The State of Water in Human and Dog Red Cell Membranes

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ABSTRACT The apparent activation energy for the water diffusion permeability coefficient, $P_d$, across the red cell membrane has been found to be $4.9 \pm 0.3$ kcal/mole in the dog and $6.0 \pm 0.2$ kcal/mole in the human being over the temperature range, $7^\circ$ to $37^\circ$C. The apparent activation energy for the hydraulic conductivity, $L_p$, in dog red cells has been found to be $3.7 \pm 0.4$ kcal/mole and in human red cells, $3.3 \pm 0.4$ kcal/mole over the same temperature range. The product of $L_p$ and the bulk viscosity of water, $\eta_w$, was independent of temperature for both dog and man which indicates that the geometry of the red cell membrane is not temperature-sensitive over our experimental temperature range in either species. In the case of the dog, the apparent activation energy for diffusion is the same as that for self-diffusion of water, $4.6-4.8$ kcal/mole, which indicates that the process of water diffusion across the dog red cell membrane is the same as that in free solution. The slightly, but significantly, higher activation energy for water diffusion in human red cells is consonant with water-membrane interaction in the narrower equivalent pores characteristic of these cells. The observation that the apparent activation energy for hydraulic conductivity is less than that for water diffusion across the red cell membrane is characteristic of viscous flow and suggests that the flow of water across the membranes of these red cells under an osmotic pressure gradient is a viscous process.

The physical state of the water in biological membranes is a matter of fundamental significance which may be studied by measurement of the temperature dependence of water fluxes across cellular membranes. Hays and Leaf (1) found the apparent activation energy for the diffusion of tritiated water (THO) across the unmodified membrane of the isolated toad bladder to be $9.8$ kcal/mole. This figure is much larger than the value of $4.6$ kcal/mole obtained by Wang et al. (2) for the diffusion of THO in bulk water. In studies of THO diffusion in peripheral nerve fibers, Nevis (3) computed apparent
activation energies of 2.5–6.6 kcal/mole but presented evidence which suggests that the temperature dependence in his studies does not satisfy the Arrhenius equation. The studies of THO diffusion in the toad bladder and across peripheral nerve are further complicated by the fact that in each case diffusion takes place across several barriers in series which makes unambiguous interpretation difficult.

A number of studies have also been made on the temperature dependence of the hydraulic conductivity across cellular membranes. Hempling (4) found that the logarithm of the hydraulic conductivity of the Erhlich ascites tumor cell varied linearly with the inverse of the absolute temperature over the range of 10° to 40°C with a slope corresponding to an apparent activation energy of 9.6 kcal/mole. In subsequent measurements apparent activation energies of 4.4 and 5.7 kcal/mole were obtained on different strains of cells (5). Hempling also computed an apparent activation energy of 3.9 kcal/mole from the temperature dependence of osmotically driven water fluxes measured by Jacobs et al. (6) in human red cells by the hemolysis method. Jacobs et al. pointed out the inherent difficulty in drawing conclusions from their data because of complications introduced by the direct effect of temperature on the hemolysis process. Lucké and McCutcheon (7) obtained apparent activation energies of 13–17 kcal/mole for the hydraulic conductivity of unfertilized sea urchin eggs and pointed out that interpretation of these results was difficult because of the possibility of temperature-induced changes in the permeability of the cell surface.

We have combined measurements of the apparent activation energy for diffusion across the red cell membrane with a study of the temperature dependence of the hydraulic permeability, \( L_p \). Such studies in the red cell offer the advantage that all the fluxes take place across a single membrane though even this relatively simple system is characterized by a complex permeability barrier (8). The temperature dependence of \( L_p \) should be a sensitive indicator of alterations in the structure of the red cell membrane. On the equivalent pore model the hydraulic conductivity is directly proportional to a high power, perhaps the fourth, of the equivalent pore radius and inversely proportional to \( \eta_w \), the viscosity of water. If the product, \( L_p\eta_w \), can be shown to be independent of temperature, it would appear that hydraulic conductivity takes place by a viscous process through apertures whose size is independent of temperature.

