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The Permeability Coefficient of Water in the Cell Membrane and the Diffusion Coefficient in the Cell Interior

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It is suggested that the water permeability properties of cells are not as diverse as they appear to be when judged by means of their widely different conventional permeability coefficients. These coefficients ignore the possible rate-limiting effect of water diffusion in the cell interior. If it is assumed that the water permeability coefficients of cell membranes are similar in the wide variety of cells for which data are available, and that the diffusion coefficients of water in the cell interior are also similar, then both these quantities may be calculated. The permeability coefficient was thus estimated to lie within the range from 3×10^{-4} to 7×10^{-3} cm/sec, and the diffusion coefficient in the range from 8×10^{-10} to 2×10^{-8} cm²/sec. The significance of these values has been discussed.

1. Introduction

It has already been shown (Dick, 1959*b*) that the conventional water permeability coefficients of the cell membranes of those cells for which data are available vary very widely, but that they appear to be correlated with the surface–volume ratio. Thus large cells, such as Arbacia eggs, which have low surface–volume ratios, have apparently low permeability coefficients, while small cells, such as erythrocytes, which have high surface–volume ratios, have apparently high permeability coefficients. It is possible, of course, that this correlation may be merely accidental and that the membrane permeabilities are in fact widely variable. On the other hand since a direct causal relationship between the true permeability of the cell membrane and the cell size or geometry seems unlikely it was suggested that permeability coefficients calculated by conventional methods may need to be revised. Thus the conventional, but usually unstated assumption that the rate of diffusion of water in the cell interior is sufficiently high for its effect on water flow to be ignored in comparison with the resistance offered by the cell membrane may well be unsound (Dick, 1959*b*, *c*; Dainty & Hope, 1959).

It is not, of course, possible to calculate both the permeability coefficient of the cell membrane and the diffusion coefficient of the interior simultaneously from data of the rate of cell swelling or shrinkage in response to a change of external osmotic pressure. However, if certain values of the diffusion coefficient are assumed it is possible to calculate corresponding values for the apparent permeability coefficient. Such pairs of values can be plotted graphically so that the water permeability of each cell can be represented by a curve connecting the pairs of values. If the assumption is then made that cells, even of widely varying types, have in general similar permeation properties for water, then the curves for all cells ought to have a common locus representing the common permeability coefficient of the cell membrane and diffusion coefficient of the interior. The present paper is an attempt to assess the available data on this basis and to see whether it provides an adequate explanation for them.

A preliminary account of this work has been given to the British Biophysical Society (Dick, 1962).

2. Nature of the Mutual Diffusion Coefficient

It must be emphasized at once that the diffusion coefficient referred to in the present calculations is a mutual diffusion coefficient as defined by Crank (1956) and not a self-diffusion coefficient. The two coefficients are quite distinct and, since unfortunately they have been frequently confused (e.g. Edelman's (1961) erroneous criticism of Dick (1959*c*) based on this confusion) it may be worthwhile to consider the difference in more detail.

The self-diffusion coefficient of water (or any other fluid) is a measure of the rate of interchange of identical molecules by reason of their thermal movements. It is approximately estimated in practice by measuring the rate of diffusion of water containing an isotope of hydrogen or oxygen, $^{2}H^{1}HO$, $^{3}H^{1}HO$ or $H_2{}^{18}O$ in ordinary water, on the assumption that the physicochemical properties of the isotopically labelled molecules are identical with those of ordinary water. It is essential, however, to notice that isotopically labelled molecules do not occur ordinarily in living systems so that the conditions of measurement of the self-diffusion coefficient are quite artificial and the coefficient itself has no relevance to many problems of physiological diffusion in living systems.

Since living processes almost always involve the interchange of quite different molecules (in osmotic systems particularly the interchange of large protein or nucleic acid molecules on the one hand with small molecules or ions of water, salts or metabolites on the other hand), the diffusion coefficients of practical importance are commonly those which measure such mixing. The mutual diffusion coefficient is a measure of the rate of interdiffusion of

two such non-identical components. Living systems, of course, contain many components, so that even the mutual diffusion coefficient measured *in vitro* in a two-component system must be used with great caution in comparisons with living systems. However, since the rate of mutual diffusion depends almost entirely on the rate of diffusion of the slow components (in cell cytoplasm either protein or nucleic acid) and hardly at all on the diffusion of the fast components (so that the rate of self-diffusion of water is almost wholly irrelevant), reasonable comparisons may be made between the mutual diffusion coefficients of proteins and nucleic acids in water and the rate of diffusion of water in the cytoplasm during osmotic experiments in living cells. Such comparisons are made in the Discussion.

