Effect of Geometrical and Chemical Constraints on Water Flux across Artificial Membranes

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ABSTRACT Studies have been made on the temperature dependence of both the hydraulic conductivity, $L_p$, and the THO diffusion coefficient, $D$, for a series of cellulose acetate membranes (CA) of varying porosity. A similar study was also made of a much less polar cellulose triacetate membrane (CTA). The apparent activation energies, $E_a$, for diffusion across CA membranes vary with porosity, being 7.8 kcal/mole for the nonporous membrane and 5.5 kcal/mole for the most porous one. $E_a$ for diffusion across the less polar CTA membrane is smaller than $E_a$ for the CA membrane of equivalent porosity. Classical viscous flow, in which the hydraulic conductivity is inversely related to bulk water viscosity, has been demonstrated across membranes with very small equivalent pores. Water-membrane interactions, which depend upon both chemical and geometrical factors are of particular importance in diffusion. The implication of these findings for the interpretation of water permeability experiments across biological membranes is discussed.

Though the temperature dependence of water fluxes in red cell membranes has been studied by Vieira, Sha’afii, and Solomon (1) similar studies have not previously been made in artificial membranes in which all the important parameters which control water flux may be measured exactly. Both steric and chemical properties need to be examined in order to understand the forces governing permeation through porous membranes, particularly since Gary-Bobo, DiPolo, and Solomon (2) have shown that hydrogen bonding is an important determinant of the permeability of artificial membranes. The rationale of the present study is to gain further insight into the nature of water-membrane interactions by studying the temperature dependence of both water diffusion and filtration across artificial membranes of known porosity and chemical properties. A series of four polar cellulose acetate membranes was prepared with graded porosity, and the temperature coefficient of both per-
meability processes across these membranes was determined. In order to assess the importance of hydrogen bonding a membrane was cast from cellulose triacetate, a much less polar compound, with porosity similar to one of the cellulose acetate membranes. Both temperature coefficients were also determined on this membrane.

The results of our studies show that chemical as well as geometrical factors affect water permeability across cellular membranes. The apparent activation energies of both diffusion and viscous flow depend on the polarity of the membrane. Water-membrane interactions are of particular importance in diffusion, even across membranes with large equivalent pore radii. Classical viscous flow has been demonstrated across membranes with very small equivalent pore radii. All the experimental results can be accounted for in terms of known properties of free water and no anomalous behavior of water needs to be postulated.

**MATERIALS AND METHODS**

Four types of membranes denoted by the symbol CA were prepared from cellulose acetate (Eastman 4644, Eastman Kodak Co., Rochester, N.Y., acetyl 39.8%) solution in acetone containing varying amounts of formamide. CA-1 and CA-4 membranes, which are respectively the "skin" and the "coarse" layers of regular Loeb membranes (3), were prepared as described previously (2) from a solution of cellulose acetate and formamide in acetone in the proportion 25/25/50 (cellulose acetate, formamide, acetone; by weight). Membrane CA-2 was prepared from a solution of cellulose acetate and formamide in acetone in a proportion of 25/5/70, and CA-3 from a solution of pure cellulose acetate in acetone, 25/0/75. Both CA-2 and CA-3 membranes were cast in layers 0.04 mm thick on a glass plate using a casting knife, allowed to dry completely in the open air, and then annealed in water at 85°C for 5 min. Cellulose triacetate membranes (CTA) were prepared from a solution of cellulose triacetate (Fluka, A. G. Chemische Fabrik, Buchs, S.G., Switzerland) in chloroform (10/90) by the method used for CA-2 and CA-3.

The hydraulic conductivity, $L_p$, and diffusion coefficient, $D$, were measured as described previously (2) in a two-compartment system consisting of chambers surrounded by a water jacket. The membrane area was 1 cm$^2$ for the $D$ measurements, and 12.75 cm$^2$ in the larger chambers used for the $L_p$ determinations. The temperature was maintained by water circulation from a temperature-controlled bath through a jacket surrounding the chambers so that the experimental system was regulated to within ±0.05°C, as measured by a calibrated thermometer placed inside the chamber before the experiment began.