In order to estimate the relative importance of water-membrane friction as compared to water-water friction in the equivalent pore we have measured the apparent activation energy for THO diffusion in both the human red cell which is characterized (9) by an equivalent pore radius of 4.3–4.5 Å and the dog red cell whose equivalent pore radius is 5.9–6.2 Å, almost 40% greater. The apparent activation energy studies will provide information about the
physical state of water in the diffusion pathway. Each kind of measurement requires different apparatus and different techniques. Consequently the paper will be divided into two sections: (1) THO diffusion in red cells; (2) hydraulic conductivity in red cells, followed by a general discussion of the results.

### I. TEMPERATURE DEPENDENCE OF THO DIFFUSION IN HUMAN AND DOG RED BLOOD CELLS

**Experimental Method**

The rapid reaction continuous flow system of Paganelli and Solomon (10), modified by Barton and Brown (11), was used to determine the THO diffusion rate constant. Blood was collected on the day of the experiment, heparinized (sodium heparin, Panheparin, Abbott, 10 mg/ml, 5 ml/liter), and kept at 4°C until the experiment began. Human blood was collected by venipuncture; dog blood was obtained by catheterization of the femoral vein in anesthetized animals (sodium pentobarbital 30 mg/kg). After the blood was centrifuged at 900 g for one-half hour, the plasma was removed and phosphate buffer was added in an amount equal to half of the plasma removed, giving an enriched cell suspension of hematocrit about 0.6. The buffer had the following composition (mM): NaCl, 117.8; CaCl₂, 1.2; Na₂HPO₄, 1.7; NaH₂PO₄, 4.2; KCl, 4.4; Na₂CO₃, 13.5, and was aerated with 5% CO₂-95% air to bring it to pH 7.4. Tritiated buffer was prepared by adding 200 μl of tritiated water (100 mCi/ml) to 1 liter of buffer solution, giving a final concentration of 0.02 mCi/ml buffer. All the experiments were performed under the same conditions and in the identical sequence both for human and dog blood.

In the rapid reaction continuous flow system, the enriched cell suspension is mixed rapidly with tritiated buffer of the same osmolality. The mixed solution flows, under an applied air pressure of about 125 lb/in², at a velocity of about 900 cm/sec, along a tube with ports that permit axial sampling of suspension solution at points spaced about 1.8 msec apart. These samples (about 10 μl each) were analyzed for THO radioactivity in a liquid scintillation counter (model 6801, Nuclear Chicago Corp., Des Plaines, Ill.). In order to determine the kinetic curve for diffusion of THO across the erythrocyte membrane, the normalized rate constant for THO exchange, \( k/v_s \), was determined from the equation:

\[
\frac{p^*}{p_e} - 1 = \left( \frac{p_0^*}{p_e} - 1 \right)e^{-k/v_s t}
\]

in which \( p^* \) is the specific activity in the external solution and the subscripts 0 and \( \infty \) refer to initial and equilibrium conditions. \( k \) is the rate constant for a specific experiment and \( v_s \) is the volume of intracellular water. Fig. 1 shows the data plotted according to equation 1 for a triple experiment carried out with blood of the same dog at 7°, 22°, and 37°C.

The experiments were performed over the temperature range of 7° to 37°C. Blood and buffer temperatures were adjusted to the desired values and the liquids were poured into reservoirs in the rapid flow apparatus. The reservoirs were kept at the
experimental temperature in a water bath, though the mixing and flow apparatus were always at room temperature. Cells were maintained in homogeneous suspension by gentle agitation until just before the experiment. Control experiments were carried out to measure the time delay required to reach constant temperature at the outlet of the apparatus. For this purpose, a small thermistor was attached at the outlet of the sampling tube to provide a continuous record of the fluid temperature during the experiment as shown in Fig. 2. When the difference between the fluid in the reservoirs and room temperature was 14°C, the steady-state temperature at the outflow was reached in 15 sec and was only 0.3°C different from the temperature in the reservoir.