An impression of the relative significance of the movement of the fast and slow component in mutual diffusion may be gained by noting that Wang, Anfinsen & Polestra (1954) obtained a value of $1{\cdot}38\times10^{-5}$ cm^2/sec for the self-diffusion of H_2O^{18} in a 10·6% solution of ovalbumin at 10°C, whereas the self-diffusion coefficient for ovalbumin in the same solution was only $3{\cdot}32\times10^{-7}$ cm^2/sec. From the data of Polson (1939) the mutual diffusion coefficient of ovalbumin and water was found to be $4{\cdot}2\times10^{-7}$ cm^2/sec (calculated by short extrapolation from data extending to 8·5% solution and by conversion from 20° to 10°C). The mutual diffusion coefficient is thus clearly seen to be comparable to the self-diffusion coefficient of the slow component, ovalbumin, rather than that of the fast component, water. The self-diffusion coefficient of water in this case merely measures the rate of movement of water *around* the albumin molecules and is similar to that obtained in free water at 10°C, $1{\cdot}68\times10^{-5}$ cm^2/sec.

It is also of interest to note that the mechanism of mutual diffusion differs radically from that of self-diffusion (Hartley & Crank, 1949) and that owing to the comparative immobility of the slow component, hydrostatic pressure gradients are set up resulting in a bulk flow of the two components.

3. Method of Calculation for Spherical Cells

Since water movement by diffusion in the cytoplasmic proteins is governed by a different mechanism from the passage of water down an osmotic pressure gradient across the semi-permeable cell membrane, serious mathematical difficulties arise in the solution of the equations involved so that an exact solution is not possible, and it is necessary to resort to approximations. While the results of the calculations cannot therefore be expected to be exact, a check (which is described in section 7) is available which suggests that the accuracy is sufficient for the present purpose. The assumption of common surface permeability and internal diffusion coefficients for all cells cannot be more than approximately true so that an indication of the correct

order of magnitude of these two parameters is in any case the most that can be hoped for.

One of the principal difficulties in devising a mathematical solution is that two distinct measures of water concentration are involved.

(1) Represented by C, where

$$C = \frac{\text{volume of water}}{\text{total volume of solution (all components)}}.$$

The rate of mutual diffusion of water and the other components of the cell is proportional to the gradient of C.

(2) Represented by C_1, where

$$C_1 = \frac{\text{volume of water}}{\text{volume of water} + \text{volume of salts in solution}}$$

i.e. the volume of any macromolecular solute is neglected. The rate of water flow through the cell membrane is proportional to the difference between the values of C_1 on its two sides. *The distinction between C and C_1 is very important as they occur in the calculations below.*

In the case of spherical cells with diffusion taking place radially, the diffusion equation takes the form

$$\frac{\partial C}{\partial t} = D\left(\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right) \tag{1}$$

where

C = water concentration (as a volume fraction)

D = diffusion coefficient

r = distance from the centre of sphere of radius a.

If the cell is initially at a uniform water concentration C_0, and is transferred to a solution in which its *internal* water concentration becomes at equilibrium C_e, the appropriate boundary conditions are

$$C = C_0, \qquad t = 0 \tag{2}$$

$$C = C_e, \qquad t = \infty \tag{3}$$

$$\frac{\partial C}{\partial r} = 0, \qquad r = 0. \tag{4}$$

The surface condition is affected by the semi-permeability of the cell membrane, i.e. permeability to water only. Let the flux across the cell membrane be J in $cm^3/cm^2 . sec$ or cm/sec measured as positive in the outward direction.

Then

$$J = P\Delta\Pi = P(\Pi_e - \Pi_a) \tag{5}$$

where P is the water permeability coefficient expressed as cm.sec^{-1}. atmos.$^{-1}$, Π_a is the osmotic pressure in atmospheres immediately inside the cell membrane at any time during osmotic swelling or shrinkage, and Π_e is the uniform *internal* osmotic pressure at equilibrium (i.e. Π_e is equal to the external osmotic pressure: but note that C_e is *not* equal to the external water concentration).

