

DIFFUSION AND MEMBRANE PERMEABILITY. I

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DIFFUSION AND MEMBRANE PERMEABILITY. I<sup>1, 2</sup>

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One of the basic physical phenomena for sustaining the growth and development of plants and organisms is that of diffusion. It has arrested the attention of investigators from the time of Fick (10) in 1855, who, drawing the analogy between conduction of heat and the transport of matter, was responsible for giving a quantitative formulation of the basic laws of diffusion. After some hundred

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years of investigation, there are aspects of the problem of diffusion which remain unexplainable. A detailed kinetic approach to diffusion is presented in the hope that it will clarify established concepts and provide impetus to a fresh approach to the existent problems in the field of biological diffusion. The absolute rate theory treatment of diffusion and membrane permeability provides a general unified point of view applicable to systems of varying degrees of complexity. It is equally adaptable to the treatment of the permeabilities of membranes to electrolytes and to non-electrolytes under the driving forces of a concentration gradient, an activity gradient, and external and internal potential gradients. Applications are made of the general equations derived to the permeabilities of egg cells of marine invertebrates and of plant cells to water and to non-electrolytes.

In Section I, Fick's first and second laws of diffusion for a two-component system are derived by use of difference equations. The general treatment of diffusion from the point of view of rate theory is given in Section II. For steady-state diffusion, the flux is defined by new equations which take explicit account of all types of potential barriers crossed by the migrating particles. The effect of external forces on the diffusing system is then taken up in Section III. In Section IV the general equations of Section II are simplified on the basis of assumed models of diffusing systems.

In Section V a somewhat detailed analysis is presented for determining the mechanism of the permeation process from studies on distribution coefficients and temperature coefficients. Section VI includes calculations of the various thermodynamic functions from permeability data.

#### I. FICK'S FIRST AND SECOND LAWS OF DIFFUSION

Molecular migration in condensed phases may be treated as point-to-point jumps of the elementary particles governed by a rate constant. The nature of the elementary jumps will show very many variations, depending on the nature of the diffusing components. It is instructive to analyze the various types of relaxations or jumps to see how they lead to the relations usually applied. We consider first a single two-component system with molecules which are sufficiently alike so that the whole may be thought of as forming a more or less perfect lattice.

In figure 1 we have a schematic potential diagram. If  $C_i$  is the concentration per cubic centimeter at the  $i^{\text{th}}$  position, then the amount of material in a square centimeter of cross-section and length  $\lambda$  (the distance between equilibrium minima in figure 1) is  $\lambda C_i$ . Let  $k$  represent the number of times per second a molecule jumps. At the steady state, let  $Q$  be the amount of  $C$  passing per second through a square centimeter of surface. Then

$$Q = k\lambda C_i - k\lambda C_{i+1} \quad (1)$$

The concentration gradient between the  $i^{\text{th}}$  and the  $(i + 1)^{\text{th}}$  position is

$$\frac{dC}{dx} = \frac{C_{i+1} - C_i}{\lambda} \quad (2)$$

Thus

$$Q = k\lambda(C_i - C_{i+1}) = -k\lambda^2 \left( \frac{C_{i+1} - C_i}{\lambda} \right) = -k\lambda^2 \frac{dC}{dx} \quad (3)$$

This is Fick's first law, ordinarily written as

$$Q = -D \frac{dC}{dx}$$

where  $D = k\lambda^2$ , a result derived earlier (9).

Fick's second law is obtained equally easily. Thus, the rate equation is

$$\frac{d(\lambda C_i)}{dt} = k(\lambda C_{i-1}) - 2k(\lambda C_i) + k(\lambda C_{i+1}) \quad (4)$$

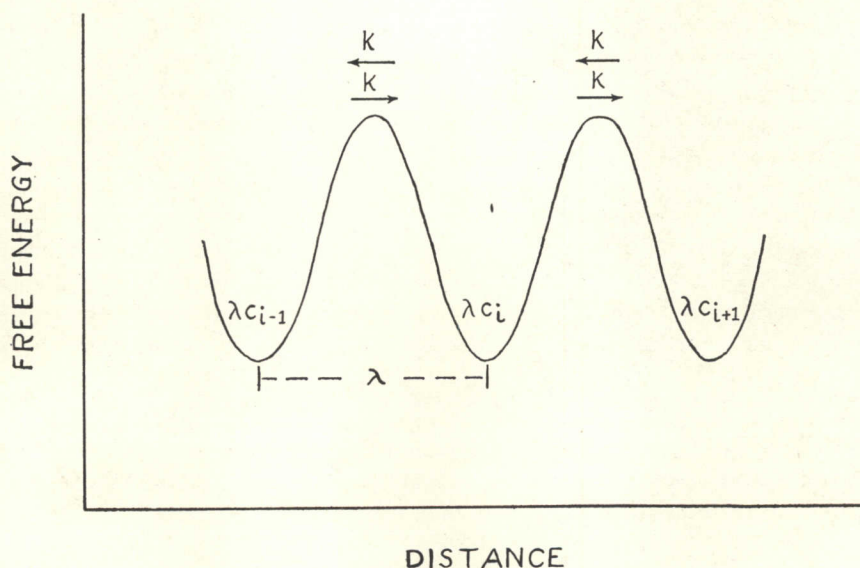


FIG. 1. Schematic potential energy profile

Here we have the rate of increase in number of molecules at the  $i^{\text{th}}$  position equal to the difference between the number of molecules jumping into the  $i^{\text{th}}$  position from the  $(i-1)^{\text{th}}$  and  $(i+1)^{\text{th}}$  positions and the number of molecules leaving the  $i^{\text{th}}$  position. Now, further, equation 3 gives the concentration gradient  $(C_{i+1} - C_i)/\lambda$  at a point halfway between point  $i$  and  $i+1$ . Similarly  $(C_i - C_{i-1})/\lambda$  is the concentration gradient halfway between the  $i^{\text{th}}$  and the  $(i-1)^{\text{th}}$  positions. The second derivative at the  $i^{\text{th}}$  position is then

$$\begin{aligned} \frac{d^2 C}{dx^2} &= \frac{1}{\lambda} \left( \frac{C_{i+1} - C_i}{\lambda} - \frac{C_i - C_{i-1}}{\lambda} \right) \\ &= \frac{C_{i+1} - 2C_i + C_{i-1}}{\lambda^2} \end{aligned} \quad (5)$$

Rearranging equation 4, we obtain:

$$\frac{dC_i}{dt} = k\lambda^2 \left( \frac{C_{i-1} - 2C_i + C_{i+1}}{\lambda^2} \right) = k\lambda^2 \frac{d^2 C_i}{dx^2} \quad (6)$$

It might be pointed out that the approximations made are valid only in the limit where

$$\lim_{\lambda \rightarrow 0} \left( \frac{\Delta C}{\lambda} \right) = \frac{dC}{dx}$$

This implies that a smooth continuous resistance is offered to the diffusing molecule, which may only be approximately true in the case of the thin natural membranes (50–150 Å.) whose complex structure of large protein and lipid molecules

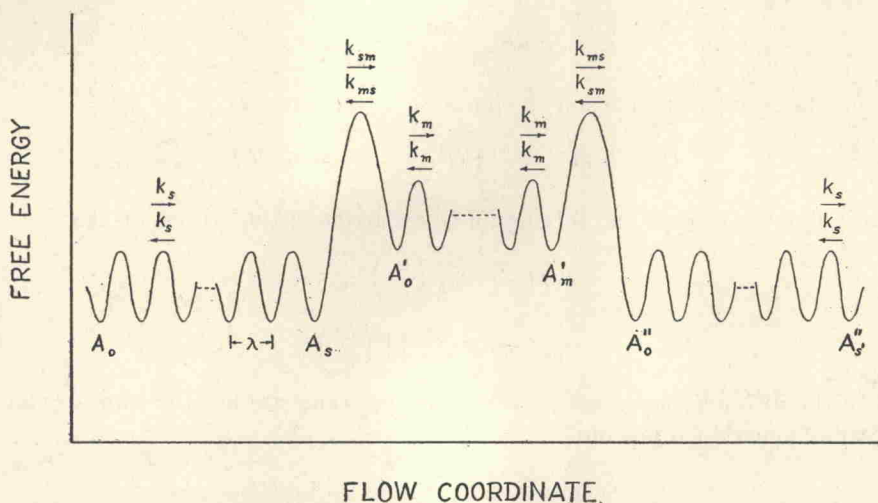


FIG. 2. An energy profile curve in which free energy is plotted against the flow coordinate may offer an irregular resistance to flow. The latter point is implicit in the derivation in the following section.