The membranes were equilibrated with water at the temperature of the experiments for at least 4 hr prior to use. The water content, $\Phi_w$, was measured on samples of the same membrane preparations used for the permeability measurements and in each instance at the same temperature used in the experiment. Two separate techniques were used. In the blotting technique, two sheets of filter paper were prepared by soaking in water at the same temperature as the membrane samples and
then rapidly but thoroughly squeezed between a thick glass rod and a glass plate. The membrane samples, after equilibration with water for 4–5 hr at the desired temperature, were quickly drawn between the two prepared filter paper sheets for blotting, and then weighed. In the centrifugation technique, the membrane sample was suspended from a rubber stopper in a centrifuge tube filled with water. The submerged membrane was allowed to equilibrate at the experimental temperature. Enough water was then removed from the tubes to uncover the membrane completely, leaving a few milliliters of water at the bottom. The tube was then centrifuged for 5 min at about 3000 rpm, and the centrifuged membrane samples were weighed. The membranes, either blotted or centrifuged, were then dried overnight at 100°C to constant dry weight. Both methods gave the same water content within the experimental error which is ± 0.7%. The results are much more representative of the true membrane water content as well as much more reproducible and constant than the lower values that would be obtained by simply blotting the membrane with dry filter paper.

**EXPERIMENTAL RESULTS**

The water content, \( \Phi_w \), was measured in at least five samples of each membrane at each temperature and found to be temperature independent between 0° and 40°C within experimental error, for both CA and CTA membranes. The measured values of \( \Phi_w \) are given in Table I. Since the water content of the very thin CA-1 membrane cannot be measured experimentally, the water content was measured in a much thicker layer of the same casting solution completely dried in the open air. The value is enclosed between brackets in Table I. The membrane thicknesses were measured by focusing a microscope on the top and bottom face of the membranes, except for CA-1 in which the figure is an estimate given by Lonsdale (4) based on electron microscopy measurements of membranes prepared by the same technique.

The measured membrane permeability coefficients are given in the third column of Table II and the hydraulic conductivities are in the fourth column. Owing to the large variations in the properties of membranes prepared with

**Table I**

| Membrane | Thickness \( \times 10^4 \) cm | Water content, \( \Phi_w \) g/g wet membrane |
|----------|-------------------------------|---------------------------------------------|
| CA-1     | 0.2                           | 0.13                                        |
| CA-2     | 16 ± 1                        | 0.140 ± 0.002                               |
| CA-3     | 20 ± 1                        | 0.148 ± 0.002                               |
| CA-4     | 200 ± 2                       | 0.600 ± 0.005                               |
| CTA      | 15 ± 1                        | 0.132 ± 0.002                               |
the same solution and casting procedures, the hydraulic conductivity and the permeability coefficient were measured successively on the same membrane sample at each temperature. Since a pressure difference of 40 cm of Hg, the highest pressure used in these experiments, was sufficient to deform the membranes permanently, the membranes in each instance were kept overnight at this pressure before beginning the experiments. All the $L_p$ measurements were made prior to the $\omega$ measurements; additional $L_p$ measurements subsequent to the $\omega$ determinations showed that the membrane properties had remained constant over the entire series of measurements. The data given in Table II are averages of at least 15 experimental runs for $\omega$ and 10 for $L_p$ at pressures varying from 4 to 40 cm of mercury. In addition to $L_p$ and $\omega$, the ratio $g = L_p/\omega\bar{V}_\omega (\bar{V}_\omega$ = partial molar volume of water) is given in the fifth column. An equivalent diffusion coefficient has been calculated in the sixth column from the equation $D_m = \omega RT\Delta x/\Phi_\omega$ to facilitate comparison between membranes of various thicknesses and water contents.

