Permeability of the Ehrlich Ascites Tumor Cell to Water

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ABSTRACT The osmotic permeability coefficient for water has been measured for the Ehrlich mouse ascites tumor cell. Measurements were made of the rate of cell shrinkage in hyperosmotic solutions of NaCl, a functionally impermeable solute. During the first 9 months of weekly serial transplantation the mean was 6.4 μ^3/μ^2/atm. $\pm$ 0.8 (S.E.). By the end of the 2nd year the permeability coefficient was much lower and averaged 1.6 $\pm$ 0.09. There were no significant differences in the volume of the tumor cells which could explain the discrepancy on the basis of a change in the volume to surface area ratio. Studies of the effect of temperature were done and Eyring's theory of absolute reaction rates was applied to the data. The apparent energy of activation was 9.6 kcal./mol and $\Delta S\ddagger$ was 39.1 entropy units. The thermodynamic data are twice as high as data reported by Wang for self-diffusion and viscous properties of water. Two alternate explanations have been advanced based on the pore hypothesis of membrane permeability. One explains the thermodynamic data from a change in the $A'/\Delta x$ available for water movement; the other assumes $A'/\Delta x$ constant and bases the results on the interaction of water dipoles with each other and the membrane.

INTRODUCTION

A characteristic of the neoplastic cell is its ability to compete successfully with the host tissues for a limited supply of nutrients. This is especially so for the rapidly growing tumors where the energy demands may be considerable. Since the membrane of the tumor cell is the first barrier which the cell shows to its environment, a functional description of this barrier will serve to elucidate the means by which the cell can regulate the constituents which enter or leave the cell. This report on the permeability characteristics of the Ehrlich ascites tumor cell to water deals with one aspect of the problem of tumor cell permeability.

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METHODS

Cell Suspensions The Ehrlich ascites tumor cell (4n) used in this investigation has been maintained in Swiss mice by intraperitoneal weekly transplantation of inocula of 0.2 ml. Studies to be reported were carried out between the 26th and the 101st transplants.

Samples were obtained by peritoneal aspiration from animals with growths ranging in age from 5 to 12 days and pooled in a K^+–Na^+ Ringer solution without glucose.[1] Cells were finally resuspended in K^+–Na^+ Ringer solution without glucose in concentrations of 10 to 15 per cent, by volume, after washing three times by gentle centrifugation. All washing procedures and resuspensions were done at temperatures ranging from 20 to 25°C.

Densimetry In general, the approach was very similar to that described by Lucké and Parpart (1). Cells were maintained in uniform suspension in a glass chamber of approximately 10 ml. capacity by gentle stirring with a motor-driven glass rod. A beam of light of constant intensity was passed through the chamber. This light impinged on a Vickers photocell and the current from the latter was detected with a Kipp torsion string galvanometer, with a period of 0.01 sec. Deflections of the galvanometer were recorded photographically on 12 cm. bromide paper. For continuous recording, timing was obtained by interrupting the light beam to the mirror of the galvanometer at half second intervals. For longer periods of recording, the shutter was opened automatically either at 15 sec. or 1 min. intervals.

In order to establish the permeability of the membrane to water, studies were carried out on the rate of shrinkage of the tumor cell. The procedure was to add 0.25 ml. of the cell suspension obtained as described above to 7.0 ml. of K^+–Na^+ Ringer solution without glucose in the densimeter chamber. Recording for 30 sec. to a minute established a base line. Then, to induce an osmotic movement of water out of the cells, 0.25 ml. of a 5.1 M NaCl solution was injected by syringe and needle into the stirred cell suspension and the changes in the galvanometer deflection recorded.

Measurements of the osmolarity of such suspensions were made with the Fiske osmometer. When the fluid injected was 0.25 ml. of 5.1 M NaCl, the osmolarity of the final suspension was 623 milliosmoles per liter. For 0.25 ml. of 5.1/2 M NaCl, the value was 467 milliosmoles per liter. The osmolarity of suspensions of cells in K^+–Na^+ Ringer solution without glucose was 322 milliosmoles per liter. In three experiments, with cells showing a high permeability to water (mean permeability = 8.4 μ^3/μ^2/min./osmotic pressure difference), the permeabilities were measured using both hyperosmotic solutions. The differences between the permeability coefficients determined with the two osmotic solutions averaged 13.6 per cent. For all other

[1] 9 gm. NaCl
40 ml. 0.154 M KCl solution
15 ml. 0.11 M NaH_2PO_4
85 ml. 0.11 M Na_2HPO_4
To a liter with distilled water. pH = 7.4.

determinations of the permeability of the membrane to water, for cells with high or low permeabilities, only the solution with final osmolarity of 623 milliosmoles per liter was used.

Cell Volumes The densimetric method permits the measurement of rapid changes in cell volume. However, the changes in cell volume are recorded as galvanometer deflections, and it is necessary to convert these deflections into their equivalent volumes. Moreover, it is essential to establish the volume of "osmotically inactive material" so that cell volume changes can be expressed in terms of net water changes and thus enable one to calculate a permeability coefficient.