## II. GENERAL TREATMENT OF DIFFUSION AND PERMEABILITY

By regarding the flow of molecules as a series of successive jumps from one equilibrium position to another, diffusion in membranes and their permeabilities are readily treated. In figure 2 an energy profile curve is constructed where free energy is plotted against the flow coordinate. Although this schematic energy diagram as drawn represents diffusion through a heterogeneous three-layer membrane or transport of matter through a solution-membrane-solution system, we shall generalize and assume a diffusion system where all the rate constants for forward and reverse flow are unequal. Concentration at the various minima  $A_i$  is defined as concentration of particles per square centimeter cross-section of thickness  $\lambda$  in the direction of flow. The specific rate constant in the forward

direction for  $A_i$  is defined as  $k_i$ . Similarly,  $k'_i$  is the specific rate in the  $(-x)$  direction from the equilibrium position  $A_i$ . It is to be noted that  $A_i = \lambda_i C_i$ . For steady-state conditions the flux at the various maxima is given by the set of rate equations:

$$\begin{aligned} Q &= k_0 \lambda C_0 - k'_1 \lambda C_1 \\ Q &= k_1 \lambda C_1 - k'_2 \lambda C_2 \\ &\dots\dots\dots \\ Q &= k_n \lambda C_n - k'_{n+1} \lambda C_{n+1} \end{aligned} \quad (7)$$

Here the flux  $Q$  represents the net flow of material through a unit cross-sectional area per second. We are assuming an ideal system and also taking all the  $\lambda$ 's, the distances between the various minima, to be equal. Solving the second equation for  $C_1$  and substituting in the first equation one obtains:

$$Q = k_0 \lambda C_0 - \frac{k'_1}{k_1} [Q + k'_2 \lambda C_2]$$

Then using the third equation to eliminate  $C_2$  one obtains:

$$Q = k_0 \lambda C_0 - \frac{k'_1}{k_1} \left[ Q + \frac{k'_2}{k_2} (Q + k'_3 \lambda C_3) \right]$$

Continuing the process and solving for  $Q$ , we arrive at the expression that

$$Q = \frac{k_0 \lambda C_0 - \prod_{i=1}^n \left( \frac{k'_i}{k_i} \right) k'_{n+1} \lambda C_{n+1}}{1 + \sum_{r=1}^n \prod_{i=1}^r \left( \frac{k'_i}{k_i} \right)} \quad (8)$$

When the distances  $\lambda_i$  between equilibrium positions are not the same, we have the more general expression:

$$Q = \frac{k_0 \lambda_0 C_0 - \prod_{i=1}^n \left( \frac{k'_i \lambda'_i}{k_i \lambda_i} \right) k'_{n+1} \lambda'_{n+1} C_{n+1}}{1 + \sum_{r=1}^n \prod_{i=1}^r \left( \frac{k'_i \lambda'_i}{k_i \lambda_i} \right)} \quad (9)$$

From the theory of absolute rate processes, a specific reaction rate constant for any process is given by

$$k' = \kappa \frac{kT}{h} e^{-\Delta F^\ddagger / RT}$$

where  $\kappa$  is the transmission coefficient, and  $kT/h$  is a frequency factor involving the Boltzmann constant,  $k$ , the absolute temperature,  $T$ , and  $h$ , Planck's constant. If we assume the transmission coefficients to be unity, which appears to be true for most processes, equation 8 simplifies to

$$Q = \frac{\frac{kT}{h} \lambda \left[ e^{-\Delta F_0^\ddagger / RT} C_0 - e \exp \left[ - \sum_{i=1}^n (\Delta F_i'^\ddagger - \Delta F_i^\ddagger) + \Delta F_{n+1}'^\ddagger / RT \right] C_{n+1} \right]}{1 + \sum_{r=1}^n e \exp \left( - \sum_{i=1}^r (\Delta F_i'^\ddagger - \Delta F_i^\ddagger) / RT \right)} \quad (10)$$

The rate of movement of matter is thus governed by the relative heights of the potential barriers, as given explicitly in equation 10. Since the specific rate constants enter as ratios, it is the difference in the free energies of activation for the individual unit processes of flow which will determine the flux.

### III. EFFECT OF EXTERNAL FORCES

How external forces act on a kinetic system can readily be incorporated into our general expression for the transport of material under steady-state conditions. The procedure to be followed has been successfully employed in interpreting rate phenomena such as plastic flow, creep, viscosity, relaxation of dielectrics, conductance in solution, and other related phenomena (11).

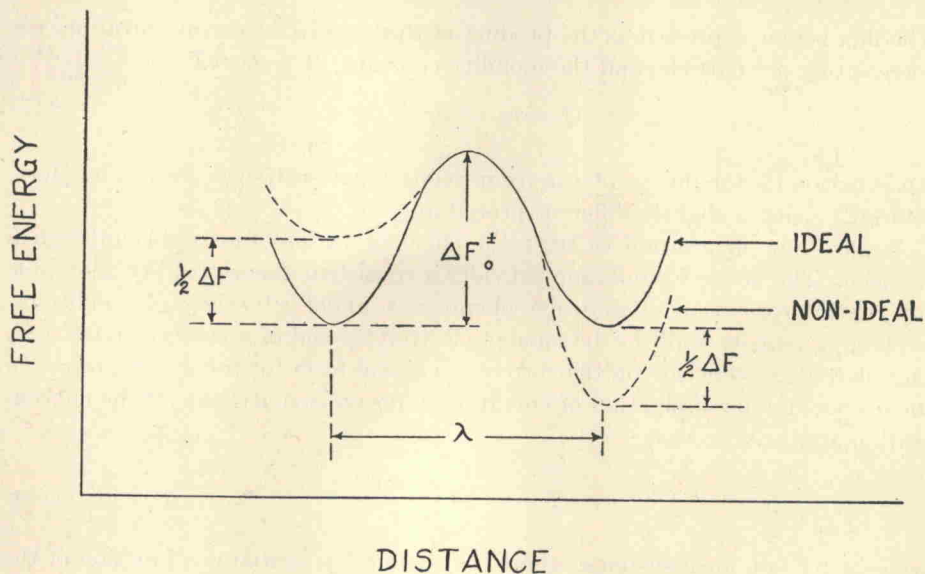


FIG. 3

For simplicity of treatment, consider a two-component diffusing system governed by a single unimolecular rate constant. The basic assumption is that the external force acting on a single unit process simply provides an additional amount of work  $W$ , which will tend to aid or hinder the process by increasing or decreasing the free energy of the initial and final positions. Assuming a symmetrical potential barrier as diagrammed in figure 3, the work done is given by the force  $f$  acting only through the distance  $\lambda/2$  between the initial and the activated states. The net linear velocity in terms of the specific rates for the forward and reverse steps is

$$v = \lambda(k_f - k_b) = k_0 \lambda (e^{W/kT} - e^{-W/kT}) \quad (11)$$

This equation can also be written in the form

$$V = \lambda k_0 2 \sinh (W/kT) \quad (12)$$

With the exception of plastic flow and creep in high-polymeric materials, it is usually found that  $W \ll kT$ . Under these conditions, it is permissible to a good approximation to retain only the first term in the power series expansion of the hyperbolic sine function, thus:

$$v \cong \lambda k_0 \left( \frac{2W}{kT} \right) \quad (13)$$

With our definition of the work term  $W$ , the above equation is readily generalized to take into account the various forces acting on the diffusing particle:

$$v = \frac{k_0 \lambda^2}{kT} \sum_i f_i = \frac{D_0}{kT} \sum_i f_i \quad (14)$$

The flux is now expressed as the product of three terms—the concentration, the force acting per particle, and the mobility constant,  $B = D_0/kT$ .

$$Q = vc = Bc \sum_i f_i \quad (15)$$

An equation for the flux similar in form to our equation 15 was derived by Herzfeld (12), using a slightly different procedure.

We are now in position to treat the effect of various forces encountered in diffusion. The above formulation provides a consistent theory readily adaptable for the interpretation of diffusion phenomena under a variety of conditions, explaining equally well the diffusion of electrolytes and of non-electrolytes. It is only necessary to decide on the correct analytical form for the driving force. In ideal systems, the dissipation of energy in diffusion is controlled by the concentration gradients so that

$$f = -kT \frac{d \ln c}{dx} \quad (15a)$$

whereas for non-ideal systems, where the chemical potential is a function of the activity  $a$ , we have:

$$f = -kT \frac{d \ln a}{dx} \quad (15b)$$

For the diffusion of charged particles of charge  $Z_i$  under a potential gradient  $\varphi$ , the force is

$$f_i = eZ_i \varphi \quad (15c)$$

Let us consider the diffusion of electrolytes in greater detail. If we assume that both the activity gradient and the potential gradient define the transport of material, then combining equations 15b and 15c with equation 15, the net transport of ions of the  $i^{\text{th}}$  species is

$$Q = D_{0i} \left\{ \frac{eZ_i \varphi_i C_i}{kT} - \frac{C_i d \ln a_i}{dx} \right\} \quad (16)$$



or it may be written

$$Q = C_i \left\{ \frac{\Lambda_i \varphi_i}{F} - D_{0i} \frac{d \ln a_i}{dx} \right\} \quad (16a)$$

where  $\Lambda_i$  is the equivalent conductance at infinite dilution and  $F$  is the faraday. Where  $C_i \neq f(x)$ , then  $C_i d \ln a_i/dx = C_i d \ln \gamma_i/dx$  and only the activity coefficient gradient is determining. Certain cases of "active diffusion" as found in biological systems are interpretable by means of the above equation. If the potential gradient term is dominant, diffusion of ions against a concentration gradient may be expected. By extension of equation 16, the Nernst-Planck equation for the diffusion of electrolytes as also the equation for the diffusion potential are obtained, as shown by Stearn and Eyring (21).

