in the receptor. Permeability constants $k_p$ were computed directly from the linear portion of the accumulation curve or when necessary by the formula:

$$\log \left( 1 - \left[ 1 + \frac{T}{\alpha} \right] \frac{C_R}{C_0} \right) = - \frac{[1+\alpha]}{2.303} \frac{A k_p t}{V_D} \quad (3)$$
The following definitions are used throughout:

- $C_R, C_D$ - concentration of receptor, donor
- $V_R, V_D$ - volume of receptor, donor; $\alpha = V_D/V_R$
- $A$ - exposed area of membrane
- $t$ - time
- $k_p$ - permeability constant
- $D$ - diffusion constant
- $J_S$ - penetration flux
- $\delta$ - membrane thickness

In this case plotting the left side of equation 3 vs. $t$ will give a straight line with slope proportional to $k_p$.

Results

Permeability of $H_2O$

Water is the principal component of any dilute aqueous solution and its diffusion into the membrane and its effect on the membrane structure and is of paramount importance. Also it is likely that the mobility of the water molecule sets the upper limit on the free diffusion of ions through skin as it is the hydrated species which diffuses.

The average permeability constant at 25°C was $1.0 \times 10^{-3}$ cm hr.$^{-1}$ corresponding to an average bulk diffusion constant $D = 2.8 \times 10^{-10}$ cm$^2$ sec$^{-1}$ ($D = k_p \delta$ ). These values are compared with corresponding ones obtained on various films and other biological membranes on Table II. Even assuming
a large degree of swelling in solution and a corresponding error in the water diffusion constant measured for stratum corneum is still as small or smaller than the most efficient cellular membranes. This value for water places it in its proper place in the series of homologous alcohols vs. Table I, i.e., the alcohol with no methyl groups.

Water flux (HTO) was measured continuously from 0° -50°C and k_p values computed. Arrhenius plots for several epidermal membranes are given in Figure III. Open triangles correspond to k_p's measured at constant temperature as this was periodically increased after each measurement. Filled triangles correspond to decreasing temperature, i.e., return measurements on the same membrane. The slope has a high initial value (at low temperature) which then decreases and becomes constant at higher temperatures. The descending curve (black points) reproduce the initial curve except a low temperatures when the final values are always greater. The constant, reproducible portion of the slope corresponds to an activation energy of about 15.0 Kcal. mole.⁻¹ and is characteristic of water diffusion through stratum corneum. This is roughly three times greater than the activation energy for the self diffusion of liquid water (4.7 Kcal.) and is responsible for the very slow diffusion of water through this tissue.

Effect of Water Immersion on Permeability

The negative curvature of the initial low temperature part of the curve, which is not reproduced in the return measurements shows than an
irreversible change in the membrane has occurred. It seems likely that the swelling and softening of the keratin filaments in water is accompanied by a partial dissolution of the membrane which opens larger "holes" through which easier diffusion may occur. This is discussed in detail elsewhere (13). According to this hypothesis the diffusion, after final equilibrium with the solution is attained, occurs through two routes, through the bulk of the intact membrane and through the newly opened "holes." The activation energy for the final diffusion at low temperature does appear to have the same activation energy as liquid water, about 6.0 Kcal. mole\(^{-1}\). Although this alteration occurs very slowly at the low temperatures employed (about 10 days were required) it appears to be an activated rate process and occurs more rapidly at room temperature as shown below.

The effect of extended aqueous immersion on the permeability constants is shown for pentanol if Figure IV. \(k_p\) values increase slowly by a factor of about two over a period of three days until a final equilibrium value is reached. This shows that at 25\(^\circ\)C about half the diffusion occurs through newly opened "holes." Raising the temperature abruptly should increase the permeability constant to a new steady state value in accord with the activation energy for diffusion. This occurs with the 0.02M solution; but there is evidence of further membrane damage with the concentrated solution. The permeability constant returned to its 25\(^\circ\)C value when the temperature was reduced in accord with an Arrhenius temperature behavior.
Permeability Constants of a Homologous Series

The permeabilities of the homologous series of normal primary alcohols $C_1$ - $C_8$ were studied (I). In this series a smooth decrease in the polar character of the molecules occurs. Other chemical properties of relevance to diffusion, e.g., the presence of a single hydroxyl group, remain constant or can be corrected for. (The molecular volume $V$ increases by a factor of 4 within the series. Its effect on the rate of diffusion which is approximately proportional to $V^{1/3}$ increases by less than a factor of 1.6).