To determine the volume per cell, measurements of the relative cell volume of the suspension were obtained by centrifuging the suspensions in capillary tubing with a uniform bore diameter of 0.8 mm. for 25 minutes at the maximum speed of the International centrifuge (*ca.* 2500 *G*). Cell counts of the cell suspensions were made in the standard Neubauer-Levy hemocytometer and one thousand or more cells were counted. The volume per cell was then calculated.

Agreement between volumes arrived at by this method and volumes obtained from direct measurements of cell diameter is good. Thus, the mean volume of cells in isotonic K^+–Na^+ Ringer solution without glucose at room temperature was found to be 1970 $\mu^3 \pm 34$ (S.E.) corresponding to a diameter of 15.6 μ for these spherical cells. Direct measurements with a filar-ocular micrometer gave values for the diameter of 15.8 μ. In addition, Lucké and Berwick (2) reported a mean cell volume of 2,188 μ^3 with a mean cell diameter of 15.9 μ. Their strain of tumor cell was the original source for the cells which have been used in this investigation and was obtained in turn from Dr. M. R. Lewis of the Wistar Institute, Philadelphia.

To determine the volume of osmotically inactive material, the volumes of the tumor cells in different hypertonic media were measured as follows: Suspensions containing a known number of cells were added to a given amount of dry NaCl. Routinely, five different degrees of hypertonicity were used. Expressed in equivalent concentration of NaCl, these were: 0.201 M, 0.234 M, 0.265 M, 0.300 M, and 0.322 M. Relative cell volumes of the suspensions were measured by centrifuging the suspensions in capillary tubing of uniform diameter as described above, and the volumes of the cells in the different hypertonic solutions were then calculated.

Galvanometer deflection was converted to cell volume as described by Lucké, Hempling, and Makler (3). There is a linear relationship between galvanometer deflection and the relative tonicity of the external solution, with the tonicity of K^+–Na^+ Ringer solution without glucose taken as 1.

When the cells shrink, light transmission decreases, and when the cells swell light transmission increases. In this respect, the ascites tumor cells behave like mammalian erythrocytes (1) or leukocytes (3).

THEORETICAL CONSIDERATIONS

Calculation of the Permeability Coefficient for Water The permeability of the Ehrlich ascites tumor cell to water has been measured as the rate of water

movement out of the cell under an osmotic gradient. The following equation of Lucké, Hartline, and McCutcheon (4) has been used to calculate the permeability coefficient:

$$k_{2a}t = \left(\frac{V_e - b}{V_0 - b}\right)\left(\frac{1}{(36\pi)^{\frac{1}{3}}P_0}\right)\left[\left(1 - \frac{b}{V_e}\right)V_e^{\frac{1}{3}}\left\{\left(\frac{1}{2}\ln\frac{V_e^{\frac{1}{3}} + (V_eV)^{\frac{1}{3}} + V^{\frac{1}{3}}}{(V_e^{\frac{1}{3}} - V^{\frac{1}{3}})^2}\right) + \sqrt{3}\arctan\frac{2V^{\frac{1}{3}} + V_e^{\frac{1}{3}}}{\sqrt{3}V_e^{\frac{1}{3}}}\right\} - 3V^{\frac{1}{3}}\right]_{V_{t=0}}^{V_{t=t}} \quad (1)$$

in which k_{2a} = permeability coefficient in μ^3/μ^2/min./osm. pressure difference, in atm.
t = time in minutes
P_0 = initial osmolarity of the cell suspension, in atm.
V_e = equilibrium volume of the cell in μ^3.
V = volume of the cell at any time, t, in μ^3.
V_0 = initial volume of the cell, in μ^3.
b = osmotically inactive material, in μ^3, obtained by extrapolation of graphs of volume *versus* 1/concentration to 1/concentration equal to 0.

This equation is the integrated form of the differential equation which states that the rate of shrinkage of the cell is proportional to the surface area of the cell and to the osmotic gradient across the cell. The "constant" of proportionality, which is equivalent to the permeability coefficient has been designated k_{2a}, in keeping with Jacobs' nomenclature (5, 6). He employs k_2 as the permeability coefficient normalized to unit cell volume and area and for a unit osmotic gradient. The coefficient, k_{2a}, on the other hand defines the numerical values based upon the volume and surface area of the particular cell studied and for the osmotic gradient used. It has the dimensions of μ^3/μ^2/min./atm. of osmotic difference.

The assumptions from which this equation is derived are adequately discussed in the paper of Lucké, Hartline, and McCutcheon (4). One of their assumptions which should be stressed, is that the membrane of the cell should be semipermeable. Three pieces of evidence may be cited in support of this assumption for the Ehrlich ascites tumor cell. First, the observation illustrated in Fig. 1, that the cell acts as an osmometer according to the Boyle-van't Hoff law. Secondly, that when concentrations of cellular electrolytes are determined in cells shrunken in hypertonic solutions, the amount of electrolyte per kilogram of cell water is found to agree with what one would calculate if the loss in cell volume came about through a loss of water alone; *i.e.*, that the cell was semipermeable. Finally, densimeter recordings of the tumor cell show no change in volume for as long as 20 minutes after reaching equilibrium in hypertonic solutions. If, however, the ability of the membrane to maintain this semipermeability is modified by an inhibitor such as Cu^{++} in concentrations

of 2.0×10^{-5} M, the cell will swell concomitant with the movement of Na^+ into the cell.