![Figure 1](image_url)

**Figure 1.** Time course of THO diffusion in dog red cells at three temperatures: 7°C, 22°C, and 37°C.

Since the elapsed time from the beginning of the experiment to the initial sample collection was always at least 25 sec, the control experiments indicated that the experiments were all performed at constant temperature.

Hematocrit determinations were carried out by the isotope dilution method, using ¹³¹I-labeled human serum albumin obtained from Abbott Laboratories (North Chicago, Ill.), and dialyzed in distilled water at 4°C for at least 48 hr prior to each experiment. ¹³¹I was counted in a scintillation counter (model 4222, Nuclear Chicago Corp., Des Plaines, Ill.). The hematocrit, \( h \), was determined using the expression:

\[
h = 1 - \left( \frac{V_s}{V} \right) = 1 - \frac{(\text{Alb}^*)_s}{(\text{Alb}^*)} \tag{2}
\]

in which \( V \) is volume, \( (\text{Alb}^*) \) is the concentration of albumin-¹³¹I (counts per unit
time and volume), and the subscripts \( e \) and \( s \) refer to extracellular fluid and total suspension, respectively. Three aliquots of the same suspension used for measurements of THO exchange were mixed with albumin-labeled and incubated for 1 hr in a water bath at 0°, 22° and 37°C, respectively and the hematocrit was calculated for each sample. The isotope dilution method was chosen because of the difficulties of making hematocrit determinations with a centrifuge when the temperature is changed over the range of 0° to 37°C.

**RESULTS**

The ratio of the rate constant, \( k \), for THO diffusion into red cells to the volume of intracellular water, \( v_i \), provides the normalized rate constant,
whose temperature dependence is to be measured. $k/v_q$ has units of msec$^{-1}$ and represents the fraction of cell volume that is exchanged in unit time. To convert $k/v_q$ to the permeability coefficient, $P_d$, it is necessary to multiply it by the volume of the cell water and divide by the cell area in suitable units to give $P_d$ in cm/sec. The permeability coefficient, $\omega = P_d/RT$ in which $R$ is given in dyne cm/mole deg.

Since the normalized rate constant includes $v_q$, it is important to study the temperature dependence of $v_q$ itself. The hematocrit was chosen as an indirect method of evaluating the relationship of $v_q$ to temperature. Samples were incubated for 1 hr at each temperature before making hematocrit measurements by isotope dilution. Table I gives the results for both human and dog blood at the three different temperatures. These data indicate that there is no systematic change of $v_q$ with temperature over the range of 0° to 37°C. Since the time that elapsed between bringing the blood to the experimental temperature and the THO diffusion experiment never exceeded 15 min, we may conclude that $v_q$ is constant and independent of temperature. Therefore the apparent activation energy we have measured is a function of the temperature dependence of $k$.

The cell volume is $87 \times 10^{-12}$ cm$^3$ for the human being and $68 \times 10^{-12}$ cm$^3$ for the dog under isosmolar conditions at 20°C. The cell surface area is $1.67 \times 10^{-6}$ cm$^2$ for the human being and $1.24 \times 10^{-6}$ cm$^2$ for the dog under the same conditions. The fractional water content in the red cells of man and dog is 0.72 (see reference 12).
Exchange of THO in Dog Red Blood Cells