But

$$\Pi = -\frac{RT}{\overline{V}_1} \ln \frac{n_1}{n_1+n_2+n_3} \tag{6}$$

(assuming ideal solutions; for a simple derivation see Dick, 1959a). The components of the solution have been divided into three groups, (1) water, (2) solutes with small molecules or ions, i.e. mainly salts, (3) solutes with large molecules or ions, i.e. mainly proteins. n_1, n_2 and n_3 are the numbers of gram-molecules of each group present in the cell and suffixes (a) and (e) have throughout the same significance as in Π_a and Π_e. It is thus reasonable to assume that

$$n_{1(a)} \gg n_{2(a)} \gg n_{3(a)}$$

and

$$n_{1(e)} \gg n_{2(e)} \gg n_{3(e)}.$$

Thus in both cases

$$\Pi \approx -\frac{RT}{\overline{V}_1} \ln \frac{n_1}{n_1+n_2} \tag{7}$$

$$\approx \frac{RT}{\overline{V}_1} \frac{n_2}{n_1+n_2}. \tag{8}$$

But

$$\frac{n_2}{n_1+n_2} = \left(\frac{\overline{V}_2 n_2}{\overline{V}_1 n_1 + \overline{V}_1 n_2}\right)\frac{\overline{V}_1}{\overline{V}_2}$$

$$\approx \frac{V_2}{V_1+V_2} \cdot \frac{\overline{V}_1}{\overline{V}_2} \tag{9}$$

(where V_1 and V_2 are the total volumes of components 1 and 2 within the cell and it is remembered that $n_1 \gg n_2$ and $\overline{V}_1$ is of the same order as $\overline{V}_2$).

Put

$$\frac{V_2}{V_1+V_2} = C_2 \qquad \text{and} \qquad \frac{V_1}{V_1+V_2} = C_1$$

(C_1 must be distinguished from C in equation (1) which is $\frac{V_1}{V_1+V_2+V_3}$ in this nomenclature).

Thus

$$\frac{n_2}{n_1+n_2} = C_2\frac{\overline{V}_1}{\overline{V}_2}$$

$$= (1-C_1)\frac{\overline{V}_1}{\overline{V}_2}. \tag{10}$$

From equations (5), (8) and (10)

$$J = P(\Pi_e-\Pi_a) = P\frac{RT}{\overline{V}_1}\frac{\overline{V}_1}{\overline{V}_2}(C_{1(a)}-C_{1(e)}) \tag{11}$$

where

$$C_{1(a)} = \frac{\mathrm{d}(V_1)}{\mathrm{d}(V_1+V_2)},\ r=a \qquad \text{and } C_{1(e)} = \frac{V_{1(e)}}{V_{1(e)}+V_{2(e)}}$$

($C_{1(a)}$ is expressed as a differential coefficient since it is the concentration at a point as distinct from an average concentration in the cell such as C_0, C_e or $C_{1(e)}$. It is analogous to a partial molar volume which although it has the dimensions of and is approximately equal to the volume of 1 g mole of a single component in a mixture, is in fact defined as $\left(\frac{\partial V}{\partial n_i}\right)_{n_j,\,n_k\ldots}$ and is thus not a simple physical concept. In the same way $C_{1(a)}$ and C_a (see below), although they can easily be pictured in terms of average concentrations, have no simple physical significance.) It is now necessary to express the osmotic pressure difference as a difference of water concentration in mole-fraction (see Løvtrup & Pigon, 1951), and another permeability coefficient α, expressed in units of cm/sec is used, given by the formula

$$\alpha = P\frac{RT}{\overline{V}_1}. \tag{12}$$

Therefore

$$J = \alpha\frac{\overline{V}_1}{\overline{V}_2}(C_{1(a)}-C_{1(e)}). \tag{13}$$

In order to use this equation as a surface condition, J must be expressed in terms of the difference of total water concentration, i.e. $C_a - C_e$; where

$$C_a = \frac{dV_1}{d(V_1+V_2+V_3)}, \qquad r = a$$

and

$$C_e = \frac{V_1}{V_1+V_2+V_3}, \qquad t = \infty.$$

Thus

$$J = \left[\alpha \frac{\bar{V}_1}{\bar{V}_2} \frac{C_{1(a)} - C_{1(e)}}{C_a - C_e}\right](C_a - C_e). \tag{14}$$

Substitute α' for the expression in square brackets,

$$J = \alpha'(C_a - C_e). \tag{15}$$

[It must be noted that $C_a - C_e$ is not the difference in concentration C across the cell membrane. Equation (15) is not therefore intended to have any physical significance, but merely to be a convenient approximate expression for the rate of water movement across the cell membrane. The exactness of the approximation depends on the actual variation with time of the expression $\frac{C_{1(a)} - C_{1(e)}}{C_a - C_e}$ which is here assumed to be constant; this variation is discussed below (see Appendix I).]