Therefore, k_{2a} is formally identical with L_p, defined by Kedem and Katchalsky (7), and is an osmotic permeability coefficient.

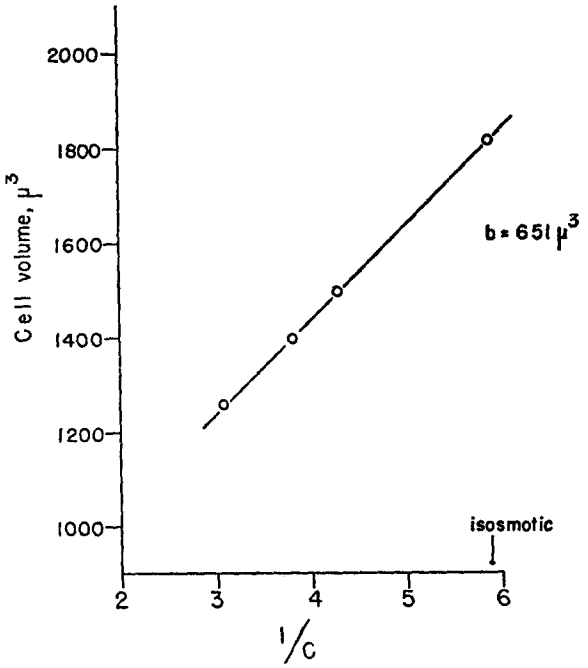

FIGURE 1. The behavior of the Ehrlich ascites tumor cell as an osmometer.

To facilitate the calculation of the permeability coefficient, the term

$$\left\{\left(\frac{1}{2}\ln\frac{V_e^{\frac{1}{3}}+(V_eV)^{\frac{1}{3}}+V^{\frac{1}{3}}}{(V_e^{\frac{1}{3}}-V^{\frac{1}{3}})^2}\right)+\sqrt{3}\arctan\frac{2V^{\frac{1}{3}}+V_e^{\frac{1}{3}}}{\sqrt{3}V_e^{\frac{1}{3}}}\right\}$$

may be expressed as:[2]

$$\left\{1.5\log_{10}\frac{1+r^{\frac{1}{3}}+r^{\frac{2}{3}}}{(1-r^{\frac{1}{3}})^2}+1.73\arctan\frac{2r^{1/3}+1}{1.73}\right\}$$

and will be referred to as $f(r)$, in which $r = V/V_e$

By solving for a number of values of r, a graph may be prepared which permits one to read off the value for this term, $f(r)$, for any value of r. This graph is

[2] The author wishes to express his thanks to Dr. W. Yamamoto, Department of Physiology, School of Medicine, University of Pennsylvania, for help with this approach.

shown in Fig. 2. Different values of r (*i.e.* V/V_e) are plotted along the abscissa while the value for the term, designated on the graph as $f(r)$, is plotted on the ordinate.