| Membrane | Temperature °C | $\omega \times 10^6$ mol dyne$^{-1}$ sec$^{-1}$ | $L_p \times 10^9$ mol dyne$^{-1}$ sec$^{-1}$ | $\phi$ | $D_m \times 10^9$ cm$^2$ sec$^{-1}$ |
|-----------|----------------|-----------------------------------------------|-----------------------------------------------|------|-----------------------------------|
| CTA       | 0              | 2.31                                         | 0.65                                         | 1.57 | 5.93                              |
|           | 10             | 3.40                                         | 0.94                                         | 1.54 | 9.09                              |
|           | 20             | 4.59                                         | 1.37                                         | 1.65 | 12.73                             |
|           | 30             | 6.62                                         | 1.85                                         | 1.56 | 18.88                             |
|           | 40             | 8.31                                         | 2.45                                         | 1.64 | 24.54                             |
| CA-1      | 4              | 100                                          | 158                                          | 0.88 | 3.70                              |
|           | 21             | 208                                          | 370                                          | 1.00 | 7.70                              |
|           | 37             | 407                                          | 705                                          | 0.96 | 15.50                             |
| CA-2      | 2              | 1.39                                         | 0.50                                         | 2.00 | 3.65                              |
|           | 10             | 2.20                                         | 0.70                                         | 1.82 | 5.95                              |
|           | 20             | 3.41                                         | 1.05                                         | 1.71 | 9.52                              |
|           | 30             | 5.20                                         | 1.50                                         | 1.60 | 15.06                             |
|           | 40             | 7.52                                         | 2.05                                         | 1.52 | 22.44                             |
| CA-3      | 2              | 1.40                                         | 1.03                                         | 4.10 | 4.32                              |
|           | 10             | 2.18                                         | 1.37                                         | 3.50 | 6.92                              |
|           | 20             | 3.30                                         | 1.94                                         | 3.20 | 10.85                             |
|           | 30             | 5.39                                         | 2.65                                         | 2.70 | 18.33                             |
| CA-4      | 4              | 17.0                                         | 153                                          | 50.0 | 130                               |
|           | 10             | 21.2                                         | 190                                          | 49.7 | 166                               |
|           | 20             | 28.0                                         | 244                                          | 48.4 | 227                               |
|           | 30             | 37.8                                         | 323                                          | 47.5 | 317                               |
|           | 40             | 50.2                                         | 412                                          | 45.6 | 435                               |
**Figure 1.** Relation of $L_p$ and $\omega RT$ to inverse temperature in membrane CA-2.

**Table III**

**APPARENT ACTIVATION ENERGY FOR DIFFUSION AND FILTRATION***

| Membrane | $g$ at 20°C | $D_m \times 10^7$ at 20°C | Diffusion $\omega RT$ | Filtration $L_p$ | Bulk flow $L_p - \omega P_q$ |
|-----------|-------------|---------------------------|----------------------|-----------------|-----------------------------|
| CA-1      | 1.00        | 7.70                      | 7.80±0.03            | 7.80±0.04       | -                           |
| CA-2      | 1.71        | 9.52                      | 7.70±0.03            | 5.90±0.04       | 4.3                         |
| CA-3      | 3.20        | 10.85                     | 8.10±0.04            | 5.00±0.03       | 4.5                         |
| CA-4      | 48.4        | 227                       | 5.70±0.03            | 4.70±0.02       | 4.7                         |
| CTA       | 1.65        | 12.73                     | 5.50±0.02            | 5.10±0.02       | 4.9                         |

* Errors are given as standard errors of the mean of the slope when the ln of the fraction is plotted against $T^{-1}$. Each point at a specific temperature represents a mean of 10 or more determinations in a single membrane; a single membrane was used in determining each apparent activation energy for both $L_p$ and $\omega$. The results of experiments with different membranes prepared under identical conditions varied by ±0.2 kcal/mole so that differences among membranes (that is, comparisons in vertical columns) are only significant when they exceed 0.6 kcal/mole. Differences along the rows represent measurements on the same membrane and are accurate to the standard error given.

The apparent activation\(^1\) energy, $E_a$, for diffusion and filtration has been computed from Arrhenius plots as shown in Fig. 1 for the CA-2 membrane.

\(^1\) The apparent activation energy for membrane diffusion, $E_a$, is to be compared to the apparent activation energy for self-diffusion of H$_2$O, so that $E_a$ is equivalent to $\Delta H^* + RT$, according to the Eyring theory of diffusion.
The apparent activation energies for diffusion are computed from $\omega RT$ in order to express the data in units comparable to self-diffusion in water. The data are given in Table III.