The above treatment for the effect of external forces can now be extended to the general expression for the flux under steady-state conditions where the individual unit rate processes are governed by their own specific rate constants. The rate constants operative in the system under the influence of external forces are defined as follows in terms of the general work term  $W_i$ . For the forward rate, we have

$$k_i = k_{0i} e^{W_i/kT} \quad (17)$$

and similarly for the reverse process (a symmetrical barrier assumed)

$$k'_i = k'_{0i} e^{-W_i/kT} \quad (17a)$$

where the  $k_{0i}$  and  $k'_{0i}$  represent the specific rate constants for the ideal system. If the equilibrium distances,  $\lambda_i$ , are taken to be equal, then  $W_i = W'_i$ , and equation 9 for the flux under the effect of external forces is

$$Q = \frac{k_{00} \lambda e^\alpha C_0 - \prod_{i=1}^n \left( \frac{k'_{0i}}{k_{0i}} \right) k'_{0, n+1} \lambda e^{-(2i+1)\alpha} C_{n+1}}{1 + \sum_{r=1}^n \prod_{i=1}^r \left( \frac{k_{0i}}{k'_{0i}} \right) e^{-2i\alpha}} \quad (18)$$

where  $\alpha = W/kT$ . In the simplest case, where all the specific rate constants are equal, we obtain the result that

$$Q = \frac{k_0 \lambda \left[ e^\alpha C_0 - \prod_{i=1}^n e^{-(2i+1)\alpha} C_{n+1} \right]}{1 + \sum_{r=1}^n \prod_{i=1}^r e^{-2i\alpha}} \quad (19)$$

The second term in the denominator is a geometric series which is readily summed and after suitable factoring, our final expression is

$$Q = 2k_0 \lambda \sinh(\alpha) \left[ \frac{1}{1 - e^{-2\beta}} + \frac{C_{n+1}}{C_0 - C_{n+1}} \right] (C_0 - C_{n+1}) \quad (20)$$

where  $\beta = (n + 1)\alpha$  and  $(n + 1)$  is the number of molecular jumps along the flow coordinate ( $+x$ ).

For large forces and thick films, i.e.,  $\alpha \sim 1$  and  $n \gg 1$ , respectively, the flux is simply

$$Q = 2k_0\lambda C_0 \sinh \alpha \quad (21)$$

When the forces are large, the reverse flow over the potential barrier becomes negligible and the net transport is thus independent of the thickness of the membrane and the final concentration. The second case of small external forces and thin films is of greater practical interest. Here, since  $\alpha \ll 1$  and  $\beta = (n+1)\alpha < 1$ , we can replace both the exponential function and the  $\sinh \alpha$  by the respective first terms in power series expansions to obtain from equation 20 that

$$Q \cong \frac{k_0\lambda^2}{(n+1)\lambda} \left\{ 1 + 2(n+1)\alpha \left( \frac{C_{n+1}}{C_0 - C_{n+1}} \right) \right\} (C_0 - C_{n+1}) \quad (22)$$

The permeability constant is then

$$P = \frac{D_0}{\delta} \left\{ 1 + 2(n+1)\alpha \left( \frac{C_{n+1}}{C_0 - C_{n+1}} \right) \right\} \quad (23)$$

where  $\delta = (n+1)\lambda$  is the film thickness. It follows that the diffusion coefficient for the non-ideal case is

$$D = D_0 \left\{ 1 + \frac{\delta f}{kT} \left( \frac{C_{n+1}}{C_0 - C_{n+1}} \right) \right\} \quad (24)$$

Now if we assume that the force is given by the activity coefficient gradient, then

$$D = D_0 \left\{ 1 - \frac{\delta C_{n+1}}{(C_0 - C_{n+1})} \frac{d \ln \gamma}{dx} \right\} \quad (25)$$

However,

$$\frac{d \ln C}{dx} = \frac{1}{C} \frac{dC}{dx} = \frac{C_{n+1} - C_0}{C_{av} \delta} \cong \frac{C_{n+1} - C_0}{\delta C_{n+1}}$$

thus,

$$D = D_0 \left\{ 1 + \frac{d \ln \gamma}{d \ln C} \right\} = D_0 \frac{d \ln a}{d \ln C} \quad (26)$$

The same thermodynamic correction to the diffusion coefficient in non-ideal cases was obtained by Onsager and Fuoss (20) and by Stearn, Irish, and Eyring (22).

#### IV. MODELS FOR DIFFUSING SYSTEMS

We may now choose to consider specific models for the diffusion systems in order to arrive at equations directly applicable to the treatment of permeability data for actual systems encountered in practice. Two cases will be considered.

*Case I:* Here all the rate constants are taken to be equal, so that we have the condition

$$k_0 = k_1 = k_2 = \dots = k_n = k'_1 = k'_2 \dots = k'_{n+1}$$

In our general equation 8 for the flux, the terms have the values

$$\prod_{i=1}^n \left( \frac{k'_i}{k_i} \right) = 1$$

and

$$\sum_{r=1}^n \prod_{i=1}^r \left( \frac{k'_i}{k_i} \right) = n$$

so that the equation reduces to

$$Q = k_0 \lambda / (n + 1) \cdot (c_0 - c_{n+1}) \quad (27)$$

The distance along the flow coördinate between the measured concentrations is  $\lambda(n + 1)$ , so that we write

$$Q = \frac{D}{\delta} \cdot (c_0 - c_{n+1}) \quad (28)$$

This is Fick's first law of diffusion for an ideal system, applicable equally well to diffusion in continuous media and through thin membranes.

*Case II:* The schematic two-dimensional potential-energy diagram for the model to be considered here is given in figure 2. This energy profile curve represents diffusion through a solution-membrane-solution system or through a composite three-layer membrane. Animal and plant cell membranes are essentially semirigid structures with proteins and phospholipoids as the main chemical building units. An excellent discussion of their structure and composition is presented in the recent text by Davson and Danielli (7). The model which is being considered corresponds to the general pattern of the cell membrane structure as proposed by Danielli (7, p. 64).

In our general equation 8 for the flux,  $c_0$  and  $c_{n+1}$  are the initial and final concentrations of the diffusing substance in the solutions on each side of the membrane. These concentrations when multiplied by  $\lambda$  correspond to the  $A_0$  and  $A'_s$  terms shown in figure 2. The diffusing system is characterized by four specific rate constants defined as follows:

$$\begin{aligned} k_s &= \text{constant for diffusion in solution,} \\ k_m &= \text{constant for diffusion in the membrane,} \\ k_{sm} &= \text{constant for diffusion through the solution-membrane interface,} \\ k_{ms} &= \text{constant for diffusion through the membrane-solution interface,} \end{aligned}$$

while all the remaining rate constants in the respective phases are equal. Further, let

$$\begin{aligned} s &= \text{number of jumps in solution on the fore side of the membrane,} \\ s' &= \text{number of jumps in solution on the back side of the membrane, and} \\ m &= \text{number of jumps in the membrane itself.} \end{aligned}$$

The total number of jumps along the flow coordinate  $n + 1 = s + m + s' + 2$ , the term 2 arising from the two solution-membrane interfaces. Since with the exception of the four specific rate constants the remaining constants are equivalent in their respective phases, the terms for both numerator and denominator in equation 8 are readily evaluated. Thus, in the numerator, we have

$$\prod_{i=1}^n \left( \frac{k'_i}{k_i} \right) = 1$$

and the denominator expands to

$$1 + \sum_{r=1}^n \prod_{i=1}^r \left( \frac{k'_i}{k_i} \right) = s + s' + 2 \frac{k_s}{k_{sm}} + m \frac{k_s k_{ms}}{k_{sm} k_m}$$

Therefore, it follows that

$$Q = \frac{k_s \lambda (c_0 - c_{n+1})}{(s + s' + 2k_s/k_{sm} + mk_s k_{ms}/k_{sm} k_m)} \quad (29)$$

where the permeability constant is now given by

$$P = \frac{k_s \lambda}{(s + s' + 2k_s/k_{sm} + mk_s k_{ms}/k_{sm} k_m)} \quad (30)$$

Other complex systems can be as readily treated as the model given for case II. We may choose to consider explicitly the adsorption and desorption of the diffusing component on the inner and outer walls of the cell membrane, which essentially consists in introducing two additional rate constants or parameters for the processes of adsorption and desorption.

#### V. APPLICATIONS TO MEMBRANE PERMEABILITIES

In the last section, an expression for the permeability constant (equation 30) was derived for a diffusing system characterized by four rate constants. Further consideration of the nature of this equation is necessary to determine under what set of conditions it is applicable.

If we limit ourselves to systems where the interior and exterior solutions bathing the membrane are of a sufficiently low viscosity (i.e., aqueous solutions), the main resistance to diffusion will be offered either by the interfaces or by the membrane proper. Since the diffusion constant in solution is several magnitudes larger than the constants associated with the membrane, the permeability constant takes the simpler form of

$$P = \frac{k_{sm} k_m \lambda}{2k_m + mk_{ms}} \quad (31)$$

The above expression will take on several different forms depending primarily on the relative magnitudes of the three specific rate constants. The derivation of this quantity is essentially based on three assumptions: (a) steady-state conditions, (b) no dependence of  $P$  on concentration, and (c) a homogeneous membrane. In its application to natural membranes, the parameter  $m$ , related to the

membrane thickness,  $\delta$ , is a constant, so that the only true variable is temperature and the only experimentally determined quantity is the permeability. As a result of the limited number of variables, an evaluation of the separate rate constants is not possible; however, a limited analysis for the determination of the rate-determining step in the transport of matter can be carried out by utilizing temperature coefficients and distribution coefficients for the diffusing component.