Fig. 3 shows the variation of $\ln (k/v_o)$ with absolute temperature for dog red blood cells. In the experimental data shown at the top of the figure each point represents a single determination of $k/v_o$ and the apparent activation energy is $4.03 \pm 0.07$ kcal/moles (se) as evaluated by the method of least squares. In order to determine whether the data obtained at each temperature fell on a straight line, as required for the use of the Arrhenius plot to determine apparent activation energies, an experimental procedure was designed in which studies could be made on the same dog blood at all three temperatures. These triple experiments were carried out at 7°, 22°, and 37°C. It is apparent from the results shown in the bottom part of Fig. 3 that the points fall on a straight line in conformity with the expectation that the apparent activation energy is independent of temperature over this range. The apparent activation energy calculated by least squares from the data in the bottom part of Fig. 3 is $4.9 \pm 0.3$ kcal/mole. Though these two values for the apparent activation energy for diffusion of THO in dog red cells are statistically different ($p < 0.01$), the value of 4.9 kcal/mole seems to be a more valid estimation of the actual value, since it is obtained in triple experiments in which the variability between different samples of blood is eliminated. The permeability coefficient,
co, at 22°C was found to be $2.3 \pm 0.3 \times 10^{-13}$ moles/dyne sec in fair agreement with the previous value (12) of $1.82 \times 10^{-13}$ moles/dyne sec.

Exchange of THO in Human Red Blood Cells

Fig. 4 shows $\ln \left(\frac{k}{v_q}\right)$ as a function of the reciprocal of absolute temperature in experiments performed with human red blood cells. Each point in the top part of Fig. 4 represents a single determination of $\frac{k}{v_q}$. In view of the limited amount of blood that can be withdrawn from each donor it is not possible to carry out experiments on the same blood at three different temperatures. Some paired experiments were carried out using blood of the same donor at 4°C and room temperature in an attempt to eliminate the variability among bloods. These results are shown in the bottom half of Fig. 4. The data were similar to those obtained in single experiments and so all have been treated together. In conformity with the results on dog red cells, we have assumed a linear relation between $\ln \left(\frac{k}{v_q}\right)$ and $1/T$. The least squares apparent activation energy for all the human red cell experiments is $6.0 \pm 0.2$ kcal/mole, significantly greater ($p < 0.01$) than the apparent activation energy for THO diffusion in dog red cells. $\omega$ for human red cells at 22°C was $1.35 \pm 0.15 \times 10^{-13}$ moles/dyne sec in excellent agreement with the previous value of $1.36 \times 10^{-13}$ moles/dyne sec (11).

II. TEMPERATURE DEPENDENCE OF HYDRAULIC PERMEABILITY IN HUMAN AND DOG RED BLOOD CELLS

Experimental Method

Immediately before an experiment, human blood was drawn from a healthy male or female donor by venipuncture. Dog blood was drawn from anesthetized dogs (Nembutal, 30 mg/kg) from the femoral vein. Heparin (10,000 units/ml, 4 ml/liter) was used to prevent clotting. The blood was diluted in an isosmolar saline buffer to form a suspension of 3% by volume of whole blood. The buffer had the following composition (mM): NaCl, 102.8; MgCl₂, 0.5; CaCl₂, 1.2; KCl, 4.4; Na₂HPO₄, 1.7; NaH₂PO₄, 4.2; Na₂CO₃, 13.5, and was aerated with 5% CO₂-95% air to bring it to pH 7.4. The osmolality of this buffer was 260 ± 5 milliosmols, as measured by freezing point depression (osmometer Model G-62, Fiske Associates Inc., Bethel, Conn.). Hypoosmolar solutions were prepared from the same buffer by changing the NaCl concentration alone. The hydraulic conductivity, $L_p$, was measured in the rapid reaction stop-flow apparatus, previously described by Sha'afi et al. (13) using the same techniques described by Rich et al. (14), which involve multiple experimental runs and multiple control runs. A digital computer is used for averaging and fitting the data to the equation by a least squares program.