**DISCUSSION**

*Viscous Flow*

In order to discuss the data it is first necessary to make some comments about both viscous flow and diffusion. Onsager (5) has made a very apt comparison in his statement: “viscous flow is a relative motion of portions of a liquid, diffusion is a relative motion of its constituents.” For our purpose we would like to introduce a definition of “classical viscous flow” as a process which fulfills the following two requirements:

1. $L_p > \omega \overline{V}_w$ In other words net flow under an osmotic pressure gradient must take place in an amount greater than that which can be attributed to the diffusion that would result from the difference in the chemical potential of water. In capillary tubes or in very large pores, the diffusion component makes a negligible contribution, but in tight membranes diffusion down the water activity gradient is quantitatively significant even in the presence of hydrostatic pressure. Thau, Bloch, and Kedem (6) have used irreversible thermodynamics to relate bulk flow to tracer flow when both are proceeding simultaneously and have derived the equation

$$\left(\frac{L_p}{\omega \overline{V}_w}\right) = 1 + \frac{f_{tw}}{f_{wm}}$$

in which tracer-water friction in the pore is denoted by $f_{tw}$ and water-membrane friction in the pore by $f_{wm}$. In a very large pore only a small fraction of the water is in contact with the membrane so $f_{tw} \gg f_{wm}$ and $L_p \gg \omega \overline{V}_w$ in accordance with the usual expectation. However, as the pore radius shrinks, $f_{wm}$ becomes of overwhelming significance for geometrical reasons and $f_{tw}$ becomes very small because more of the nearest neighbors are membrane than water. Thus in the limit, $(f_{tw}/f_{wm}) \to 0$ and $L_p \cong \omega \overline{V}_w$. This shows that a diffusional component is always present in hydraulic flow though it is only significant for membranes with small pores.

2. $L_p \eta_w = \text{constant}$ This means that the hydraulic conductivity of classical viscous flow of water is inversely proportional to the bulk viscosity of water at any temperature. Therefore, the apparent activation energy of $L_p$ is equal to the inverse of the activation energy for bulk water viscosity. It should be noted that this criterion implicitly requires that the water properties in the membrane be the same as in the bulk phase.

In the case of membranes characterized by small pores, the criterion has to be modified because the measured $L_p$ represents a combination of both filtra-
tion and diffusion. A correction may be made for the diffusion component, measured by \( \omega \bar{V}_w \), by forming the expression \((L - \omega \bar{V}_w)\) assuming the two processes to be additive and neglecting interaction between them. Thus, the modified second criterion becomes \((L - \omega \bar{V}_w) \eta_w = \text{constant}\).

These two conditions which define classical viscous flow are satisfied by a wide variety of artificial and biological membranes, as discussed by Solomon and his colleagues (1, 7). However, there are other forms of bulk flow that do not satisfy these criteria. One such example is afforded by the results of Debye and Cleland (8) who found the second condition to be violated by the flow of \(n\)-decane through porous Vycor tubing. They attributed their results to the absence of a stationary layer of solvent at the solvent membrane interface, which is a necessary condition for classical viscous flow.

The second condition requires water-water friction, \(f_{ww}\), to be much greater than water-membrane friction, \(f_{wm}\), in order for the kinetics to be governed by bulk water viscosity. This means that the effective contribution of \(f_{wm}\) to the total friction must be small, but it can be small either for geometrical reasons when viscous flow takes place in very large channels or for energetic reasons because the water-membrane interactions are very weak.

It should be pointed out that our definition of classical viscous flow does not require that flow through small apertures satisfy Poiseuille’s law. As Mikulecky (9) has stated, Poiseuille’s law is a limiting case in which the water-membrane friction drops off very sharply at the wall. Many other cases may be considered in which the value of \(f_{wm}\) is dependent upon position within the equivalent pore.

**Chemical Interactions with Membranes**

The most important consideration in discussing diffusion is the nature of the interaction between the diffusing water molecules and the membrane matrix. Since the water content of the least porous membrane is 13\%, each water molecule has a significant opportunity for hydrogen bonding with other water molecules, as well as with the hydroxyl groups of the membranes. No clear distinction can be made as to whether the partners are other water molecules, membrane hydroxyl groups, or both. Since hydroxyl groups do not alter water structure much, if at all (10), the behavior of water in the membrane might be expected to be similar to that of water in bulk. The apparent activation energy for self-diffusion in water is 4.8 kcal/mole as given by Wang (11). However, the hydrogen bond between water and alcoholic groups has a somewhat greater activation energy than the water-water bond, as judged by the measurements of Schneider and Reeves (12) on alcoholic solutions. Thus, the effect of binding with hydroxyl groups in the CA membranes should be an increase in the apparent activation energy.
Cellulose Acetate Membranes

Cellulose acetate differs chemically from cellulose triacetate, because there are about 1.5 free hydroxyl groups acetylated per residue in CA whereas all three free hydroxyl groups are acetylated in CTA. As a consequence, CTA membranes are far less polar, and have only about half of the hydrogen-bonding possibilities of CA membranes.