The distribution or partition coefficient,  $K$ , is defined as the ratio of the rate constants for diffusion through the solution-membrane interface, i.e.,  $K = k_{sm}/k_{ms}$ . Introducing the distribution coefficient and rearranging equation 31, we have

$$\frac{1}{P} = \frac{2}{k_{sm}\lambda} + \frac{m}{k_m\lambda K} \quad (32)$$

which can be written in the more compact form

$$\frac{1}{P} = \frac{2\lambda}{D_{sm}} + \frac{\delta}{D_m K} \quad (33)$$

where  $\delta \cong \lambda m$ . The membrane thickness and the diffusion coefficients follow from our previously derived relation that  $D_i = k_i\lambda^2$ . The use of this relation with the distribution coefficient considered as a variable can be utilized for determining the slow step in diffusion only for certain defined classes of compounds. It requires that the permeability of a membrane to a series of compounds is primarily determined by their distribution coefficients while the variations in their specific rate constants  $k_{sm}$  and  $k_m$  are relatively small. This condition may be fulfilled by the lower members of certain homologous series of compounds. If the latter is true, a graphical plot of  $1/P$  vs.  $1/K$  will establish the rate-determining step. Equation 33 may also be written as

$$\frac{1}{P} = A + \frac{B}{K} \quad (34)$$

where  $A$  and  $B$  are regarded as constants.<sup>4</sup>

For natural membranes bathed by aqueous solutions, the distribution coefficient for most non-electrolytes is much less than unity. With this added condition that  $k_{sm} \ll k_{ms}$ , three cases can be distinguished, namely,

- I.  $k_m \ll k_{sm} \ll k_{ms}$
- II.  $k_m \gg k_{ms} \gg k_{sm}$
- III.  $k_m \approx k_{ms} \gg k_{sm}$

The fourth possibility that  $k_m \approx k_{sm} \ll k_{ms}$  is highly unlikely, in view of the contrasting difference in the structure and composition of membrane materials as compared with that of aqueous solutions, making the relation  $k_m \approx k_{sm}$  seldom if ever true.

<sup>4</sup> No improvement in the rectilinearity of the plots was obtained by correcting for molecular size on the basis of the Einstein-Stokes equation for diffusion, i.e., plotting  $1/PV_m^{1/3}$  against  $1/K$ , where  $V_m$  is the molar volume.

In the first case where the rate-determining step is diffusion in the membrane, plot of  $1/P$  vs.  $1/K$  for a series of related compounds will give rise to a straight line through the origin, for the constant  $A$  is negligible. The permeability constant is then given by the relation

$$P = \frac{KD_m}{\delta} \quad (35)$$

For the second case, the slow step is diffusion through the solution-membrane interface. A series of compounds characterized by this mechanism will exhibit very closely a horizontal line for the  $1/P$  vs.  $1/K$  plot, since  $B$  is very small. The equation of this line is then

$$P = \frac{D_{sm}}{2\lambda} \quad (36)$$

When the transport of material is regulated by diffusion through the interface, the permeability constant will be found to be independent of the distribution coefficient and membrane thickness. Unfortunately a direct test of this relation for natural membranes presents almost insurmountable experimental difficulties. In the third case, the rate-determining step is again diffusion through the solution-membrane interface modified to the extent that the rate constants for diffusion in the membrane and through the membrane-solution interface are equal. Plotting  $1/P$  vs.  $1/K$  gives rise to a straight line with an intercept on the ordinate axis. Since  $k_m = k_{ms}$ , the permeability constant is given by

$$P = k_{sm} \frac{\lambda}{m + 2} = D_{sm} \frac{\lambda}{\delta} \quad (37)$$

Here, in contrast to case II as represented by equation 36, the permeability constant is a function of the membrane thickness.

The data available in the literature on the permeability of natural membranes are fairly inadequate to give a conclusive answer as to the reliability of the above procedure based on distribution coefficients for determining the mechanism of the permeation process. An extensive series of measurements involving forty-five organic compounds were carried out by Collander and Bärilund (4) on the plant cell *Chara ceratophylla*. This work was extended by G. Marklund (18) to nine other plant cells. Unfortunately, only a few cases could be picked where the compounds (more than two in number per class) were sufficiently chemically and physically related to permit the above analysis to be carried out.

In figure 4 the required plot of the function  $1/P$  vs.  $1/K$  is given for the aliphatic amides for two specimens,—the plant cell *Chara* and the marine eggs *Arbacia*. Following the suggestion of Collander and Bärilund, the olive oil-water partition coefficients were used as a substitute for the membrane-solution coefficients. This plot is characteristic of case III, where  $k_m \approx k_{ms} \gg k_{sm}$ , i.e., the slow step is diffusion across the solution-membrane interface. The linearity of the plots indicates that the various rate constants  $k_m$ ,  $k_{ms}$ , and  $k_{sm}$  are fairly constant for the members of this series of compounds. On this assumption,

Marklund's data for several plant cells are given in figure 5. With the exception of the three plant cells *Oedogonium*, *Pylaiella*, and *Melosira*, whose plots appear to pass through the origin as required in case I, the remaining six plants appear to have the same mechanism of permeation as the plant cell *Chara*. The polyhydroxy alcohols ethylene glycol, glycerol, and erythritol offer another class for comparison. With the exception of the plant cell *Melosira*, given in figure 6, the  $1/P$  vs.  $1/K$  plots for the other eight plant specimens studied by Marklund exhibit a marked curvature; however, they seem to approximate case I ( $k_m \ll$

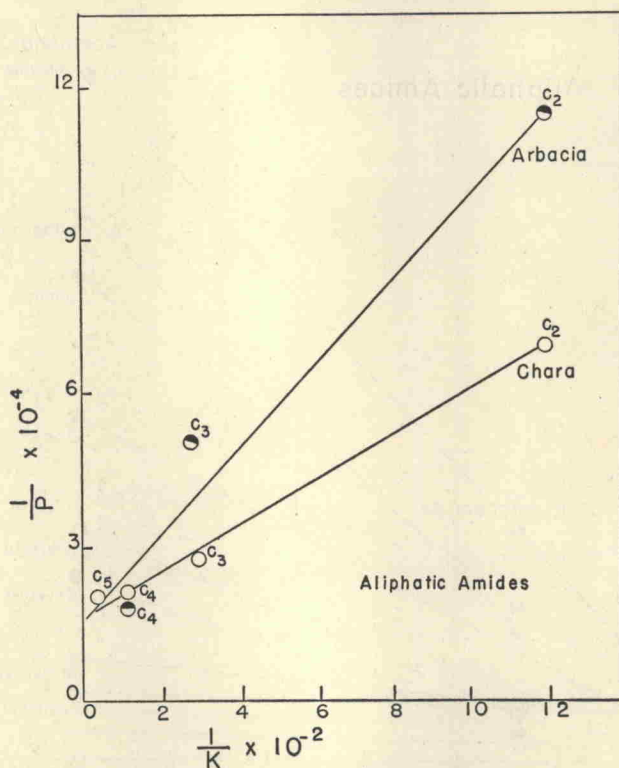


FIG. 4. Plot of the function  $1/P$  versus  $1/K$  for aliphatic amides for *Chara* and *Arabacia*

$k_{sm} \ll k_{ms}$ ), with the curve passing through the origin indicating that diffusion in the membrane proper is the regulating step.

Of the remaining compounds studied by Collander and Bärlund, about twelve classes could be differentiated where the compounds were sufficiently alike to permit an analysis. Inasmuch as only two compounds were found per class, the data are too meager to warrant any conclusions. Typical plots are demonstrated by the alkyl-substituted ureas in figure 7. These compounds, like the polyhydroxy alcohols, appear to belong to case I. Of the twelve cases studied, they appeared to be evenly distributed as belonging to either case I or case III. Urethylan and urethan (methyl and ethyl esters of carbamic acid) were the only compounds

belonging to case II, i.e.,  $k_{sm} \ll k_{ms} \ll k_m$ , where diffusion through the solution-membrane interface is the slow step. Further work is required before an unequivocal answer can be given. More precise measurements of olive oil-water partition coefficients are badly needed, or, perhaps, in view of the indefiniteness of olive oil as a solvent regarding its origin, uniformity, composition, and purity,