Figure V shows that the permeability constant increases as the molecular weight increases. This seemingly anomalous result is a consequence of the increasing alcohol-membrane solubility which results from the decreasing polar character of the alcohols. The selectivity shown by the membrane evidences the chemical interaction between the penetrating molecule and the lipid parts of the membrane. It also shows that "holes" which have developed in the membrane due to immersion do not obviate the far greater effect of lipid solubility. The large increase in permeability shows that the stratum corneum is a strongly lipophilic membrane and that these lipids can aid in overcoming the "barrier." Their presence decreases the "barrier" function of the skin as far as a small molecular weight, non polar, non electrolytes are concerned. This is shown quantitatively below in terms of the membrane partition coefficient.
Partition Coefficients and Membrane Permeability

It has long been recognized that the permeability of non-electrolytes through many biological membranes increases as the membrane solubility of the penetrating molecule increases \( (14) \). More precisely it is the solubility of the penetrating molecule within the membrane relative to the solubility in the solvent, i.e., the membrane partition coefficient, which directly influences the permeability. Unfortunately the direct measurement of membrane partition coefficients is usually impossible or very difficult. It has been customary to estimate the actual partition coefficients of lipophilic membranes by approximating their lipophilic solubility using olive oil as a representative chemical substitute and use olive-oil-water partition coefficients \( (15, 16) \). This is not always adequate as shown below for the stratum corneum membrane. True aqueous membrane partition coefficients \( K_m \) have been measured for the alcohols and are compared with the olive-oil values \( K_o \) in Table I. These data show the large differences between the two partition coefficients. The effect of this disparity on the analysis of permeability data is shown in Figure VI where permeability constants are plotted as a function of both partition coefficients separately. It is seen that the permeability constant varies approximately linearly with the partition coefficient in agreement with the theoretical formula, equation \( (1) \), only when the true membrane coefficient \( K_m = K \) is used. One can, therefore, approximate the penetration rate of the alcohols by \( J_s = K_p^o K_m \triangle C_s \), where \( K_p^o \) is the
membrane permeability coefficient at an hypothetical partition coefficient of unity. The olive-oil-water coefficient $K_0$ is not nearly as proportional to the observed permeability. The very limited range over which this approximation is useful is shown in Figure VII where $K_m$ is plotted against $K_0$. Perfect proportionality between the two constants would result in a straight line; this "ideal line" is drawn tangent to their actual region of correspondence in the Figure. The olive-oil-water coefficient is seen to be a poor approximation to $K_m$ except in a narrow range near a value of $K_0 = K_m = 10.0$. The actual curve deviates enormously at both extremes as shown. The deviation near the origin (enlarged in the inset) stems from the fact that while $K_m$ can not be less than 0.3 - the approximate weight fraction of water in the membrane, $K_0$ for water and the polar alcohols approaches 0.0 - the limiting solubility of water in olive-oil. The finite membrane coefficient represents the obvious physical situation that both polar as well as non-polar sites are present in the membrane. Water and alcohols are strongly absorbed although in lesser amount. From the deviation at higher $K$ values it is clear that stratum corneum is less potent an absorbant for strongly non-polar molecules than is olive-oil.