Since

$$J = -D \frac{\partial C}{\partial r}, \qquad r = a \tag{16}$$

(from Fick's equation).
Then

$$-D \frac{\partial C}{\partial r} = \alpha'(C_a - C_e), \qquad r = a \tag{17}$$

which is the surface condition required.

The solution of equations (1), (2), (3), (4) and (17) is

$$\frac{C_e - C}{C_e - C_0} = \frac{2La}{r} \sum_{n=1}^{\infty} \frac{\exp(-\beta_n^2 Dt/a^2)}{\beta_n^2 + L(L-1)} \cdot \frac{\sin(\beta_n r/a)}{\sin \beta_n} \tag{18}$$

where $L = \frac{\alpha' a}{D}$ and β_n's are roots of the equation

$$\beta_n \cot \beta_n + L - 1 = 0 \tag{19}$$

(see Carslaw & Jaeger, 1959; values of β_n given on p. 492). Equation (18) may be integrated between the limits $r = 0$ and $r = a$, to give

$$\frac{C_e - C_t}{C_e - C_0} = \sum_{n=1}^{\infty} \frac{6L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L[L-1])} \tag{20}$$

where C_t is the *average* concentration of water in the cell at time t, i.e.

$$C_t = \left(\frac{V_1}{V_1 + V_2 + V_3}\right)_t.$$

In the case of a cell swelling or shrinking after transfer to a hypotonic or hypertonic solution

$$C_0 = \frac{V_0 - b}{V_0}, \qquad C_t = \frac{V_t - b}{V_t}, \qquad C_e = \frac{V_e - b}{V_e} \tag{21}$$

where V_0, V_t and V_e are the total volumes of the cell, initially, at time t, and when in equilibrium with the external solution respectively. b is the non-solvent volume of the cell, i.e. $b = V_2 + V_3$. It is also necessary to treat the cell as though the diffusion boundary were fixed although, of course, it actually moves outwards or inwards during osmotic swelling or shrinking (see Dick, 1959*b*). Since, however, the increase of radius is only the cube root of the increase of cell volume, if the value of a is selected as midway between the initial and final radius of the cell the error involved is comparatively small.

Thus the final equation used is

$$\frac{V_e - V_t}{V_e - V_0} \cdot \frac{V_0}{V_t} = \sum_{n=1}^{\infty} \frac{6L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L[L-1])}. \tag{22}$$

Two methods were used for evaluating D and α from this equation.

(1) The ratio of term 2 to term 1 on the R.H.S. is

$$\exp(-[\beta_2^2 - \beta_1^2]Dt/a^2) \cdot \frac{\beta_1^2(\beta_1^2 + L[L-1])}{\beta_2^2(\beta_2^2 + L[L-1])}. \tag{23}$$

It may be shown that provided Dt/a^2 exceeds 0·1 this ratio does not exceed 0·02 for any value of L.

Thus for $Dt/a^2 > 0{\cdot}1$, the second and subsequent terms were neglected and the resultant equation solved for D giving

$$D = \frac{a^2}{\beta_1^2 t} \ln\left[\frac{6L^2}{\beta_1^2(\beta_1^2 + L[L-1])} \cdot \frac{V_e - V_0}{V_e - V_t} \cdot \frac{V_t}{V_0}\right]. \tag{24}$$

Various values of L are substituted in equation (24) to give values of D. The corresponding values of the permeability coefficient, α, are calculated as

$$\alpha = \frac{LD}{a}\frac{\bar{V}_2}{\bar{V}_1}\left(\frac{C_a - C_e}{C_{1(a)} - C_{1(e)}}\right) \tag{25}$$

from equations (14) and (15), and the definition of L (see equation (18)).

The expression within brackets in equation (25) may be evaluated as follows

$$C_a - C_e = (C_0 - C_e)2L \sum_{n=1}^{\infty} \frac{\exp(-\beta_n^2 Dt/a^2)}{\beta_n^2 + L[L-1]} \tag{26}$$

(from equation (18), setting $r = a$).