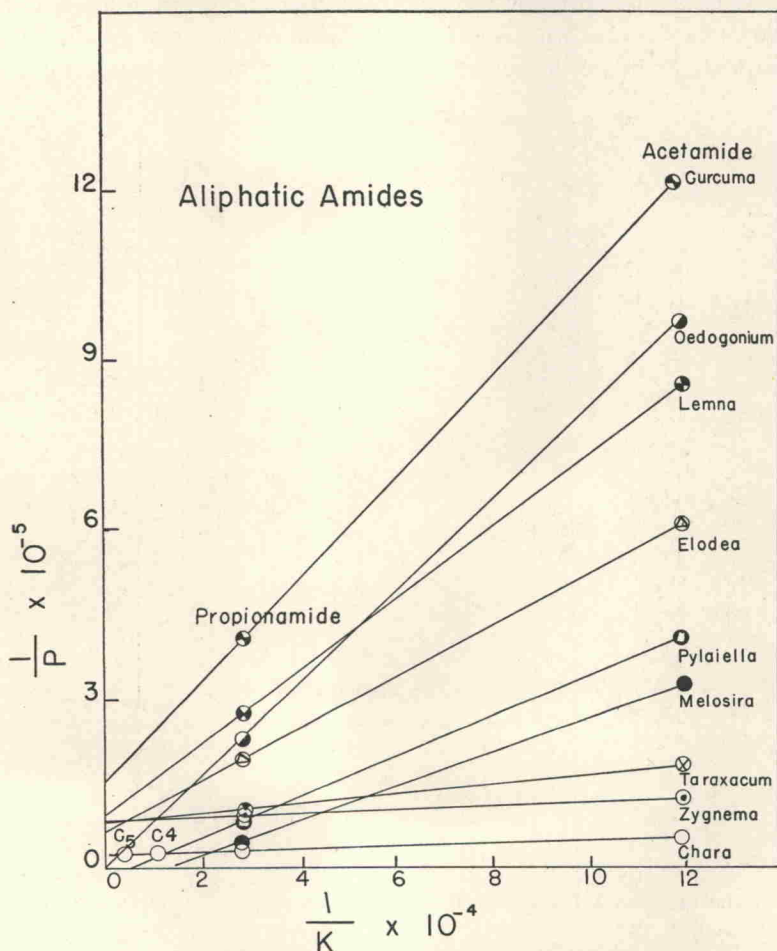


FIG. 5. Plot of Marklund's data for several plant cells

a better solvent should be recommended to replace the solution-membrane partition coefficients.<sup>5</sup>

Danielli (6) derived expressions for the permeability constant similar in form to equations 35 and 36, for the two distinct rate-determining steps in the mechanism of permeation. For very rapidly penetrating molecules, he assumes that the rate-determining step is diffusion through the membrane as given by equation 35, whereas for slowly penetrating molecules, the principal barrier to be sur-

<sup>5</sup> Meyer (19) recommended oleyl alcohol as a model substance in preference to olive oil.



mounted by the diffusing particle is presented by the solution-membrane interface, so that the correct form for the permeability constant is given by equation 36. The calculations carried out to prove this point appear to be questionable (8). There is no *a priori* reason for differentiating between rate-determining steps on the basis of the magnitudes of the permeability constants; only the relative values of the specific rate constants involved decide on the form of the equation

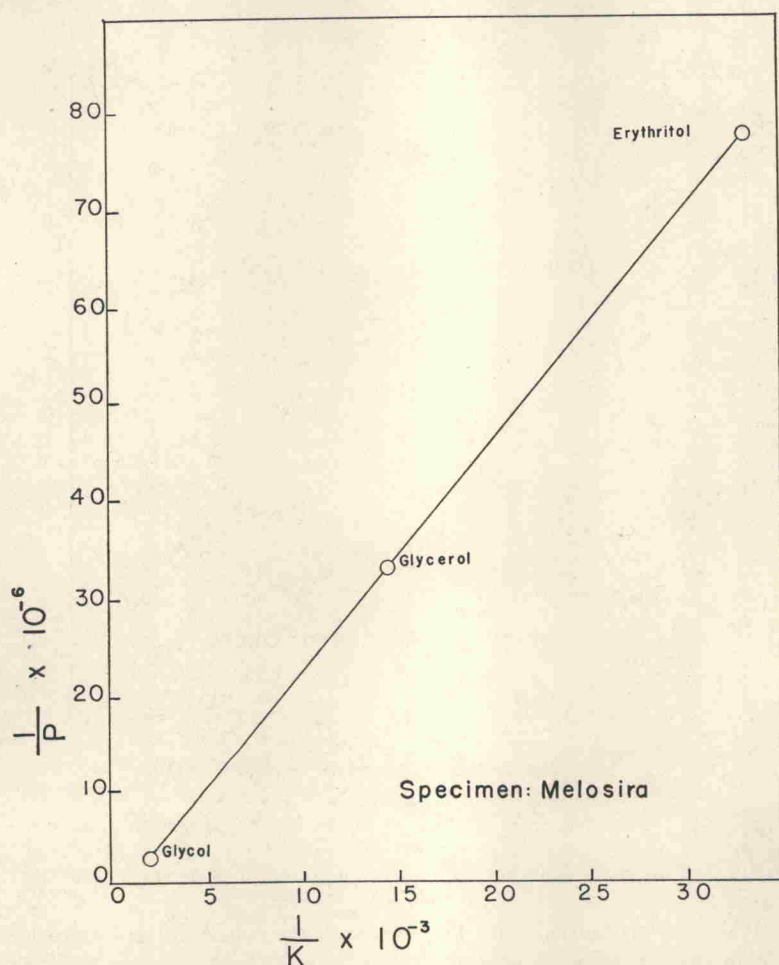


FIG. 6. Plot of the function  $1/P$  versus  $1/K$  for polyhydroxy alcohols and *Melosira*

for the permeability constant. The analysis carried out on the basis of the distribution coefficients, though sketchy in nature, appears to justify this point. Also it was found for the limited number of compounds investigated that no relation can be drawn between the magnitude of permeability constants and the mechanism of permeation.

Temperature coefficients do not provide a ready solution to the problem of

the mechanism of permeation. If we disregard for the moment the question of reversibility of the membrane structure to changes in temperature, the following

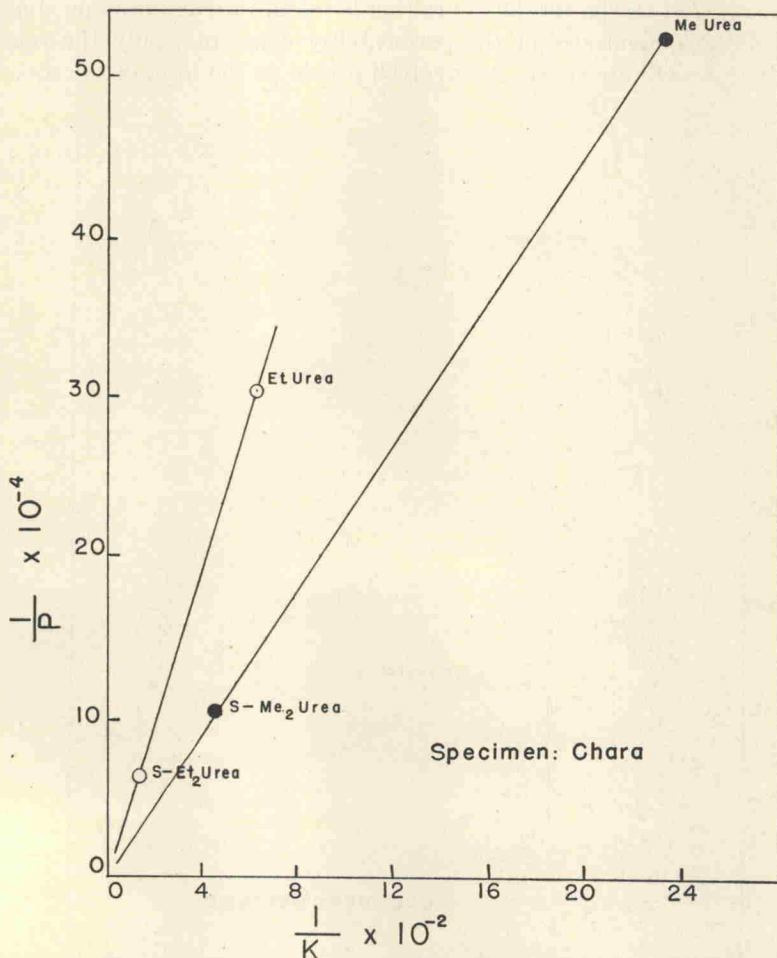


FIG. 7. Plot of the function  $1/P$  versus  $1/K$  for alkyl ureas and *Chara*

limited analysis can be carried out. The specific rate constants are expressed in the usual form:

$$k_i = \frac{kT}{h} e^{-\Delta F_i^\ddagger/RT}$$

To determine the heats of activation,  $\Delta H^\ddagger$ , the function  $\ln (Ph/kT)$  is plotted against the reciprocal of absolute temperature. For the three limiting cases discussed in relation to the distribution coefficients, as exemplified by equations 35, 36, and 37, a straight-line relation will be obtained between  $\ln (Ph/kT)$  and the reciprocal of absolute temperature. A differentiation of the three cases on the

basis of the magnitude of the slopes, i.e., the heats of activation, is also not possible; thus temperature coefficients are of little aid for elucidating the mechanism of permeation. In one particular case a temperature study would be of help. Consider the case that  $k_m > k_{ms} \gg k_{sm}$ , which is a modification of our third case where  $k_m$  and  $k_{ms}$  were taken to be equal. In this case the original expression for the permeability constant applies as given by equation 31. The effect of an extra specific rate constant in the denominator would be to destroy the linearity between the  $\ln(Ph/kT)$  vs.  $1/T$  relation. The plot would exhibit a definite concave curvature to the abscissa axis, especially in the lower temperature range.