Discussion

Morphological Diffusion Pathways

The multicellular structure of the stratum corneum and the invariable presence of a variety of skin appendages might appear to provide a ready
made, anatomical network of potential short-circuit diffusion pathways through which penetrating molecules could diffuse rapidly. The low permeability of the skin has been considered by some investigators to arise principally from limited diffusion through these ready made pores. Low overall permeability would result from the relatively small diffusion volume available within the pores, where molecules would diffuse rapidly, rather than from an intrinsically low diffusivity throughout a homogeneous tissue. Considering for simplicity, only intercellular spaces the two contending routes of penetration are illustrated in Figure VIII. That ordinary liquid diffusion between cells can not be the predominant diffusion process in skin, even in fully hydrated membranes, is strongly implied by the high activation energies obtained. This conclusion can be further strengthened by the following line of argument. From the data presented above the principal diffusion mechanism of water and the polar alcohols is characterized by an activation energy near 15 Kcal. mol.\(^{-1}\) and a diffusion constant of about \(3.0 \times 10^{-10}\) cm.\(^2\) sec\(^{-1}\). This diffusion constant is lower than for most biological membranes and is really remarkably low when it is remembered that it characterizes a structure several microns thick. (The special bimolecular leaflet model invoked to explain the low permeability of 100 Å. cell membranes cannot apply here.) Diffusion between cells would obviously
imply a lower diffusion constant for the cell walls than for the space between them. It is easily seen that this requires the diffusivity of the cell walls to be several orders of magnitude too low. Assuming for example a fraction, \( f \), of the membrane allows intercellular diffusion characterized by a diffusion constant \( D_i \) and the remainder, \((1-f)\), allows more difficult bulk diffusion with diffusivity \( D_B \); we have adding fluxes:

\[
D_m = D_i + (1 - f) D_B
\]  

(4)

where \( D_m \) is the observed, overall diffusion constant. A maximum value of \( 10^{-2} \) for \( f \) can be derived for abdominal stratum corneum from known morphological data (17).

This very likely is a gross overestimate as both intercellular spaces, sweat ducts and hair follicles are to some degree filled with fibrous material or swollen shut in aqueous solution. In either case we can safely assume \((1 - f) = 1.0 \) if \( D_i \) is to be characteristic of a rapid diffusion between cells it must be substantially larger than the value observed for \( D_m \) which is conservatively \( = 10^{-9} \) cm\(^2\) sec.\(^{-1}\). We can reasonably assume \( D_i \) is between \( 10^{-7} \) and \( 10^{-5} \) cm\(^2\) sec.\(^{-1}\), the approximate diffusion constants for water through hydrated protein (18), and liquid water respectively (19). Using the estimate for \( f \), the measured value of \( D_m \) and equation (4) we can obtain the resulting values for \( D_B \) and \( D_i \) and compare them with physically reasonable estimates.

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For intercellular diffusion to predominate we can assume $J_i = 100 J_B$. From equation (4) we see that $D_i = 10^{-9} / f = 10^{-7}$ cm$^2$ sec.$^{-1}$. This just falls within the acceptable range. However, using this value for $D_i$, equation also implies a value of $10^{-11}$ cm$^2$ sec.$^{-1}$ for $D_B$, which is a hundred times too small for biological membranes. Effective diffusivities for various membranes are given in Table II. Large scale intercellular, or appendageal diffusion, is therefore, very unlikely. The evidence is far more consistent with diffusion directly through cell walls and cells alike without discrimination, i.e., transcellular.

It is possible that these localized diffusion pathways constitute some or even all of the pores found after extended immersion of the membrane, but even in hydrated membranes this is not the predominant mode of diffusion at normal temperatures and these "holes" are absent in fresh, unhydrated membranes. From physical evidence (the low activation energy) we can say with reasonable certainty only that the "holes" are large enough to permit ordinary liquid diffusion. A generalized concept of "holes" is illustrated in Figure IX. The low diffusion constant and high activation energy obtained for water and the polar alcohols and the selective diffusion exhibited by molecules of varying polar character suggests that extensive hydration does not drastically effect the "barrier" function of the stratum corneum. The hydrated stratum corneum is one of the most water impermeable
biological membranes found in nature; though it is slightly more efficient before extensive hydration and presumably in vivo.