If one substitutes $f(r)$ in Equation 1 and integrates between $t = t$ and

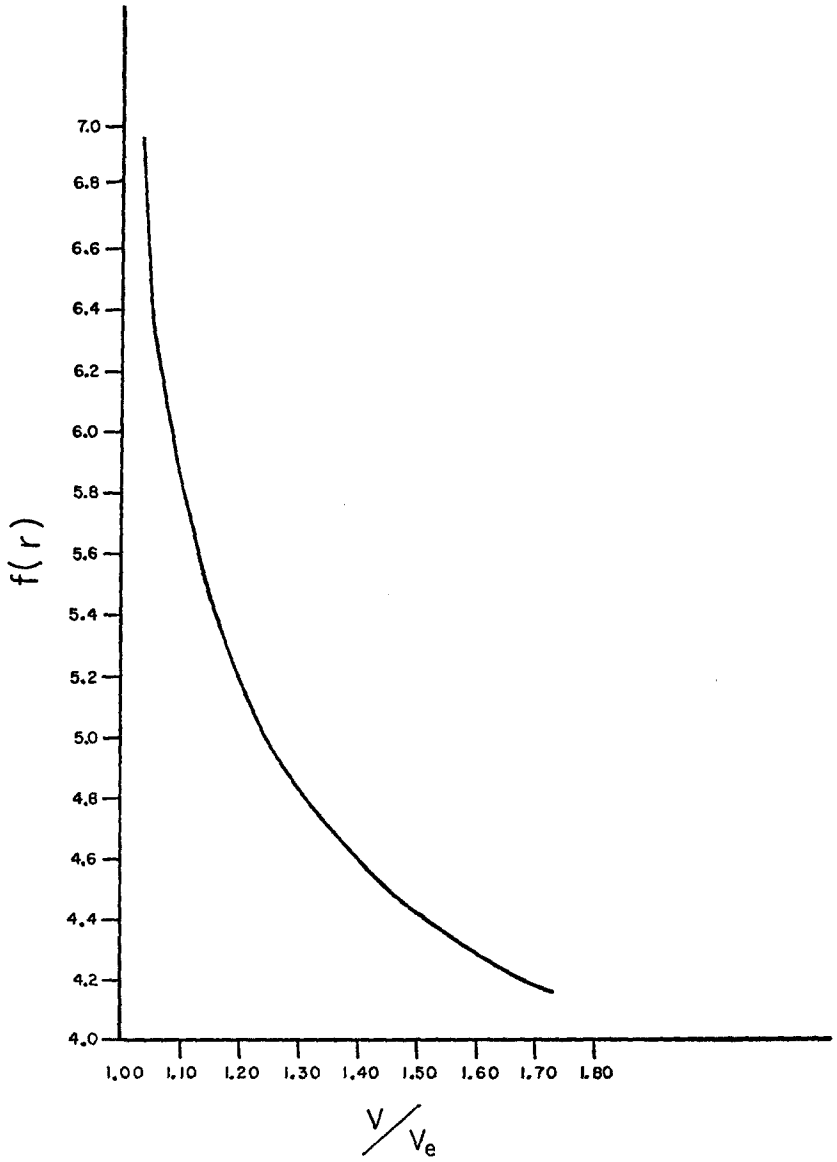

FIGURE 2. The relation between V/V_e, or r and the term:

$$\left\{1.5 \log \frac{1 + r^{1/3} + r^{2/3}}{(1 - r^{1/3})^2} + 1.73 \arctan \frac{2r^{1/3} + 1}{1.73}\right\}$$

in the permeability equation of Lucké, Hartline, and McCutcheon.

$t = 0$, then

$$k_{2a}t = \left(\frac{V_e - b}{V_0 - b}\right)\left(\frac{1}{(36\pi)^{\frac{1}{3}}P_0}\right)\left[\left\{\left(1 - \frac{b}{V_e}\right)V_e^{\frac{1}{3}}(f(r))_{V_{t-t}} - 3V_{t-t}^{\frac{1}{3}}\right\} - \left\{\left(1 - \frac{b}{V_e}\right)V_e^{\frac{1}{3}}(f(r))_{V_{t-0}} - 3V_{t-0}^{\frac{1}{3}}\right\}\right] \tag{2}$$

and finally

$$k_{2a}t + C = \left(\frac{V_e - b}{V_0 - b}\right)\frac{1}{(36\pi)^{\frac{1}{3}}P_0}\left[\left(1 - \frac{b}{V_e}\right)V_e^{\frac{1}{3}}(f(r))_{V_{t-t}} - 3V_{t-t}^{\frac{1}{3}}\right] \tag{3}$$