When $Dt/a^2 > 0{\cdot}1$, the second and subsequent terms in the summations may be neglected, and then after substituting a value of $(C_0 - C_e)$ obtained from equation (20),

$$C_a - C_e = (C_t - C_e)\frac{\beta_1^2}{3L}. \tag{27}$$

Also

$$C_t - C_e = \frac{b(V_t - V_e)}{V_t V_e} \tag{28}$$

(from equations (21)), and so

$$C_a - C_e = \frac{b(V_t - V_e)}{V_t V_e}\frac{\beta_1^2}{3L}. \tag{29}$$

It is now necessary to introduce equations similar to (26), (27), (28) and (29) with the difference that water concentrations are measured in terms of $C_1 = \dfrac{V_1}{V_1 + V_2}$.

From equation (13) the appropriate permeability coefficient relating flux J and concentration difference $(C_{1(a)} - C_{1(e)})$ across the membrane is $\alpha\dfrac{\bar{V}_1}{\bar{V}_2}$ and the appropriate diffusion coefficient D^* relates water flux in the cytoplasm to the appropriate concentration gradient $\dfrac{\partial C_1}{\partial r}$.

Thus

$$J = -D^* \frac{\partial C_1}{\partial r}. \tag{30}$$

(It must be noted that as in equation (15), equation (30) is not intended to represent a physical process, since the diffusional water flux is not strictly proportional to $\frac{\partial C_1}{\partial r}$. Equation (30) must be regarded merely as a convenient approximation.)

On comparison of equation (15) with equation (16)

$$D^* \frac{\partial C_1}{\partial r} = D \frac{\partial C}{\partial r}. \tag{31}$$

But

$$\frac{\partial C_1/\partial r}{\partial C/\partial r} = \frac{dV_2}{dV_3}$$

(by an argument similar to that used to obtain equation (4') of Appendix I, without the restriction of $r = a$).

Then, since at any point within the cell the volume of macromolecular solute of component 2 always greatly exceeds the volume of salts of component 3,

$$\frac{dV_2}{dV_3} < 1$$

so that

$$\frac{\partial C_1}{\partial r} < \frac{\partial C}{\partial r},$$

and therefore, from equation (31),

$$D^* > D$$

so that if $Dt/a^2 > 0{\cdot}1$, then $D^*t/a^2 > 0{\cdot}1$.

Then by analogy with equation (26) (when $D^*t/a^2 > 0{\cdot}1$),

$$C_{1(a)} - C_{1(e)} = (C_{1(t)} - C_{1(e)}) \frac{\beta_1^*}{3L^*} \tag{32}$$

where

$$L^* = \frac{a\alpha \dfrac{\overline{V}_1}{\overline{V}_2}}{D^*}$$

and

$$\beta_1^* \cot \beta_1^* + L^* - 1 = 0$$

(by analogy with equation (19)).

But

$$C_{1(t)} = 1 - \frac{V_{2(0)}}{V_t - b} \quad \text{and} \quad C_{1(e)} = 1 - \frac{V_{2(0)}}{V_e - b} \tag{33}$$

(since $V_{2(0)} = V_{2(t)} = V_{2(e)}$)

$$C_{1(t)} - C_{1(e)} = C_{2(e)} \frac{V_t - V_e}{V_t - b} \tag{34}$$

so that equation (32) becomes

$$C_{1(a)} - C_{1(e)} = C_{2(e)} \cdot \frac{V_t - V_e}{V_t - b} \cdot \frac{\beta_1^{*2}}{3L^*}. \tag{35}$$

Combining equations (29) and (34)

$$\frac{C_a - C_e}{C_{1(a)} - C_{1(e)}} = \frac{b}{V_e C_{2(e)}} \left(1 - \frac{b}{V_t}\right) \left[\frac{\beta_1^2}{3L} \cdot \frac{3L^*}{\beta_1^{*2}}\right]. \tag{36}$$

But from equations (13), (15), (16) and (30)

$$J = \alpha \frac{\overline{V}_1}{\overline{V}_2} (C_{1(a)} - C_{1(e)}) = - D^* \left(\frac{\partial C_1}{\partial r}\right)_{r=a} \tag{37}$$

and

$$J = \alpha'(C_a - C_e) = - D \left(\frac{\partial C}{\partial r}\right)_{r=a}. \tag{38}$$

Thus

$$\frac{\alpha \dfrac{\overline{V}_1}{\overline{V}_2}}{\dfrac{D^*}{\alpha'}} \cdot \frac{D}{\alpha'} = \frac{L^*}{L} = \frac{\left(\dfrac{\partial C_1}{\partial r}\right)_{r=a} \Big/ \left(\dfrac{\partial C}{\partial r}\right)_{r=a}}{(C_{1(a)} - C_{1(e)})/(C_a - C_e)}$$