**Summary and Conclusions**

1. The penetration rate for small molecular weight alcohols is approximately given by the expression:

   \[ J_s = K_p^0 K_m \Delta C_s \]

   where \( K_p^0 = 1 \times 10^{-3} \text{ cm hr}^{-1} \) is essentially the permeability constant for water in the membrane, \( K_m \) is the membrane-water partition coefficient for the alcohol and \( \Delta C_s \) is the concentration difference across the membrane.

2. The activation energy for the penetration of water and the polar alcohols, through hydrated stratum corneum is approximately 15 Kcal mole\(^{-1}\).

3. Extensive immersion in water effects the "barrier" function of stratum corneum only slightly; principally by opening "pores" which contribute to the diffusion a parallel flux which is relatively small above room temperature.

4. Penetration through the stratum corneum is not primarily intercellular or appendageal. The hydrated stratum corneum seems to be best described as a dense, effectively homogeneous phase into which small molecular weight polar non-electrolytes dissolve with strong chemical interaction and through which diffusion occurs remarkably slowly.

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REFERENCES


A - clamping arrangement. B - perforated plate in recess.

Pyrex diffusion cell for measuring permeability of stratum corneum.

Figure 1
Figure II  Micro-Solubility cell for measuring membrane-water partition coefficients.
Figure III  Arrhenius plots for the penetration of water (HTO) and ethanol (CH₃CHTOH) through immersed epidermal membranes.

Figure IV  Increase in the membrane permeability of pentanol due to extended aqueous immersion. A true steady state flux is achieved only after about three days.
Figure V  Permeability constant at 25°C for the homologous primary alcohols as a function of molecular weight.

Figure VI  Permeability constants vs. partition coefficients both at 25°C. Membrane-water partition coefficient $K_m$ (filled circles), Olive-oil-water partition coefficient $K_0$ (open circles).
Figure VII  Comparison of membrane-water partition coefficients with olive oil-water partition coefficients. The "ideal" line would be followed if the two coefficients were proportional to each other.

SCHEMATIC OF THE STRATUM CORNEUM MEMBRANE

Figure VIII  Two simplified contending routes of penetration: I- Through the bulk tissue (transcellular penetration). II- Between cells (intercellular penetration).
Figure IX  Conception of "holes" in hydrated fibrous protein tissue. Much of the water is strongly bound and may not be able to diffuse rapidly.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$k_p$</th>
<th>$k_pV^*$</th>
<th>$K_m$</th>
<th>$K_m$</th>
<th>$k_p$</th>
<th>$M$</th>
<th>$V$</th>
<th>$K_o$</th>
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<td>Water</td>
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<td>8.65</td>
<td>0.3</td>
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<td>1.0</td>
<td>18.02</td>
<td>18.02</td>
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<td>1.0</td>
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<td>157.4</td>
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</table>

Table I Permeability and partition coefficient data. $V$-molecular volume, $M$-molecular weight.
### Table II

Comparison of permeability constants and diffusion constants for monolayers, films, cellular and multicellular membranes. (at room temperature).

**Note 1.** Permeability constants $k_p$ and diffusional resistance values $R$ are inversely related:

$$k_p = \frac{1}{R}$$

**Note 2.** Diffusion constants $D$ are computed from permeabilities using approximate effective membrane thickness according to:

$$D = \delta k_p$$

**Note 3.** Isotopic and osmotic permeability differences and anisotropic permeability are ignored. All values are approximate.