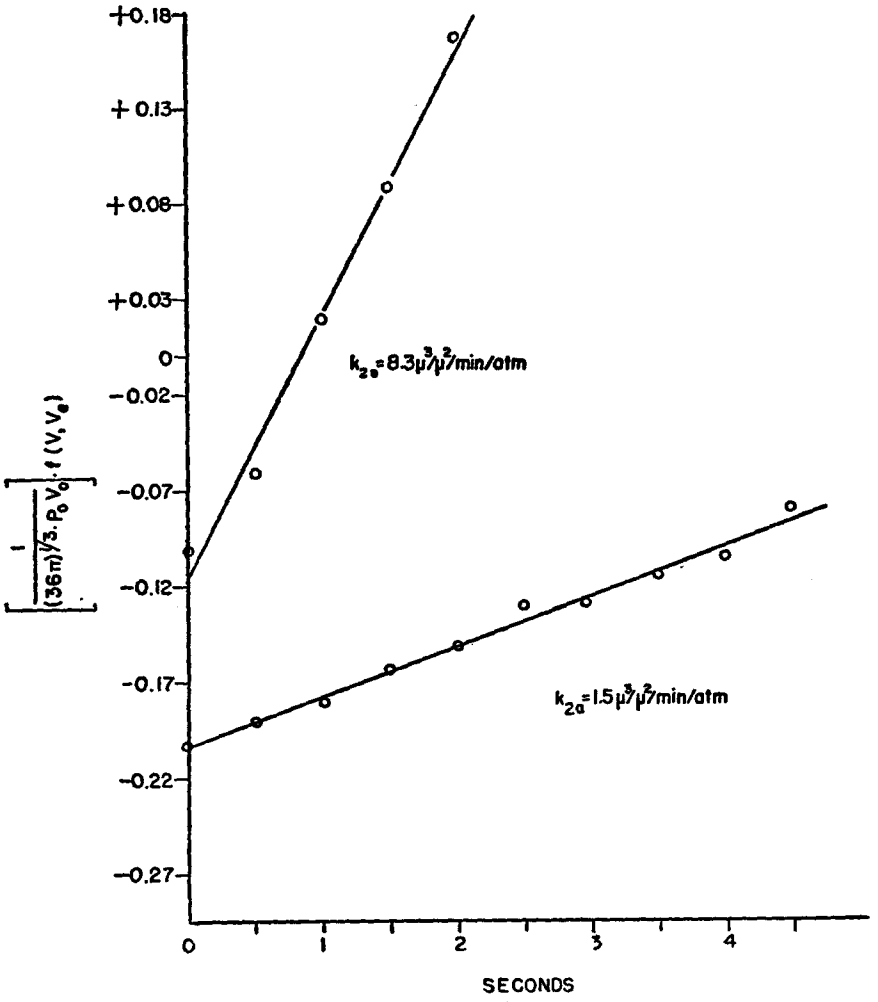

FIGURE 3. The application of the equation of Lucké, Hartline, and McCutcheon to the calculation of the permeability of the membrane of the Ehrlich ascites tumor cell to water. The permeability coefficient is obtained from the slope of the line. Upper curve is for typical data from "early" transplants. Lower curve, for "later" transplants.

in which

$$C = \frac{(V_e - b)}{(V_0 - b)} \frac{1}{(36\pi)^{\frac{1}{3}} P_0} \left[\left(1 - \frac{b}{V_e} \right) V_e^{\frac{1}{3}} (f(r)) V_{t=0} - 3V_{t=0}^{\frac{1}{3}} \right]$$

This constant, C, has been evaluated and found to be (-20.29) for the example illustrated in Fig. 3 (lower) and Table I. The terms on the right side of Equation 3 are evaluated in a sequential fashion in Table I for several times, t, and the resulting products are seen in the last column. Plotting this last column as the dependent variable of t, we have the plot in Fig. 3 (lower), where the slope of the line is k_{2a}, the permeability coefficient. In the interest

TABLE I

DATA AND CALCULATIONS TO OBTAIN A PERMEABILITY COEFFICIENT FOR WATER

$V_0 = 2110\ \mu^3$ $b = 749\ \mu^3$ $V_e = 1440\ \mu^3$ $P_0 = 7.4$ atm.

Time	Volume	$r = V/V_e$	$f(r) \times (1 - b/V_e)V_e^{1/3}$	$V_{t=0}^{1/3} \times (V_e - b)/36\pi)^{1/3} P_0(V_0 - b)$	$-3V^{1/3} \times (V_e - b)/36\pi)^{1/3} P_0(V_0 - b)$
sec.					
0	2110	1.46	4.48	24.21	−14.28	−20.29
1	1990	1.38	4.64	25.10	−12.64	−17.95
2	1860	1.29	4.86	26.29	−10.61	−15.06
3	1770	1.23	5.05	27.30	−9.00	−12.78
4	1690	1.18	5.26	28.45	−7.28	−10.34

of conserving tabular space, only a few representative calculations are shown in Table I. A complete plot is shown for these data in Fig. 3 (lower).

The value for "b," the volume taken by osmotically inactive material, was obtained by plotting the volume of the cells *versus* the reciprocal of the concentration. Extrapolation of the graph to $1/C$ equal to 0 provides a value for "b." Fig. 1 shows such a plot. The value for "b" in this case was 651 μ^3. In twenty-one experiments, the "b" value was found to average 33.2 per cent ± 3 (S.E.) of the cell volume.

RESULTS

Tumor Strain and the Permeability to Water As a routine procedure, samples as obtained from the peritoneum of the mouse were taken periodically and frozen at −50°C. In January, 1956, one such sample was prepared and stored at −50°C. Two months later this sample was reinstituted in Swiss mice and has been maintained in continuous transplant since then.

Between the 26th and 35th transplants, a number of studies were carried out on the permeability of the cells to water. Permeability coefficients were calculated for eleven such experiments. The mean was 6.4 μ^3/μ^2/min./atm. ±0.8

(S.E.), at a mean temperature of 24°C. (range, 20–26°C.). An example from this group is shown in Fig. 3 (upper curve). Many other experiments carried out at this time, but in which the permeability coefficient was not calculated, gave values of 0.9 to 1.1 seconds to reach half-equilibrium and provided a qualitative index of permeabilities of the same order as those in which the coefficients were calculated.

The permeability of the cells to water was examined again between the 86th and 101st transplant, and it was found that the mean permeability coefficient of the cells to water was much lower and averaged 1.61 μ^3/μ^2/min./atm. $\pm$0.09 (S.E.), in seven experiments, at a mean temperature of 23°C. (range, 20–26°C.). The lower curve of Fig. 3, already referred to above, is an example of data from such a transplant.