The R.H.S. of this equation is shown in Appendix I to be approximately unity, so that

$$L \approx L^* \quad \text{and} \quad \beta_1^1 = \beta_1^* \tag{39}$$

Equation (36) may now be rewritten

$$\frac{C_a - C_e}{C_{1(a)} - C_{1(e)}} = \frac{b}{V_e C_{2(e)}} \left(1 - \frac{b}{V_t}\right). \tag{40}$$

(A small error will arise from the fact that the R.H.S. of equation (39) is a function of time although the L.H.S. is treated as a constant in equation (14). However, since the limits of b/V_t are b/V_0 and b/V_t and the largest variation of these is (for the ox erythrocyte) from 0·38 to 0·18, the largest variation of $(1 - b/V_t)$ is from 0·70 to 0·82, which introduces variation of only 8% from the mean. The error from this source in regarding the L.H.S. of equation (40)

as a constant is therefore small.) Since the quantity in brackets on the R.H.S. of equation (40) does not differ greatly from 1·0, it is thus seen that the calculated value of $\frac{C_a-C_e}{C_{1(a)}-C_{1(e)}}$ is largely determined by $\frac{b}{V_e}\frac{1}{C_{2(e)}}$. The range of this quantity is from 11·1 for cell 1 (see Table 1) to 441 for cell 5, so that α is very much larger than α' and the correction applied by the use of the ratio $\frac{C_a-C_e}{C_{1(a)}-C_{1(e)}}$ is of great importance. This is due to the osmotically effective difference of water concentration across the semi-permeable cell membrane being much smaller than the true difference of water concentration since the presence of the cell colloids has in this instance a negligible effect on the water transfer across the cell membrane.

In evaluating $\bar{V}_2/\bar{V}_1$ for substitution into equation (25), $\bar{V}_2$ is the mean partial volume/g ion of solute. Since the partial molar volumes of NaCl and KCl in 0·1 M solution are 17·4 ml. and 27·6 ml. $\bar{V}_2$ was taken as approximately half of the mean of these values, i.e. 11·3 ml. Since $\bar{V}_1$ is 18·0 ml, $\bar{V}_2/\bar{V}_1 = 0{\cdot}63$.

(2) When $Dt/a^2 < 0{\cdot}1$, use was made of the graphical values of

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{6L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L[L-1])}$$

for various values of Dt/a^2 and L given by Crank (1956). (The L.H.S. of equation (22) is equivalent to $(1-M_t/M_\infty)$ in Crank's notation.) By assuming various values for L, the corresponding value of Dt/a^2 was found from the graph, taking the value of $(1-M_t/M_\infty)$ as equal to the value of the L.H.S. of equation (22). Then

$$D = \left(\frac{Dt}{a^2}\right) \cdot \frac{a^2}{t}.$$

α is calculated from equation (25), but in this case

$$\frac{C_a-C_e}{C_t-C_e} = \frac{\sum_{n=1}^{\infty} \frac{2L \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2 + L(L-1)}}{\sum_{n=1}^{\infty} \frac{6L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L[L-1])}}. \tag{41}$$

(since the second and subsequent terms in the summations cannot be neglected). The R.H.S. of this equation was calculated numerically using a Mercury computer. It was then shown (Fig. 1) to be approximately independent of the value of Dt/a^2 (i.e. independent of both D and t), when $Dt/a^2 > 0{\cdot}01$, a condition always satisfied in the present calculations. Thus