<table>
<thead>
<tr>
<th>Membrane, etc.</th>
<th>$k_p$ (cm$^2$ sec$^{-1}$)</th>
<th>$R$ (sec cm$^{-1}$)</th>
<th>$\delta$ (Angstroms)</th>
<th>$D$ (cm$^2$ sec$^{-1}$)</th>
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</thead>
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<td><strong>MONOLAYERS AND FILMS</strong></td>
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<td></td>
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<td>Clean air-water interface</td>
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<td>$2 \times 10^{-3}$</td>
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<td>$1.5 \times 10^{-8}$</td>
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<td>$(1-2) \times 10^{-4}$</td>
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<td>$2.4 \times 10^{-8}$</td>
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<td>Sestern water layer 10 µ thick</td>
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<td>50</td>
<td>$10^{4}$</td>
<td>$2 \times 10^{-8}$</td>
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<td>Phospholipid bilayer (tetradecane lecithin)</td>
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<td>$3 \times 10^{3}$</td>
<td>61</td>
<td>$2 \times 10^{-10}$</td>
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<td>Human erythrocytes</td>
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<td>80</td>
<td>50</td>
<td>$6.5 \times 10^{-9}$</td>
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<tr>
<td>Fibroblasts in tissue culture</td>
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<td>$6 \times 10^{3}$</td>
<td>100</td>
<td>$6.2 \times 10^{-9}$</td>
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<td>$3-5 \times 10^{3}$</td>
<td>100</td>
<td>$2.8 \times 10^{-9}$</td>
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<td>Arbecla eggs</td>
<td>$(2-6) \times 10^{-4}$</td>
<td>$(1-5) \times 10^{3}$</td>
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<tr>
<td><strong>MULTICELLULAR MEMBRANES</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Frog skin</td>
<td>$7 \times 10^{-6}$</td>
<td>$1-4 \times 10^{4}$</td>
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<tr>
<td>Human epidermis</td>
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<td>Human dermis</td>
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<td>$2-0 \times 10^{4}$</td>
<td>$4 \times 10^{7}$</td>
<td>$2.5 \times 10^{-8}$</td>
</tr>
</tbody>
</table>
APPENDIX B

Calculation of Thermodynamic Quantities

The permeability constant is related to the diffusion constant \( D \) by the expression:

\[
k_{p} = \frac{Km D}{\delta} \quad (1)
\]

where \( \delta \) is the membrane thickness. \( D \) can be expressed in terms of the specific rate constant \( k \) by

\[
D = \lambda^2 k \quad (2)
\]

where \( \lambda \) is the average distance between successive equilibrium positions of a diffusing molecule. Since

\[
k = \frac{k T}{h} e^{\Delta F^\pm/RT} \quad (3)
\]

we have, combining (1), (2) and (3):

\[
k_{p} = \frac{Km \lambda^2 k T}{\delta h} e^{\Delta F^\pm/RT} \quad (4)
\]

Or equivalently:

\[
k_{p} = \frac{Km \lambda^2 k T}{\delta h} e^{\Delta S^\pm/RT - \Delta H^\pm/RT} \quad (5)
\]
This equation can be related to the empirical expression,

\[ k_p = A \ e^{-E/RT} \]  \hspace{1cm} (6)

by a consideration of the appropriate standard states involved and by using the thermodynamic relation:

\[ E = \Delta H^* + RT - \rho \Delta v^* \]  \hspace{1cm} (7)

combining (5), (6) and (7) we have

\[ A = \frac{k_m A e^{kt}}{\delta h} e^{\Delta S^*/R} \]  \hspace{1cm} (8)

If we compute \( \lambda \) from the molecular volume of the diffusion media (water in the case of polar molecules) (8) becomes

\[ A = \frac{k_m}{\delta} \left( \frac{v}{N} \right)^{\frac{3}{2}} \ e^{kt} \frac{e^{\Delta S^*/R}}{h} \]  \hspace{1cm} (9)

Assuming \( \delta = 2 \times 10^{-3} \) cm

\[ \left( \frac{v}{N} \right)^{\frac{1}{3}} = \lambda = 2.10 \times 10^{-8} \text{ cm} \]

\[ \frac{e^{kt}}{h} = 1.689 \times 10^{-13} \text{ sec}^{-1} \]

This correction to $\Delta S^\ddagger$ is equivalent to recognizing that the partition equilibrium between the membrane and solution occurs much more rapidly than diffusion (after steady state). We, therefore, can remove the factor $K_m$ and treat the system as if the original concentration difference was $K_m \Delta C_s$ instead of $\Delta C_s$.

For the free energy we use the equation

$$\Delta F^\ddagger = \Delta H^\ddagger - T \Delta S^\ddagger$$  \hspace{1cm} (11)