The differences observed in the mean values for the permeability coefficients are not likely to be due to technical error for two reasons. First, observations on the rates of shrinkage for red blood cells, which were being carried out concomitantly, were unchanged. Secondly, the permeability of the cell to penetrating solutes, using the same osmotic techniques, did not change for the two sets of experiments. There were no significant changes in the volume of the tumor cells which could explain the differences on the basis of a change in the volume to surface area ratio. For the present, the hypothesis has been adopted that the population of tumor cells has assumed altered permeability characteristics associated with prolonged transplantation. The basis for alteration requires further investigation.

Permeability of the Membrane to Water at Different Temperatures

Wang (8, 9) has shown that an increase in temperature increases the self-diffusion coefficient for water and decreases the viscosity coefficient for water. Each process has the same energy of activation of 4.4 to 4.6 kcal./mol.

A study of the effect of temperature on the permeability coefficient for the Ehrlich ascites tumor cell was undertaken to determine whether a similar energy of activation held for the passage of water across this cell membrane or whether other properties of the membrane were also involved.

Since what is being measured is the response of the permeability coefficient to a temperature change, and since this coefficient consists of several factors which contribute to it, and which will be discussed below, the energy of activation which is calculated will represent an integrated response of all of these.

The temperature of the mixing chamber was kept constant for the period of the measurement by maintaining a continuous flow of water in an outer chamber surrounding it. The cell suspension was brought to this temperature prior to the injection of the hypertonic (5.1 M NaCl) solution. The tem-

perature studied ranged from 10 to 37.2°C. Calibration curves between galvanometer deflection and cell volume were made at each of the different temperatures. This was necessitated not so much by possible changes in cell volume at the different temperatures as by alterations in the optical properties

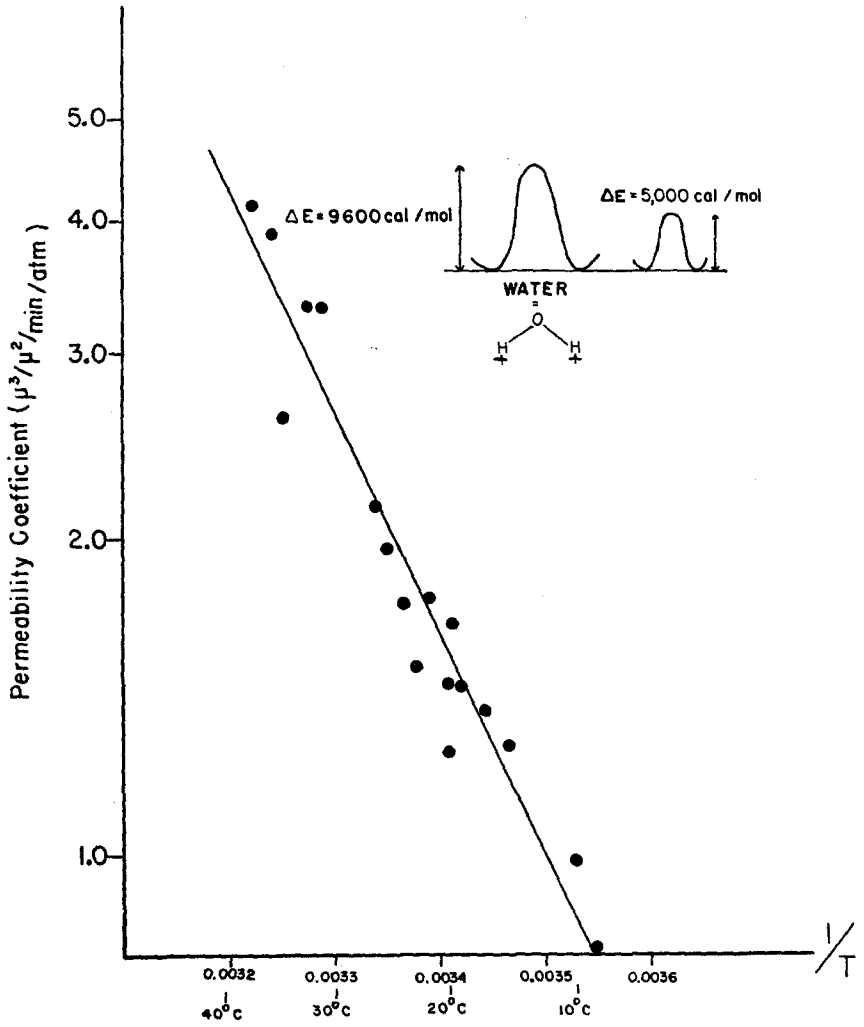

FIGURE 4. The effect of temperature on the permeability of the membrane of the Ehrlich ascites tumor cell to water. Heats of activation have been diagrammed as barriers and compared to the barrier for the diffusion of mannitol in ideal aqueous solutions.

of the recording system. This usually came about because different flow rates around the mixing chamber were necessary for different temperatures and in the process of making these adjustments, light transmissions altered, requiring, therefore, a recalibration of the recording system.

A preliminary experiment run at 17 and 33°C. indicated that there was no detectable volume change when the cell suspension at 25°C. was brought to either of these temperatures by mixing in the chamber. Measurements of cell volume showed a 4 per cent increase between 17 and 37°C.