Temperature Measurements

The fluid flow system (two syringes, two motors, the stopcock, mixing chamber, prism, light source, and two photomultipliers) was enclosed in a large temperature-
controlled air bath (environmental proportioning refrigeration control system [Harris Refrigeration S. and S., Inc., 6 Lowell Avc., Winchester, Mass.]). The dynamic characteristics of the temperature control system are given in Table II. \( L_p \) was measured over the temperature range of 5°C to 39°C.

In order to make observations on the same blood at 10 different temperatures, 10 aliquots each of the blood suspension, the isosmolar, and the hyperosmolar solutions were put into 10 sets of 3 flasks. Each triplet of flasks—the first containing the blood suspension, the second, the hyperosmolar solution, and the third, the isosmolar solution—was incubated at temperatures that approximated those to be used in the temperature-controlled system. When the runs for a given temperature were completed, the system was set for the next temperature and the three flasks which were at this temperature were brought into the system. It was possible to carry out measurements at 10 different temperatures in about 3 hr. Control runs were carried out at each temperature. In these runs the blood was mixed rapidly in the stop-flow apparatus with isosmolar solutions at the same temperature.

The temperature was measured by placing two thermistors (Fenway subminiature matched thermistor K1195–GA45SM2, Fenwal Electronics, Inc., Framingham, Mass.) in the two inflow tubes. Each thermistor was mounted in a Lucite block in which a well was drilled to within 0.001 inch of the flowing solution. The sensing bead of the thermistor was immersed in mercury to ensure good thermal conductivity. Silastic embedding compound (Dow Corning 3110 RTV, Dow Corning Corp., Midland, Mich.) was then poured around the thermistor to seal it in the mounting block. The temperature-sensing assembly can detect a change of 0.05°C and has a full scale response time of 0.5 sec.

The output of each thermistor is fed to a bridge-amplifier arrangement and recorded on a Dual Channel servo type recorder (Texas Instruments Inc., Houston, Texas). A simplified drawing of the thermistor temperature bridge is shown in Fig. 5. The actual circuit contains a number of additional items, such as amplifier fine zero control and range controls which have been omitted for the sake of clarity. The particular amplifier arrangement chosen uses a low-drift chopper stabilized amplifier rather than a differential input type. The over-all gain of the system is set by the feedback arrangement shown in Fig. 5. Under normal conditions the gain is adjusted

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**Table II**

CHARACTERISTICS OF THE TEMPERATURE-CONTROLLED SYSTEM

| Temperature range | System regulation | Time for 1° change |
|-------------------|-------------------|--------------------|
| °C                | °C                | min                |
| 6.5 – 9.3         | 0.48              | 10                 |
| 9.0 – 16.5        | 0.60              | 8                  |
| 16.3 – 21.2       | 0.70              | 10                 |
| 21.0 – 25.1       | 0.89              | 15                 |
| 26.0 – 32.5       | 1.00              | 3                  |
| 32.5 – 38.0       | 1.00              | 2                  |

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to yield a full scale output of ±10 v for a temperature change of ±1°C to ±2°C. Calculations indicate that the bridge output should be linear to better than 5% for a ±2°C change from initial balance. The actual range of linearity has been observed to be somewhat better than calculated. In practice, two identical units are used either operating independently, or switched into a single bridge for making differential temperature measurements.

Self-heating errors are minimized by operating with a bridge input voltage of only 0.2 v. At room temperature, this represents a self-heating power input to the thermistor of approximately one-fifth μw. Since the dissipation constant of the thermistor used is about 3000 μw/°C in still water, the error due to self-heating is negligible.

![Figure 5](image)

**FIGURE 5.** Simplified circuit of thermistor bridge amplifier. The bridge is a standard configuration in which $R_1$ represents the nominal value of thermistor resistance at a given initial balance point and $\alpha$ represents the percentage change in $R_1$ from balance. The open circuit bridge output voltage is easily calculated by standard technique to be $[\alpha/(4 + 2\alpha)]E$. For the thermistor used the nominal value of $\alpha$ is 4.4%/°C.