$$C_a - C_e = (C_t - C_e).f(L). \tag{42}$$

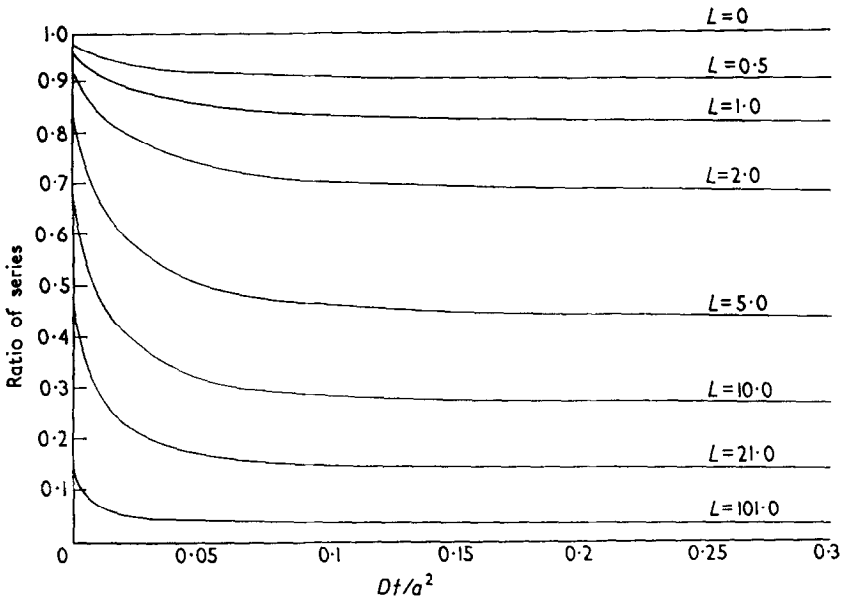

FIG. 1. The ordinate is the ratio of the two series forming the R.H.S. of equation (41) plotted against Dt/a^2 in the abscissa.

Similarly

$$\begin{aligned} C_{1(a)}-C_{1(e)} &= (C_{1(i)}-C_{1(e)})f(L^*) \\ &\approx (C_{1(i)}-C_{1(e)})f(L) \end{aligned} \tag{43}$$

(from equation (39)).

On combining equations (42) and (43), we again ultimately obtain equation (40), so that the final equation for α is unchanged.

4. Method of Calculation for Flattened Cells

Certain cells can be regarded as plane sheets. These are fibroblasts and the human and beef erythrocytes (in two experiments, cells 18 and 19) in which the cells swelled only up to 1·09 times their original volume during the experiment. (Another experiment on cell 16, in which the erythrocyte swelled to 1·7 times the original volume, was treated only by the spherical model, since for a great part of the time of swelling the cell was effectively spherical. The values obtained by the two methods of calculation are different, but this is mainly due to a discrepancy in the original data of the authors concerned.) The symbols used here are identical to those of the previous section, except where otherwise stated.

In this case the fundamental diffusion equation is

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{44}$$

and the boundary conditions are

$$C = C_0, \qquad t = 0 \tag{45}$$

$$C = C_e, \qquad t = \infty \tag{46}$$

$$\frac{\partial C}{\partial x} = 0, \qquad x = 0 \tag{47}$$

$$-D\frac{\partial C}{\partial x} = \alpha'(C_a - C_e), \qquad x = l \tag{48}$$

where the sheet extends in thickness from $-l$ to $+l$ and so that l is the half-thickness of the sheet.

The solution of equations (44), (45), (46), (47) and (48) is

$$\frac{C_e - C}{C_e - C_0} = \sum_{n=1}^{\infty} \frac{2L\exp(-\beta_n^2 Dt/l^2)}{\beta_n^2 + L(L+1)} \cdot \frac{\cos(\beta_n x/l)}{\cos\beta_n} \tag{49}$$

where $L = \dfrac{\alpha' l}{D}$ and the β_n's are the roots of the equation

$$\beta_n \tan\beta_n = L \tag{50}$$

(see Carslaw & Jaeger, 1959).

Equation (49) may be integrated between the limits $x = -l$ and $x = +l$, to give

$$\frac{C_e - C_t}{C_e - C_0} = \sum_{n=1}^{\infty} \frac{2L^2\exp(-\beta_n^2 Dt/l^2)}{\beta_n^2(\beta_n^2 + L[L+1])}. \tag{51}$$

The equation similar to equation (22) is then

$$\frac{V_e - V_t}{V_e - V_0} \cdot \frac{V_0}{V_t} = \sum_{n=1}^{\infty} \frac{2L^2\exp(-\beta_n^2 Dt/l^2)}{\beta_n^2(\beta_n^2 + L[L+1])}. \tag{52}$$

Again there are two methods of solution.

(1) When $Dt/l^2 > 0{\cdot}1$

$$D = \frac{l^2}{\beta_1^2 t}\ln\left[\frac{2L^2}{\beta_1^2(\beta_1^2 + L[L+1])} \cdot \frac{V_e - V_0}{V_e - V_t} \cdot \frac{V_t}{V_0}\right] \tag{53}$$

and

$$\alpha = \frac{LD}{l}\frac{\overline{V}_1}{\overline{V}_2}\left(\frac{C_a - C_e}{C_{1(a)} - C_{1(e)}}\right) \tag{54}$$

where the expression in brackets has the same value as in equation (40).