The point has been emphasized because of the well known effect of temperature on the volume of the red blood cell, when decreases in temperature produce significant increases in volume. Permeability coefficients based upon hemolysis times are, therefore, strongly influenced by the initial volume of the cell (10, 11). This is not a problem here, not only because there is little rapid volume change of the tumor cell with temperature, but, also, because the permeability coefficient is calculated from an equation (Equation 1) which takes into account not the initial volume alone, but, rather, the ratio of equilibrium volume to initial volume. This ratio has been found to remain a constant for all the temperatures studied.

A summary of the effect of different temperatures on the permeability coefficient of the membrane to water is shown in Fig. 4. These data all refer to cells from late transplants; *i.e.*, those with a lower permeability coefficient to water.

The logarithm of the permeability coefficient in μ^3/μ^2/min./atm. is plotted against the reciprocal of the absolute temperature. The equation of the line, arrived at by a calculation of least squares is:

$$y = (-2.1 \times 10^3)x + 7.35 \tag{5}$$

in which

$$y = \log k_{2a} \quad \text{and} \quad x = 1/T$$

In terms of an Arrhenius type of interpretation,

$$k_{2a} = Ae^{-\mu/RT} \tag{6}$$

in which k_{2a} is the permeability coefficient for water, A is a constant, μ is the Arrhenius energy of activation, R is the gas constant, and T is the absolute temperature.

Since

$$\log k_{2a} = (-\mu/2.3R)1/T + \log A \tag{7}$$

then μ may be calculated from equation (5) and is equal to 9.6 kcal./mol. ± 0.02 (S.E.).

This initial calculation serves to indicate that the energy of activation for the movement of water across the Ehrlich ascites tumor cell membrane is more than double the value which would be predicted from the effect on the diffu-

sion coefficient and viscosity coefficient components of the permeability coefficient.

DISCUSSION

The permeability to water and the energy of activation for this process have been studied in several cells and tissues. Thus, Pappenheimer (12), studying the permeability of the capillary wall to water, found that the flow changes with temperature were compatible with the effect of temperature on the viscosity component of the Poiseuille resistance. Nevis (13) measured the effect of temperature on the efflux of THO from invertebrate peripheral nerve fibers and obtained an apparent activation energy of 3 to 5 kcal./mol. In the human red cell, Jacobs, Glassman, and Parpart (10) measured the effect of temperature on hemolysis times and an apparent energy of activation of 3.9 kcal./mol can be calculated from their data. In all these biological systems, the apparent energies of activation are not considerably different from those reported by Wang (8) for either the self-diffusion coefficient for water or for the viscosity coefficient for water.

In contrast, Lucké and McCutcheon (14) have reported energies of activation of 13 to 17 kcal./mol for the permeability of the *Arbacia* egg to water. We see also, from Fig. 4 that the Ehrlich ascites tumor cell falls into this same category. The apparent energy of activation of 9.6 kcal./mol is twice that found by Wang for the self-diffusion of water.

With regard to their permeability to water, these cells may be arranged in the order: erythrocyte > tumor cell > *Arbacia* egg cell. The reverse order holds when apparent energies of activation are compared: *Arbacia* egg cell > tumor cell > erythrocyte. These results can be explained by mechanisms proposed independently by Danielli (15) and Eyring (16). If the apparent energies of activation can be interpreted as barriers to the diffusing molecule and that to move along the diffusion path, the individual molecules must acquire sufficient energies to pass over the barrier, then, the more slowly the molecules permeate, the higher will be the apparent energy of activation.

There has been much discussion of the means by which an osmotic gradient brings about the net movement of water. Durbin, Frank, and Solomon (17) have reported for the frog gastric mucosa that an osmotic gradient produced a bulk flow of water in excess of that measured for the simultaneous diffusion of THO. These observations differed from those of Chinard (18) who has stated that all flows result from a diffusion of water molecules arising from activity gradients across a semipermeable membrane.

Chinard's thesis has been seriously questioned by Mauro (19) who found ratios of 700 to 1 between the osmotic permeability coefficient and the diffusion coefficient in coarse artificial membranes, and more recently by Robbins and Mauro (20).

At the level of the cell membrane, where the relative water content is low, one would expect to lose the distinction between bulk flow and diffusion flow. Nevertheless, Paganelli and Solomon (21), Sidel and Solomon (22), and more recently, Villegas, Barton, and Solomon (23), have used data of diffusion and osmotic movements of water across the red cell membrane to arrive at "effective pore sizes" ranging from 3.5 A in man to 7.4 A in the dog. Values such as these are compatible with the restrictive properties of the red cell membrane to non-electrolytes of small molecular size. However, the latter authors point out that differences in pore size will not account for differences in permeability to non-electrolytes exhibited by the several species.