There is a graded difference in porosity in the more polar CA membrane series as shown by the stepwise increments in \( g \) at 20°C which increases from a value of 1.0 for CA-1 to 48.4 for CA-4 as shown in Table III. Membrane CA-1 is entirely nonporous, whereas CA-4 is very porous with an equivalent pore radius of about 24 A at 20°C. Though the water content of CA-1, CA-2, and CA-3 does not vary by more than a few per cent (between 0.13 and 0.148 g/g wet membrane), \( g \) varies by more than a factor of three (from 1.0 to 3.20 at 20°C). It is likely that this variation in \( g \) is related to the size of the water clusters in the membranes, which is known to vary with the casting procedure and the composition of the casting solution. In the case of CA-4, the very large value of \( g \) is clearly related to its high water content (\( \Phi_w = 0.6 \)).

The apparent activation energy for water diffusion in membrane CA-1 is 7.8 kcal/mole (Table III), appreciably greater than the 4.8 kcal/mole characteristic of self-diffusion in water (11). This reflects the contribution of the water-membrane interactions that have already been discussed. The apparent activation energy for filtration is exactly equal to that for diffusion, an observation which gives strong support to the conclusion that the only flux process is diffusion, in accordance with the conclusions of Lonsdale, Merten, and Riley (13) who studied water transport through similar membranes.

Membrane CA-4 is at the other end of the spectrum since the primary diffusion process in this membrane is free diffusion through the water channels. The diffusion coefficient in the membrane given in Table III is \( 2.27 \times 10^{-5} \) cm\(^2\) sec\(^{-1}\), in fortuitously close agreement with the coefficient for self-diffusion in bulk water of \( 2.27 \times 10^{-5} \) cm\(^2\) sec\(^{-1}\) (11). Nonetheless the apparent activation energy of 5.7 kcal/mole is a little higher than that for free diffusion. This may easily be explained on a geometrical basis since some 30% of the water in the equivalent pores is within 1.5 A of the membrane surface. In this very porous membrane, we would expect that the filtration process would exhibit the characteristics of classical viscous flow. The apparent activation energy for filtration is 4.7 kcal/mole, slightly but not necessarily significantly, above the value of 4.2 kcal/mole for the inverse of the apparent activation energy for water bulk viscosity.

Both intermediate membranes, CA-2 and CA-3, are relatively tight membranes as shown by the small values of both \( g \) and \( D_m \) in Table III. The low value of \( g \) for both CA-2 and CA-3 is an indication that, on geometrical con-
siderations alone, water-membrane interaction should be of much greater importance for these membranes than for membrane CA-4. Diffusion is still dominated by dissolution in the membrane fabric as is apparent from a comparison of the values of $D_m$ for these membranes in column 3 of Table III with $D_m$ for the nonporous CA-1. As would be expected, both activation energies for diffusion are also in close agreement with the figure for CA-1.

The apparent activation energy for filtration across membrane CA-2 is clearly higher than that for bulk water viscosity and an intermediate value is found for membrane CA-3. Thus, at first sight, these membranes do not apparently fulfill the second condition for classical viscous flow. However, for these tight membranes, the diffusion component of the measured $L_p$ cannot be neglected, as previously discussed. The high $g$ value for CA-4 is an indication that $\omega\bar{V}_w$ makes a small contribution to the observed bulk flow in this very porous membrane; the low value of $g$ for CA-2 and CA-3 indicates that $\omega\bar{V}_w$ is of great importance for these membranes, contributing about 59 and 32% of the total observed flow, respectively. The apparent activation energy of $(L_p-\omega\bar{V}_w)$ used in the modified second criterion for classical viscous flow, is given in the last column of Table III. The values for CA-2 and CA-3 are in fair agreement with the 4.2 kcal/mole figure for the inverse of the apparent activation energy for water viscosity. Thus, these very tight membranes in which water-membrane interactions are of great geometrical importance, appear to satisfy the modified second criterion for classical viscous flow even though it would seem unlikely that cooperative processes between water molecules would be of sufficient magnitude to produce this result.