**RESULTS**

**Effect of Temperature on Hydraulic Conductivity**

The equation that relates the hydraulic conductivity, $L_p$, to the time course of cell volume and the technique by which $L_p$ is calculated, have been extensively discussed in previous papers by Rich et al. (14) and Sha’afi et al. (13). For each experiment the least squares line was drawn on a plot of $\ln L_p$ against the inverse absolute temperature. $L_p$ at 20°C was then calculated and the $L_p$'s for all the other temperatures were normalized to this $L_p$ in order to minimize experimental and donor variability. In the top part of Fig. 6 all the data of five experiments for the $L_p$'s in human red cells are plotted logarithmically against relative $T^{-1}$. The apparent activation energy calculated from the slope of the least squares line in Fig. 6 is 3.3 ± 0.4 kcal/mole for human red cells. The corresponding value for dog red cells is 3.7 ± 0.7 kcal/mole computed from the least squares line in the bottom part of Fig. 6; each point is the average of two experiments.
Fig. 7 shows that the product of the osmotic permeability coefficient for water, \( L_p \), at each given temperature and the corresponding bulk viscosity of water, \( \eta_w \), remains virtually independent of temperature for both dog and man. If Poiseuille's law describes viscous flow through equivalent pores in the membrane, 
\[
L_p = \frac{n \pi r^4}{8 \eta_w \Delta x},
\]
in which \( r \) is the equivalent pore radius and \( \Delta x \) the path length through the membrane. The only directly temperature-sensitive variable in \( L_p \) is \( \eta_w \). Since the Poiseuille coefficient contains the
fourth power of the equivalent pore radius divided by the path length, the data in Fig. 7 provide strong support for the view that changes in temperature over the range of 5° to 39°C do not affect the geometrical restraint offered by the equivalent pore. It is quite possible for the dependence of flow velocity on the radius to be different from Poiseuille flow, but even so there will be a dependence on some power of the radius between the square and the fourth. Hence the conclusion that temperature does not affect the impedance offered

\[ \text{TABLE III} \]

**EFFECT OF TEMPERATURE ON WATER DIFFUSION IN DOG RED CELLS**

| Experiment | T (°C) | \( k/q \) (cm/sec) | \( \sigma RT \) (cm/sec \times 10^8) | \( \omega \eta \) (dyn/cm deg \times 10^4) |
|------------|--------|---------------------|----------------------------------|------------------------------------|
| 25         | 25     | 0.064               | 2.50                             | 1.28                               |
| 22         | 0.123  | 4.86                | 1.58                             |
| 37         | 0.176  | 6.95                | 1.56                             |
| 26         | 7      | 0.087               | 3.43                             | 1.75                               |
| 22         | 0.138  | 5.43                | 1.77                             |
| 37         | 0.186  | 7.34                | 1.64                             |
| 27         | 7      | 0.074               | 2.92                             | 1.49                               |
| 22         | 0.116  | 4.58                | 1.49                             |
| 37         | 0.169  | 6.59                | 1.48                             |
| 28         | 7      | 0.098               | 3.87                             | 1.93                               |
| 22         | 0.146  | 5.76                | 1.87                             |
| 37         | 0.228  | 7.00                | 2.02                             |
| 29         | 7      | 0.069               | 2.72                             | 1.39                               |
| 22         | 0.110  | 4.34                | 1.41                             |
| 37         | 0.155  | 6.12                | 1.37                             |

\[ \text{Mean}^* \]

| T (°C) | \( k/q \) (cm/sec) | \( \sigma RT \) (cm/sec \times 10^8) | \( \omega \eta \) (dyn/cm deg \times 10^4) |
|--------|---------------------|----------------------------------|------------------------------------|
| 7      | 3.1 ± 0.3           | 1.58                             |
| 22     | 5.0 ± 0.3           | 1.63                             |
| 37     | 7.2 ± 0.5           | 1.61                             |

* The mean has been obtained by averaging all the measured values at 22°C, and computing the points at the other temperatures from the slope of a straight line drawn by eye for all three points in each separate experiment.

by the equivalent pore does not rest on the validity of the application of Poiseuille's law to flow in small equivalent pores. In a study of the hydraulic permeability of a cellophane membrane with an equivalent pore radius of about 15 A, Madras et al. (15) have also found the product of hydraulic conductivity and \( \eta \) to be essentially independent of temperature between 20° and 40°C.