#### VI. ANALYSIS OF DATA

The data which have been selected for analysis pertain to the permeabilities of the cell walls of the eggs of several marine invertebrates, including *Arbacia punctulata* (a sea urchin), *Chaetopterus pergamentaceus* (a marine annelid), and *Cumingia tellenoides* (a mollusc). The egg cells of these marine animals, together with the plant cell *Chara ceratophylla*, represent a class of specimens whose permeabilities are determined primarily by a thin membrane enveloping the cell. In the case of the plant cell *Chara*, the rigid cellulose wall is very permeable to most substances, and it is the exterior plasma membrane of the cytoplasm which offers the main resistance to transport of material into and out of the cell sap or vacuole.

The permeabilities of these marine specimens to water and various non-electrolytes<sup>6</sup> represent primarily the studies of Lucké (14, 15, 16), Jacobs (24), and their collaborators.<sup>7</sup> Their measurements on water permeability were carried out by observing the changes in the diameter of the spherical egg cells during swelling when placed in hypotonic solutions of sea water. Jacobs (13) developed a special method for determining permeability values for the non-electrolytes. The eggs, initially in sea water, are placed in sea water made hypertonic by addition of the non-electrolyte. Under these conditions the cell reaches a minimum volume from which the permeability constants for the non-electrolyte, as also that for water, can be calculated. Since the cells of the plant *Chara ceratophylla* are sufficiently large, Collander and Bärlund (3, 4) used a direct chemical method for the determination of the permeabilities.

To permit the calculation of the various thermodynamic quantities listed in tables 1 through 4, it is necessary to make certain assumptions as to the equations which express the permeability. In addition, the quantities involved in the final expression have to be defined, such as the solubility of the penetrating substance in the cell membrane, the thickness of the cell wall, and the distance between equilibrium positions ( $\lambda$ ) in the transport of the material. In the preceding section the difficulties encountered in the determination of the mechanism of the permeation process were pointed out. The analysis of the permeability data for non-electrolytes seems to show that all three possible mechanisms do

<sup>6</sup> Compounds stable to ionization in the physiological pH range.

<sup>7</sup> For permeability to water see references 14, 15, and 16; for permeability to non-electrolytes see reference 24.

exist; however, the majority of the compounds appear to have their permeation rates determined by diffusion through the bulk membrane. In view of this analysis, the assumption is made that both water and non-electrolytes belong to class I, whose rate-determining step is diffusion in the bulk membrane. The equation for the permeability constant then corresponds to the expression:

$$P \left( \frac{\text{cm.}}{\text{sec.}} \right) = \frac{KD}{\delta} \quad (38)$$

as developed in the preceding section for case I. This form of the equation was used in the treatment of the available data on the permeability of the egg cells of marine invertebrates and the plant cell *Chara*.

From the theory of absolute rates of reaction, we may write

$$P = \frac{K\lambda^2}{\delta} \frac{kT}{h} e^{-\Delta F^\ddagger/RT} = \frac{kT}{h} \frac{\lambda^2}{\delta} e^{-\Delta F'/RT} \quad (39)$$

where  $\Delta F'$  is the free energy of activation for permeability and  $\Delta F^\ddagger$  is the free energy of activation for diffusion in the membrane. The free energy,  $\Delta F'$ , represents the difference in free energy of the diffusing component between its initial position in the aqueous solution surrounding the cell and the top of the highest potential barrier the diffusing molecule must pass over within the membrane. If the variation of the permeability constant and the partition coefficient with temperature is known, it is possible to calculate both  $\Delta H'$  and  $\Delta H^\ddagger$ , the heat contents for permeation and diffusion, by plotting  $\ln(P_h/kT)$  and  $\ln(P_h/kTK)$  vs.  $1/T$ , respectively.

The entropies of activation for permeability ( $\Delta S'$ ) and diffusion ( $\Delta S^\ddagger$ ) are calculated from the Gibbs-Helmholtz equation:

$$\Delta F = \Delta H - T\Delta S \quad (40)$$

All the thermodynamic quantities refer to the diffusing substance in its standard state of unit concentration. The usual zero superscript is omitted to simplify the presentation. Since pressure has a small effect on diffusion, i.e.,  $p\Delta V^\ddagger$  is negligible, the heat of activation,  $\Delta H^\ddagger$ , for diffusion is related to the Arrhenius energy of activation by the equation:

$$\Delta E = \Delta H^\ddagger + RT \quad (41)$$

In calculating the free energy terms of activation,  $\Delta F'$  and  $\Delta F^\ddagger$ , it is necessary to assume values for the cell wall thickness ( $\delta$ ) as also for the distance ( $\lambda$ ) between equilibrium positions in the membrane. Measurements of wall thickness for cells of various animals and plants give values in the range of 100–200 Å. For the diffusion of substances in condensed phases (11), the values for the size of molecular jumps,  $\lambda$ , are in the limits of 3–10 Å. In the calculations the maximum value for  $\delta$  of 200 Å. and a mean value for  $\lambda$  of 5 Å. were used. The maximum variation in the value of the term  $\ln(\lambda^2/\delta)$  for the defined limits of  $\lambda$  and  $\delta$  corresponds to 6.2 E.U., if absorbed in the entropy term for permeability.

In view of the large entropy changes which are calculated, this approximation is significant but not serious.

No experimental values are available for  $K$ , the distribution coefficient for the diffusing substance between the cell membranes and the solution in which it is immersed. Because of the lipid nature of the cell membranes, the procedure commonly followed is to assume that the true coefficient is directly proportional to that found for the substance partitioned between olive oil and water. Values for this quantity,  $K = (\text{concentration in olive oil}) \div (\text{concentration in water})$ ,

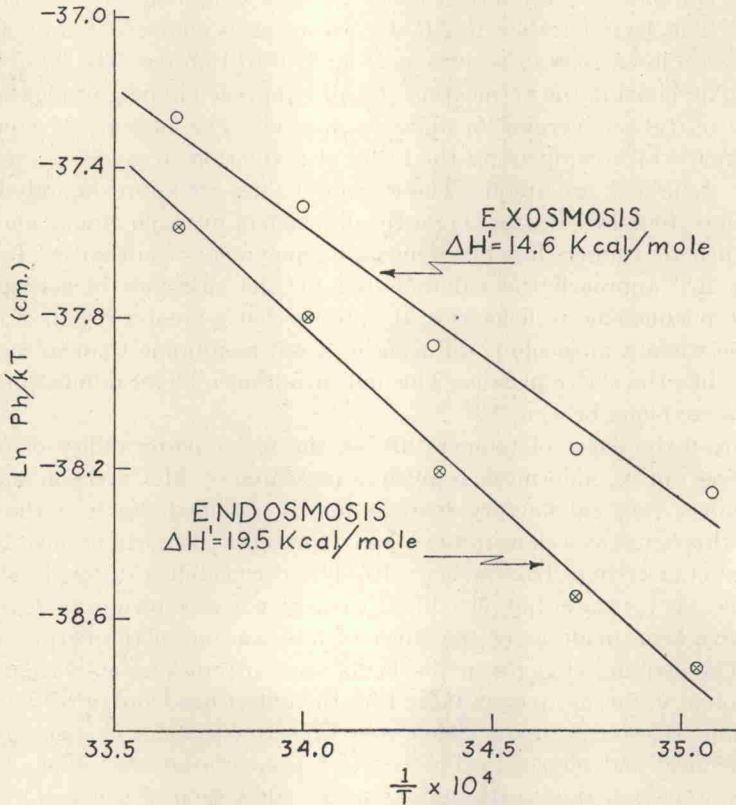


FIG. 8. Permeability of *Arbacia* eggs (unfertilized) to water

were taken from a paper by Collander and Bärlund (5). Since no literature data are available on the temperature variation of the olive oil-water coefficients, it is only possible to calculate the heats of activation for permeability,  $\Delta H'$ . The permeability constants reported in the literature for substances diffusing in biological systems are not given in the standard units of  $\text{cm.} \cdot \text{sec.}^{-1}$ . The factors used in converting the reported constants to the units of  $\text{cm.} \cdot \text{sec.}^{-1}$  as given in the tables are discussed in the appendix.

In figure 8, the data of Lucké, Hartline, and McCutcheon (14) on the water

permeability of unfertilized *Arbacia* eggs during endosmosis and exosmosis are plotted as a function of temperature. By plotting  $\ln (Ph/kT)$  against reciprocal temperature, the heats of activation for permeability are calculated from the slopes of the lines. Values for the permeability constants are uncorrected for the non-solvent volume, i.e., the osmotically inactive materials of the egg cells. The non-solvent volume correction is on the average about 10 per cent (17), which if absorbed in the entropy term corresponds to less than one entropy unit. In the calculation of the free energies given in table 1 for water, the uncorrected permeability constants were employed. Though the permeation of water during exosmosis is higher by only 30 per cent, the heat of activation,  $\Delta H'$ , is smaller by 5 kcal. This large decrease in  $\Delta H'$  for exosmosis is compensated by a similar large decrease in entropy of activation from 31.6 to 15.5 E.U. The large heats of activation involved in the permeation of water approach in magnitude the values found for activation energies for diffusion in solids. The large positive entropies of activation which compensate the heats of activation to give free energies of the order of 10 kcal. are unique. The entropy values are approximately twice as large as those found by Barrer (1) for the diffusion of nitrogen, argon, and hydrogen in synthetic rubbers like neoprene and copolymers of butadiene. In magnitude, the  $\Delta S'$  approach the values found for the entropies of activation for relaxation phenomena in dielectrics. It appears that a greater region of disorder must arise when a molecule is diffusing in a cell membrane than in more rigid structures like the above plastics. This indicates that a larger number of secondary bonds are being broken.