Although we have developed the argument by separating the viscous component from the diffusional component by assuming them to be additive, the conclusion that the filtration process differs from the diffusion process does not rest on these grounds alone. The distinction may be made on grounds of activation energies since Table III shows the apparent activation energy for filtration to be significantly below that for diffusion for all three of the porous CA membranes studied. Furthermore, the general trend of the three apparent activation energies for filtration is consistent with the geometry of the system since the apparent activation energy decreases regularly as the porosity of the membrane increases.

**Cellulose Triacetate Membranes**

The important difference between the CTA and CA-2 membranes lies in the difference in the ability to form hydrogen bonds. The value for $g$ is approximately equal in these two membranes, and insofar as $g$ is primarily a measure of equivalent pore geometry, we would expect differences in apparent activation energies between the two membranes to reflect hydrogen bond formation primarily. This notion is supported by the apparent activation energy for dif-
fusion which is 5.5 kcal/mole for the CTA membrane as compared to 7.7 kcal/mole for CA-2. Even though the activation energy for diffusion is smaller than for CA-2, it remains higher than the 4.8 kcal/mole characteristic of self-diffusion in bulk water. This indicates that the hydroxyl groups in the CTA membrane probably form some hydrogen bonds with water molecules. The apparent activation energy for filtration is significantly lower than that for diffusion, and the difference approaches the 0.6 kcal/mole difference between diffusion and viscous flow in free solution. In the CA-2 membrane the higher apparent activation energy for $L_p$ of 5.9 kcal/mole reinforces the view that the apparent activation energy for filtration in membranes with small equivalent pores includes an important contribution from water-membrane interactions. The apparent activation energy for bulk flow in the CTA membrane, 4.9 kcal/mole, fits moderately well with the values obtained for the other CA porous membranes.

**Comparison of Filtration and Diffusion**

The acceptance of the ratio of filtration to diffusion as a measure of the geometrical dimensions of an equivalent pore involves a number of implicit conditions. The apparent activation energies for these two processes are different in each case in Table III, so the ratio itself is temperature dependent as shown in Table II. The water content of the membrane is independent of temperature for each membrane studied which suggests that the temperature dependence of $g$ may not be attributed to a change in membrane dimensions. Furthermore the diffusion coefficients fall on a straight line in the Arrhenius plot in Fig. 1, as would be expected for diffusion through a membrane whose dimensions are unaffected by temperature. These findings are consistent with a membrane whose dimensions and water content are temperature independent over our experimental range.

Nonetheless it is important to examine the possibility that our results may be attributed to a temperature-induced dimensional change. The membrane may still expand with temperature in the range of 2°C to 40°C, notwithstanding its having been annealed at 85°C. If so, we would expect it to expand isotropically so that the pores would increase both in length and diameter. This possibility is excluded by the temperature independence of the water content. Alternatively the membrane might expand differentially so that an increase in pore length would be balanced by a decrease in pore diameter to keep the water content unchanged. A comparison of the data for membranes CA-2 and CA-3 in Table II shows that $\omega$ is relatively insensitive to changes in pore diameter. Thus the increased pore length would predominate so that the effect of anisotropic expansion on $\omega$ would be to cause a decrease in the apparent activation energy.

Table II shows that $L_p$ is very sensitive to pore diameter so that a decrease
in diameter would cause a decrease in $L_p$. The impedance to hydraulic flow is inversely proportional to the pore length and directly proportional to some power of the pore diameter, which in the case of Poiseuille flow is the fourth. Thus shrinkage in pore diameter with increased temperature would not be compensated for by the increased pore length. In this case, also, we would therefore expect anisotropic swelling to cause a decrease in apparent activation energy. These arguments lead us to conclude that the effect of swelling, if it occurs, would be to make the computed activation energies too small, rather than too large. Hence it does not seem probable that the observed differences in apparent activation energies for $L_p$ and $\omega$ in Table III may be attributed solely to temperature-induced dimensional changes.