Evidence has been presented by Sha'afi et al. (8) and by Passow (16) that the membrane presents a series barrier to permeability. The barrier at the outer face, denoted by \( a \), is apparently thin and has a smaller aperture than the inner barrier, \( b \), whose thickness and location are as yet undetermined.
Thus it is possible that barrier $a$ is the primary determinant of $L_p$ whereas barrier $b$ plays the more important role for $w$. Even so, $L_p,\text{total}^{-1} = L_p,\text{a}^{-1} + L_p,\text{b}^{-1}$ so that the impedance of both barriers is reflected in the composite $L_p$. It is clearly apparent that the dominant term, $L_p,\eta_w$, is temperature-independent, and it seems quite probable that the same conclusion holds for $L_p,\eta_w$ both for mathematical reasons and because it is unlikely that a temperature-induced deformation could exist in one part of the membrane and be totally absent in an adjacent region.

Wang (17) has pointed out that $D_n,\eta_w/ T$ remains constant for self-diffusion of H$_2^{18}$O over the temperature range of 5° to 25°C and has used this as evidence that the self-diffusion of water may be described in terms of the movement of individual water molecules, notwithstanding the tetrahedrally bonded structure of water. In the case of the dog red cells the parameter analogous to the diffusion coefficient of water, $D_w$, which is expressed in units of cm$^2$/sec, is the diffusion permeability coefficient, $P_d$, which has units of cm/sec, since the thickness of the permeability barrier which cannot be measured explicitly is included in the permeability coefficient. We have therefore computed $P_d,\eta_w/ T = \omega R \eta_w$ over the temperature range of 7° to 37°C in the five triple experiments on dog red cells. The results for each experiment are given in the last column of Table III. It will be seen that this index is temperature-insensitive over the entire range. The conclusion is even more apparent from consideration of the mean data of all five experiments given at the bottom of the table. The invariance of $P_d,\eta_w/ T$ provides convincing evidence that the diffusion of water across the dog red cell membrane takes place by the same mechanism as in free solution. Furthermore, the apparent activation energy of THO diffusion of 4.9 ± 0.3 kcal/mole is in excellent agreement with the data of Wang et al. (2) of 4.6 kcal/mole for the diffusion of THO in free solution and the more recent value (17) of 4.8 kcal/mole for H$_2^{18}$O. Thus there is no evidence for the presence of extraordinarily structured water within the diffusion pathway across the dog red cell membrane.

In the case of human red blood cells the apparent activation energy for THO diffusion is 6.0 ± 0.2 kcal/mole which, as previously stated, is significantly greater than that for the dog. The equivalent pore radius of the dog red cell is some 40% greater than that in man, so the equivalent pore area for the human erythrocyte is just about half of that for the dog. Thus it is entirely reasonable to suppose that water-membrane interactions are appreciably more important in the human being and to ascribe the higher activation energy to such interactions, possibly arising from steric considerations or possibly resulting from chemical reactions with the molecules in the membrane. As has already been discussed, the complex nature of the permeability barrier obscures the interpretation of an equivalent pore radius as an exact measure of the diffusion permeability area, but the present data are entirely in agree-
ment with the notion that the water diffusion path through the human red cell membrane is appreciably more constrained than is the case for the dog.