In figure 9 the effect of temperature on the water permeability of fertilized *Arbacia* eggs during endosmosis is given as measured by McCutcheon and Lucké (16). Again a very satisfactory straight line is obtained. Each of the plotted points in this figure as well as in the others represents the mean of measurements on a maximum of five *Arbacia* egg cells. The permeability of fertilized eggs is higher not only for water but also for ethylene glycol, as shown in table 3. Speculations have been made as to the effect of fertilization on the nature of membranes. The striking changes in the heats and entropies of activation for the permeation of water, as given in table 1 for the unfertilized and fertilized *Arbacia* eggs during endosmosis, leave little doubt that a permanent change must occur in the chemical and physical properties of the membrane. Any changes in the membrane thickness due to stretching and/or adsorption of new compounds on the inner wall surface of the cell appear to be of secondary importance. Fertilization causes an increase in the heat of activation which is overcompensated by a larger increase in the entropy, resulting in higher permeability rates. Whatever changes occur in the membrane on fertilization, whether denaturation or rearrangement of the protein and lipid units, the thermodynamic values indicate that the activation process in the diffusion of a water molecule involves formation of a larger number of stronger bonds made possible by the greater amount of disarrangement that takes place. This view is supported by the work of Cole and Spencer (2), who found that the electrical capacity of the membrane of fertilized *Arbacia* eggs is increased by almost a factor of four.

The effect of temperature on the permeability of ethylene glycol through unfertilized *Arbacia* eggs was thoroughly investigated by Stewart and Jacobs (23). Their results are plotted in figure 10. The value for  $\Delta H'$  of 23.6 kcal. is of the same order of magnitude as that found for water. Since ethylene glycol permeates only one-fifth as rapidly as water, it appears that here also a large positive entropy of activation is involved. At 22.5°C. it amounts to 36.8 E.U.

In table 1, the permeabilities to water of several species of marine invertebrates are compared. The free energies of an activation are all of the same magni-

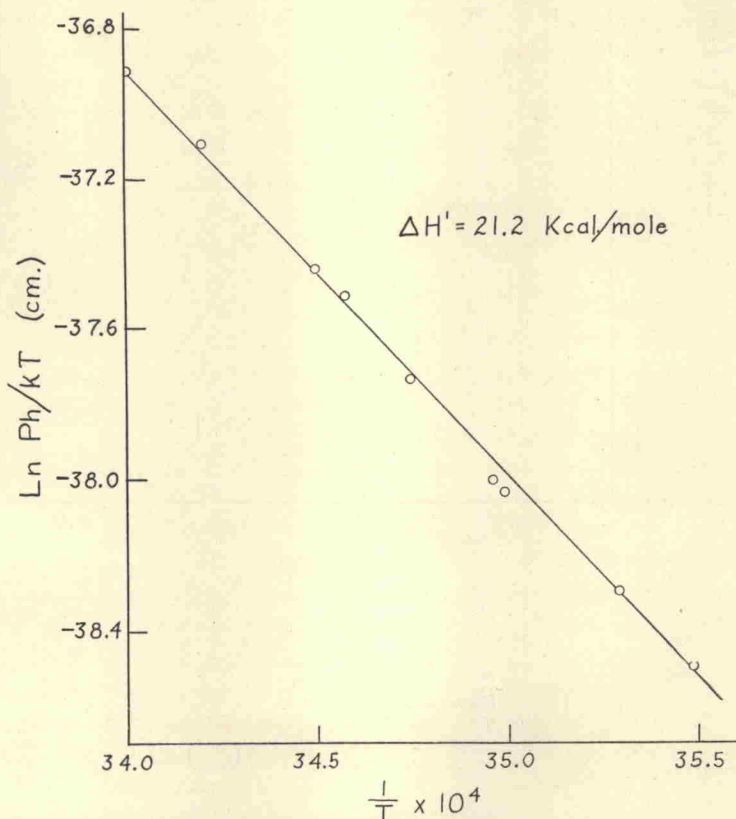


FIG. 9. Permeability of *Arbacia* eggs (unfertilized) to ethylene glycol during endosmosis

tude—about 10 kcal. Similarly, in table 2, the values are given for the permeability and diffusion constants of three aliphatic amides. The  $\Delta H'$  and  $\Delta F'$  are of the same magnitude as those found for water permeation of the *Arbacia* eggs. It is interesting to note that as we progress up in the homologous series leading to greater solubility in the cell membrane, the free energies for permeation decrease, whereas those for diffusion increase. This indicates that, although the permeability constants increase with solubility in agreement with Overton's theory, the diffusion constants actually decrease. The same relation was found

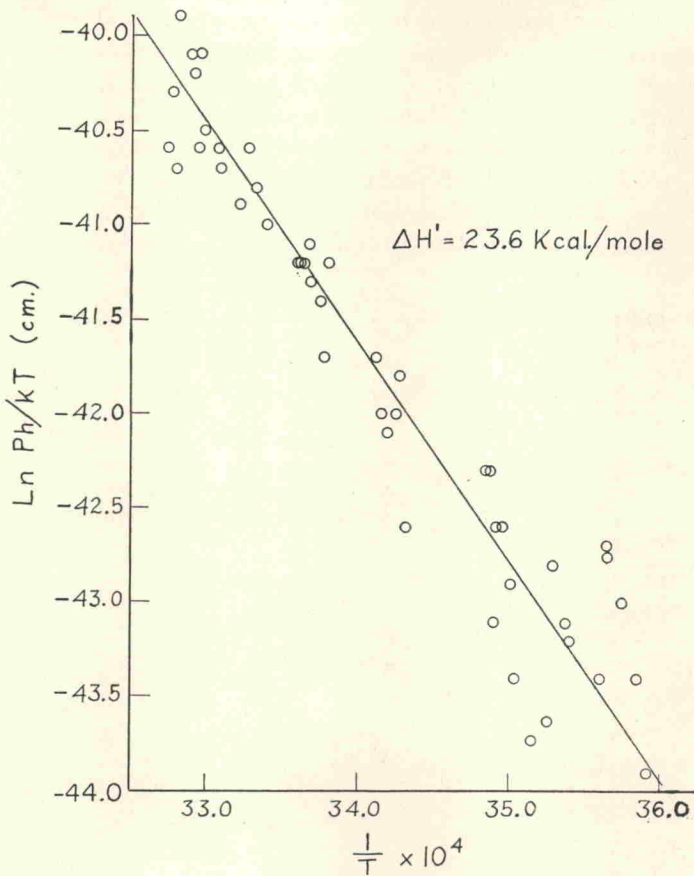


FIG. 10

TABLE 1  
Permeability to water

SPECIMEN: EGGS OF MARINE INVERTEBRATES	TYPE	PROCESS	$P \left( \frac{\text{cm.}}{\text{sec.}} \right) \times 10^6$ AT 24°C.	$\Delta H'$	$\Delta F'^*$	$\Delta S'$
				kcal.	kcal.	E.U.
<i>Arbacia</i> .....	Unfertilized	Endosmosis	30.5	19.5	10.1	31.6
<i>Arbacia</i> .....	Unfertilized	Exosmosis	39.5	14.5	9.9	15.5
<i>Arbacia</i> .....	Fertilized	Endosmosis	55.6	21.2	9.7	38.8
<i>Cumingia</i> .....	Unfertilized	Endosmosis	92.		9.4	
<i>Chaetopterus</i> .....	Unfertilized	Endosmosis	103.		9.3	

$$* P = \frac{KD}{\delta} = \frac{Kk'\lambda^2}{\delta} \text{ where } \lambda = 5 \text{ \AA. and } \delta = 200 \text{ \AA.}$$

to hold true for the two pairs of substances, propylene glycol-glycerol and the methyl and ethyl ethers of glycerol. These calculated values are given in table 4.



TABLE 2  
Permeability of *arbacia* eggs (unfertilized) to aliphatic amides

SUBSTANCE	$P \left( \frac{\text{cm.}}{\text{sec.}} \right) \times 10^6$ AT 22°C.	$K = \frac{[\text{C. Oil}]}{[\text{C. H}_2\text{O}]}$ $\times 10^3$	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right)$ $\times 10^8$	$\Delta H'$	$\Delta F'$	$\Delta S'$	$\Delta F' \dagger$	$\Delta F_s^*$
				kcal.	kcal.	E. U.	kcal.	kcal.
Acetamide.....	9.7	0.83	2.3		12.1		7.9	4.2
Propionamide.....	23.7	3.6	1.32	21.6	11.5	34.	8.2	3.3
Butyramide.....	60.	9.5	1.3	22.8	11.0	40.	8.2	2.8

\*  $\Delta F_s$  = free energy of solubility.