We therefore conclude that the ratio, $g$, though dominated by a geometrical term, includes an important energetic component which represents the strength of the water-membrane interaction. For example, $g$ is essentially identical for the CTA and the CA-2 membranes. However, there are important and significant differences in the strength of the water-membrane interactions as shown in the differences in the apparent activation energies. Hence it would be wrong to conclude that the geometrical equivalent pore radii for the two membranes are identical, because $g$ is a composite of geometric and energetic factors which cannot be separated from one another on the basis of our present information alone.

Solomon (7) has pointed out that the product, $D_\omega \eta_w$, appears in the derivation of the equation for the equivalent pore radius. According to the Stokes-Einstein derivation of the diffusion coefficients, the ratio $D_\omega \eta_w / T$, should be temperature independent and Wang (11) has shown this to be true for bulk water. Solomon suggested that a similar relation should hold within the equivalent pore so that $D_\omega \eta_w$ should be less dependent on local interactions within the pore than either of the separate terms. However, the present result indicates that this is not the case in porous CA membranes. Water-membrane interactions affect the diffusion process much more than filtration. The high apparent activation energies for diffusion for membranes such as CA-2 and CA-3 may perhaps be attributed to the large fraction of diffusion which goes through the matrix of the membranes rather than the porous regions. However, diffusion through much more porous membranes is also sensitive to water-membrane interactions, as shown most clearly by the CA-4 membrane in which the equivalent pore radius is 24 A at 20°C, and the diffusion coefficient at 20°C is indistinguishable from that in bulk solution. Nonetheless the apparent activation energy for membrane diffusion is significantly greater than that for self-diffusion in free solution which underlines the importance of water-membrane interactions in affecting the diffusional process. These interactions exercise a very much smaller effect on filtration as shown by the 1.0 kcal/mole difference between apparent activation energies for diffusion and filtration in this membrane.
Similar considerations may also be applied to biological membranes using the data obtained by Vieira, Sha’afi, and Solomon (1) for the red cells of man and dog which are reproduced in Table IV. For the dog the ratio, $D \omega \eta_w / T$, is temperature independent so that the water-membrane interactions are very similar to those in free solution, and very much less than those in porous CA membranes. In the case of man the apparent activation energy for diffusion is almost double that for filtration, so that the equivalent pore radius determined at $20^\circ$C is necessarily different from that obtained from the $g$ value at another temperature. Hence the water-membrane interactions are more important in this species and the difference in equivalent pore radius between man and dog at $20^\circ$C is affected by energetic considerations. Thus, in addition to geometric considerations, chemical factors play an important role in species differences.

Thau, Bloch, and Kedem (6) have observed values of $g$ greater than unity in liquid membranes and have pointed out that our first criterion that $L_p \omega$ be greater than $\omega \bar{V}_w$ is not sufficient to establish the presence of bulk flow of water across a membrane. The temperature dependence of the permeability processes has enabled us to impose a second criterion for classical viscous flow, namely that $L_p \eta_w = \text{constant}$ or, for tight membranes, that $(L_p - \omega \bar{V}_w) \eta_w$ be temperature independent. It is our view that satisfaction of both criteria for classical viscous flow of water is sufficient to establish the porous nature of a membrane, whether artificial or biological. Such a conclusion is in full accord with Onsager’s statement, quoted at the beginning of the Discussion.

Classical viscous flow persists in membranes characterized by very small equivalent pore radii, even down to a value of $g$ of 1.7 at $20^\circ$C. The bulk flow component, given by $(L_p - \omega \bar{V}_w)$, has an apparent activation energy approximately equal to that for bulk water viscosity. Thus there is no evidence of anomalous behavior of water in the permeation processes of the membranes we have studied. The present results show quite clearly that the relationship between diffusion and filtration is dependent upon at least two factors. Though the geometrical factor appears to be dominant, water-membrane

| Species | $g$ at $20^\circ$C | Apparent activation energies | Diffusion | Filtration |
|---------|------------------|----------------------------|-----------|-----------|
| Man     | 1.85             | kcal/mole                  | 6.0       | 3.3       |
| Dog     | 4.5              | kcal/mole                  | 4.9       | 3.7       |
interactions are of importance. Both factors must be included in order to provide an accurate physical description of membrane permeation.

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