Eyring has used the theory of absolute reaction rates to explain both the diffusion and viscous properties of water. To see how far one can apply Eyring's thesis to the ascites tumor cell, the total water movement across the tumor cell membrane will be assumed to come about through diffusion. This may be to some a radical assumption in view of the objections raised against Chinard's concept, but most of these objections have been based upon studies with membranes having pore radii greater than 20 A. Below 20 A, the observation that the osmotic permeability coefficient is greater than the diffusion permeability coefficient does not distinguish bulk flow from diffusion. It is evidence only that a continuum for water movement exists across the cell, *i.e.* that water-filled pores exist in the membrane, a construct with a wide acceptance. Thus Hodgkin and Keynes (24) proposed a membrane with long narrow pores to explain the interrelationship they found between the bidirectional fluxes for K in the *Sepia* axon, and noted the similarity of this phenomenon to water movements. Edwards and Harris (25) applied the model to water specifically and proposed that when discrepancies exist between tracer measurements and net water movements, one could interpret the results in terms of an alignment of water molecules in long narrow pores. This same point of view has been developed from the theory of irreversible thermodynamics by Kedem and Katchalsky (7) and more recently by Nims (26).

According to the theory of absolute reaction rates (16),

$$D = C\lambda^2 e^{-\frac{\Delta E}{RT}} e^{\Delta S^{\ddagger}/R} \tag{8}$$

in which D is the self-diffusion coefficient for water in cm.^{2}/sec.; C is a constant, equal to kTe/h in which k is Boltzmann's constant; T is absolute temperature, e is the natural logarithm, and h is Planck's constant. C has the dimensions of sec.$^{-1}$. The mean free path along the diffusion coordinate is λ, in centimeters; ΔE is the apparent energy of activation as obtained in Fig. 4. R, the universal gas constant has the dimensions of cal./mol/°A. The entropy change $\Delta S\ddagger$ associated with the formation of the activated complex, has the dimensions cal./mol °A (entropy units).

This equation has a useful function. It permits the calculation of $\Delta S\ddagger$, the

entropy of activation, which is a measure of the extent to which molecular alterations have occurred during the transfer process. With the choice of a reasonable value for λ of 1.5×10^{-8} cm., a value of D from Wang's paper of 2.59×10^{-2} cm.^{2}/sec.,[3] and the use of ΔE from Fig. 4, $\Delta S\ddagger$ may be calculated. At 23°C., C has the value of 0.621×10^{13} sec.$^{-1}$.

The value for $\Delta S\ddagger$ thus calculated is positive and equal to 39.1 cal./mol/°A.

The Significance of the Entropy of Activation for Water Movements

The entropy of activation for transfer of water across the ascites tumor cell membrane is approximately twice the entropy of activation which can be calculated with the use of the same diffusion coefficient, D, but with a ΔE from Wang's studies of 4.5 kcal./mol.

Two possible explanations for these results come to mind. The energy of activation, ΔE, of 9,600 cal./mol was calculated from the effect of temperature on the permeability coefficient for water. The permeability coefficient for water k_{2a}, as it derives from the equation of Lucké, Hartline, and McCutcheon has the dimensions of μ^3/μ^2/min./1 atm. osmotic pressure difference. It is related to the diffusion coefficient for water by the relation:

$$k_{2a} = D\left(\frac{A'}{\Delta x}\right)\left(\frac{C_w \bar{v}_{H_2O}}{A}\right)\frac{60}{10^{-4}} \tag{9}$$

in which D = diffusion coefficient in cm.^{2}/sec.; A = surface area, in cm.^{2}; $\bar{v}$, the partial molal volume for water, equal to 18.02 cm.^{3}/mol; and C_w the concentration difference for water associated with an osmotic effect of 1 atmosphere, equal to 41.0×10^{-6} moles/cm.^{3}; and $A'/\Delta x$ is the area to thickness ratio for the pathway for water movement, in centimeters.

One explanation is based on the tacit assumption that the diffusion properties of water remain unchanged during movement from one aqueous medium across the tumor cell membrane into another aqueous medium. The higher energies and entropies of activation would come about then through a change in the value of $A'/\Delta x$. The entropy change could reflect the structural alteration of pore geometry to permit a greater diffusing pathway for water. Structural changes would occur in the membrane which defines the pore.

A second explanation would take the alternate position that the value, $A'/\Delta x$, remains constant, and that D and its associate parameter, $\bar{v}_{H_2O}$, contribute to the temperature effect on the permeability coefficient, k_{2a}. The corollary to this would be that the structural properties of water as it exists in the membrane are different from the structural properties in an unconfined state. Because of the greater entropy change one would deduce that

[3] The dimensions of Equation 8 require that the diffusion coefficient be expressed in terms of a driving force of moles/cm.^{3} rather than the more frequently used driving force of moles/liter.

during diffusion the quasicrystalline water lattice in the membrane became less oriented and more random in structure. This second thesis would still include the pore construct but would focus attention on the interaction of the water dipoles with each other and with the components of the membrane.

A satisfactory answer will depend upon a determination of $A'/\Delta x$ which will not require a comparison between the osmotic and the tracer movements of water, since, as was pointed out above, this method also starts from ambiguous assumptions at pore sizes which approach those of the transported solvent.

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