In theory it would also be possible to analyze these data in terms of the apparent enthalpy and entropy of activation as has been done by Eyring et al. (see reference 18) and applied to biological systems by Hempling (4) and Hays and Leaf (1). Such analysis involves parameters which depend upon specific knowledge of the exact area for diffusion. Our uncertainty in this regard has kept us from further exploration along this avenue. However, it is possible to eliminate all the geometric parameters if the temperature effect on diffusion is coupled with that on viscous flow. Eyring’s equations (18) for the temperature dependence of diffusion and viscosity in bath solution are

\[
D = \lambda^2 (kT/h)(F^2/F)e^{-E/RT} \tag{3}
\]

\[
\eta = (\lambda_1/\lambda_2\lambda_3)(h/\lambda^3)(F/F)e^{E/RT} \tag{4}
\]

in which \(k\) is Boltzmann’s constant; \(h\), Planck’s constant; \(\lambda\) is the distance between two equilibrium positions in the direction of motion; \(\lambda_3\), the distance between neighboring molecules in the same direction; \(\lambda_2\), the distance between two adjacent molecules at right angles to the direction of motion; and \(\lambda_1\), the distance between two layers of molecules. \(F^2\) and \(F\) are partition functions of the molecule in the activated and initial states, \(E\) is the activation energy, and \(R\) and \(T\) have their usual meanings.

Many of the coefficients are eliminated by forming the product \(D\eta = TX\) (constant) and the rest disappear by forming the derivative

\[
-\frac{d \ln D}{d (1/T)} = -\frac{d \ln (1/\eta)}{d (1/T)} - \frac{d \ln T}{d (1/T)} \tag{5}
\]

So that

\[
R \left[ -\frac{d \ln D}{d (1/T)} + \frac{d \ln (1/\eta)}{d (1/T)} \right] = RT \approx 0.6 \text{ kcal/mole} \tag{6}
\]

Graphical evaluation of \(R[ d \ln (1/\eta)/d(1/T)]\) for viscous flow in water over the temperature range of 0°C to 37°C leads to a value of 4.2 kcal/mole which is approximately 0.6 kcal/mole less than the apparent activation energy for water for diffusion of 4.6–4.8 kcal/mole given by Wang and his collaborators (2, 17).

Table IV includes a compilation of all the apparent activation energies that have been measured in the present study. Water fluxes in the dog have already been shown to conform to expectations based on free solution behavior. The difference in apparent activation energies for osmotic and diffusion flow in Table IV is 1.2 ± 0.8 kcal/mole for dog red cells which is in reasonable agreement with the figure of 0.6 kcal/mole in equation 6. The
direction and magnitude of the difference are entirely consonant with expectations based on the Eyring treatment and strongly support the view that viscous flow takes place across the dog red cell membrane when an osmotic pressure gradient is applied.

One further piece of information may be extracted from the observation that, for both dog and man, the apparent activation energy for $L_p$ is apparently less, though not necessarily significantly so, than the 4.2 kcal/mole activation energy for bulk water viscosity. Such a difference would be compatible with some slip at the water-membrane interface in the equivalent pore, in contrast to the zero velocity at the interface layer in Poiseuille flow in glass capillaries in which viscosity is normally measured.

The most striking fact that emerges from the present experiments is that water movement across the red cell membrane is similar in most important respects to diffusion and viscous flow in pure liquid. In the case of water diffusion in the dog red cell, there appears to be no distinction from bulk solution. The hydraulic conductivity data suggest some slippage at the water-membrane interface, but even in this case the difference in the apparent activation energies may not be significant. In the human red cell, the situation is slightly modified by significant evidence of water-membrane interaction, consistent with the presence of more constrained diffusion pathways.

|          | Activation energies |
|----------|---------------------|
|          | Osmotic kcal/moles | Diffusion kcal/moles |
| Human    | 3.3 ± 0.4           | 6.0 ± 0.2            |
| Dog      | 3.7 ± 0.7           | 4.9 ± 0.3            |

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