TABLE 3  
Permeability of animal and plant membranes to polyhydroxy alcohols  
A. Substance: ethylene glycol

SPECIMEN	$P \left( \frac{\text{cm.}}{\text{sec.}} \right) \times 10^6$ AT 22.5°C.	$K = \frac{[\text{C. Oil}]^*}{[\text{C. H}_2\text{O}]}$ $\times 10^4$	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right) \dagger$ $\times 10^8$	$\Delta F'$	$\Delta F' \ddagger$
				kcal.	kcal.
<i>Arbacia</i> (unfertilized).....	7.3	4.9	3.0	12.2	7.7
<i>Arbacia</i> eggs (fertilized).....	14.	4.9	5.7	11.8	7.4
<i>Chaetopterus</i> (unfertilized).....	23.8	4.9	9.7	11.7	7.2
<i>Cumingia</i> (unfertilized).....	26.0	4.9	10.5	11.5	7.0
<i>Chara ceratophylla</i> .....	12.	4.9	4.9	11.9	7.4

B. Substance: propylene glycol

SPECIMEN	$P \left( \frac{\text{cm.}}{\text{sec.}} \right) \times 10^6$ AT 22.5°C.	$K = \frac{[\text{C. Oil}]^*}{[\text{C. H}_2\text{O}]}$ $\times 10^3$	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right) \dagger$ $\times 10^8$	$\Delta F'$	$\Delta F' \ddagger$
				kcal.	kcal.
<i>Arbacia</i> (unfertilized).....	13.	5.7	0.46	11.9	8.8
<i>Arbacia</i> (fertilized).....	21.7	5.7	0.76	11.6	8.5
<i>Chara ceratophylla</i> .....	24.	5.7	0.84	11.5	8.4

C. Substance: glycerol

SPECIMEN	$P \left( \frac{\text{cm.}}{\text{sec.}} \right) \times 10^6$ AT 22.5°C.	$K = \frac{[\text{C. Oil}]^*}{[\text{C. H}_2\text{O}]}$ $\times 10^5$	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right) \dagger$ $\times 10^8$	$\Delta F'$	$\Delta F' \ddagger$
				kcal.	kcal.
<i>Arbacia</i> (unfertilized).....	0.05	7.0	0.14	15.	9.5
<i>Chaetopterus</i> (unfertilized).....	10.3	7.0	29.	12.0	6.4
<i>Chara ceratophylla</i> .....	0.21	7.0	0.60	14.3	8.7

\* The olive oil-water distribution coefficient assumed to represent the cell membrane-water coefficients.

†  $\delta$  = Assumed cell thickness of 200 Å.

‡  $\lambda^2/\delta = 1.25 \times 10^{-9}$ .

In table 3 are summarized the values for the permeability constants of the polyhydroxy alcohols ethylene glycol, propylene glycol, and glycerol through

different marine invertebrates and the plant cell *Chara*. Though the permeabilities vary widely, it is seen that this is primarily due to their varied solubilities in the cell membrane. All of the heats, free energies, and entropies of activation are of approximately the same magnitude. In table 4 a comparison is made of the relative permeabilities of alcohols and ethers through the plant cell *Chara*. In contrast to the amides, here it is to be noticed that as we introduce more polar groups into the molecule, the free energies for permeability and diffusion both

TABLE 4

*Cell permeability of the plant Chara ceratophylla to oxygen-containing aliphatic compounds*

SUBSTANCE	$P \left( \frac{\text{cm.}}{\text{sec.}} \right) \times 10^6$ AT 22°C.	$K = \frac{[\text{C. Oil}]}{[\text{C. H}_2\text{O}]} \times 10^8$	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right) \times 10^8$ *	$\Delta F'$	$\Delta F^\ddagger$
Methanol.....	280.	7.8	7.20	10.1	7.2
Ethylene glycol.....	12.	0.49	4.9	11.9	7.4
Propylene glycol.....	24.	5.7	0.84	11.5	8.4
Glycerol.....	0.21	0.07	0.60	14.3	8.7
Erythritol.....	0.014	0.03	0.093	15.9	9.8
Glycerol methyl ether.....	12.	2.6	0.92	11.9	8.4
Glycerol ethyl ether.....	21.	7.4	0.57	11.6	8.7

\* Assumed values of cell thickness 200 Å. and  $\lambda$  as 5 Å.

TABLE 5

*Diffusion constants in water and plant cell membranes (Chara ceratophylla)*

SUBSTANCE	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right) \times 10^5$ WATER (15°C.)	$D \left( \frac{\text{cm.}^2}{\text{sec.}} \right) \times 10^9$ AT 22°C.
Methanol.....	1.28	72.0
Ethanol.....	1.00	14.5
Acetamide.....	0.96	36.
Ethylene glycol.....	0.93	49.0
Propylene glycol.....	0.83	8.4
Glycerol.....	0.72	6.0
Erythritol.....	0.68	0.93

increase. This indicates that as the substances become more soluble, it is easier for them to diffuse in the membrane. The values of the diffusion constants for non-electrolytes in membranes are of the order of 10,000 to 100,000 times smaller than their values for diffusion in aqueous solutions. This is primarily due to the higher energies of activation for diffusion in membranes. A few of the values for the diffusion constants are summarized in table 5. Their magnitudes occupy an intermediate position in the spectrum of diffusion constants in solids and liquids which bespeak a semisolid structure for natural membranes.

## SUMMARY

A new theory has been developed for the diffusion and permeability of membranes by considering molecular migration as a series of molecular jumps from one equilibrium position to another governed by a rate constant.

A simple method has been developed for deriving Fick's first and second laws by considering difference equations.

The theory is applicable to the diffusion of electrolytes and non-electrolytes in homogeneous and heterogeneous membranes, either in the presence or absence of external forces such as electrical fields, concentration gradients, or activity gradients.

The theory has been applied to the permeability data of water, aliphatic alcohols, ethers, and amides through animal and plant cells. An analysis of the mechanism of the permeation process is made on the basis of distribution coefficients and temperature coefficients. By assuming diffusion in the bulk membrane as the slow step, free energies, heat energies, and entropies for permeation are calculated and discussed in terms of the nature of these membranes. The heats of activation were found to range from 14.5 to 23.6 kcal., with corresponding large values for the entropy changes from 15.5 to 38.8 e.u. Values for the diffusion constants of the non-electrolytes in the bulk membrane are calculated.

## APPENDIX

*Conversion factors for the permeability constants*

(1) *Permeability of water:* The permeability constants for water as reported in the literature are calculated from the following equation expressing the swelling of an egg cell in hypertonic sea water. Osmotic pressure is assumed to be the sole driving force:

$$\frac{dV}{dt} = k_2 A (\pi - \pi_e) \quad (1)$$

Here  $k_2$  is given in  $\mu^3/\mu^2\text{-min.-atm.}$ ,  $A$  is the area of the egg cell in square microns, and  $\pi$  and  $\pi_e$  are the osmotic pressures of the internal and external solutions, respectively. In order to use the values of these constants it is necessary to convert them to units of  $\text{cm.-sec.}^{-1}$ , the conventional label for a permeability constant. It is easily shown that the flux is

$$Q = \frac{1}{A} \frac{dn}{dt} = \frac{k_2}{\bar{V}_1} (\pi - \pi_e) \quad (2)$$

where  $n_1$  = number of moles of water penetrating,

$\bar{V}_1$  = partial molar volume of water in cubic centimeters per mole, and

$Q$  = moles of water per square centimeter per second.

For dilute solutions, the osmotic pressure is given by

$$\Pi = \frac{p_1^0 - p_1}{p_1^0} \frac{RT}{\bar{V}_1} \quad (3)$$

where the mole fraction  $N_2$  of the solute is expressed in terms of the partial pressures of the solution and solvent. If we substitute in equation 2, the following result expresses the transport of water into the egg cell:

$$Q = k_2 \frac{RT}{\bar{V}_1} \left[ \left( \frac{p_1^e - p_1}{p_1^0} \right) \frac{1}{\bar{V}_1} \right] \quad (4)$$

where  $p_1^e$  and  $p_1$  are the vapor pressures of water above the external solution (sea water) and the internal cell sap solution, respectively. The permeability constant is given by

$$P = k_2 \frac{RT}{\bar{V}_1} \cong k_2 \frac{RT}{V_m} \quad (5)$$

where  $V_m$  is the molal volume of water. Thus to convert the reported values of the permeability constant,  $k_2$ , for water into units of  $\text{cm.} \cdot \text{sec.}^{-1}$ , we use the factor:

$$\begin{aligned} P \left( \frac{\text{cm.}}{\text{sec.}} \right) &= k_2 \frac{RT}{V_m} \times \frac{1 \text{ min.}}{60 \text{ sec.}} \times 10^{-4} \text{ cm./micron} \\ &= k_2 7.57 \times 10^{-6} T(^{\circ}\text{K.}) \end{aligned}$$

(2) *Permeability of non-electrolytes*: The values for the permeability constants ( $k_1$ ) for egg cells as reported by Jacobs, Danielli, and others are expressed in the units of moles of substance diffusing per unit cell area of 1 square micron per minute per a unit concentration gradient in moles per liter of solution. To convert to our values of the permeability constants in units of centimeters per second, the following factor was used:

$$P (\text{cm./sec.}) = k_1 \times 10^{-4} \text{ cm./}\mu \times 1/60 \text{ min./sec.} \times 10^{15} \mu^3/\text{liter}$$

which simplifies to

$$P (\text{cm./sec.}) = k_1 1.67 \times 10^9$$

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