(2) When $Dt/l^2 < 0{\cdot}1$, graphical values of

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2L^2 \exp(-\beta_n^2 Dt/l^2)}{\beta_n^2(\beta_n^2 + L[L+1])} \tag{55}$$

for various values of Dt/l^2 and L given by Crank (1956) were used to calculate Dt/l^2 from M_t/M_∞ as described in the previous section.

α was calculated from

$$\alpha = \frac{LD}{l}\frac{\bar{V}_2}{\bar{V}_1}\left(\frac{C_a - C_e}{C_{1(a)} - C_{1(e)}}\right).$$

In this case

$$\frac{C_a - C_e}{C_t - C_e} = \frac{\sum_{n=1}^{\infty} \frac{2L \exp(-\beta_n^2 Dt/l^2)}{\beta_n^2 + L[L+1]}}{\sum_{n=1}^{\infty} \frac{2L^2 \exp(-\beta_n^2 Dt/l^2)}{\beta_n^2(\beta_n^2 + L[L+1])}} \approx f(L). \tag{56}$$

The R.H.S. of this equation was again evaluated numerically. Again this is approximately a function of L only. Similarly

$$\frac{C_{1(a)} - C_{1(e)}}{C_{1(t)} - C_{1(e)}} \approx f(L^*) \approx f(L) \tag{57}$$

so that equation (40) applies once again in this case.

5. Method of Calculation for Cylindrical Cells

The giant axons of *Loligo* and *Sepia* and the single muscle fibre were regarded as infinite cylinders. Again the symbols used here are identical with those of the previous two sections, except where otherwise stated.

The fundamental diffusion equation is

$$\frac{\partial C}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left(rD\frac{\partial C}{\partial r}\right) \tag{58}$$

and the boundary conditions are as given in equations (2), (3), (4) and (17), giving as a solution

$$\frac{C_e - C}{C_e - C_0} = \sum_{n=1}^{\infty} \frac{2L \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2 + L^2} \cdot \frac{J_0(r\beta_n/a)}{J_0(\beta_n)} \tag{59}$$

where $J_0(x)$ is the Bessel function of the first kind of order zero, $L = \dfrac{\alpha' a}{D}$

and the β_n's are the roots of the equation

$$\beta_n J_1(\beta_n) = L J_0(\beta_n) \tag{60}$$

where $J_1(x)$ is the Bessel function of the first order (see Carslaw & Jaeger, 1959).

Equation (59) may be integrated between the limits $r = 0$ and $r = a$ to give

$$\frac{C_e - C_t}{C_e - C_0} = \sum_{n=1}^{\infty} \frac{4L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L^2)}. \tag{61}$$

The final equation is therefore (cf. equation (22))

$$\frac{V_e - V_t}{V_e - V_0} \cdot \frac{V_0}{V_t} = \sum_{n=1}^{\infty} \frac{4L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L^2)}. \tag{62}$$

In all cases graphical values of

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4L^2 \exp(-\beta_n^2 Dt/a^2)}{\beta_n^2(\beta_n^2 + L^2)} \tag{63}$$

for various values of Dt/a^2 and L, given by Crank (1956) were used to calculate Dt/a^2 from M_t/M_∞ as described in the previous two sections.

α was calculated from equation (25)

$$\alpha = \frac{LD}{a} \cdot \frac{\bar{V}_2}{\bar{V}_1} \cdot \left(\frac{C_a - C_e}{C_{1(a)} - C_{1(e)}} \right).$$

Again the expression in brackets was found to be approximately a function of L only, so that equation (40) was found to apply once more.

6. Results

The data used in the present calculations consisted of all available data of osmotic swelling or shrinkage of cells in which adequate data of cell volume against time and cell dimensions were given or could be calculated. These are shown in Table 1, Section A. The values of α and D for various assumed values of L are shown plotted against one another in Fig. 2. It may be seen that the graphs from even this wide variety of cells can be fitted within a common locus in which α ranges from 3×10^{-4} to 7×10^{-3} cm/sec and D ranges from 8×10^{-10} to 2×10^{-8} cm^2/sec. (In order to equalize the range of α and D the locus was chosen as the smallest square capable of intersecting all the curves of cells of Section A.)

Four curves, constructed from the data of cells 20 to 23 in Section B of Table 1, have not been used in finding the common locus, and there were several reasons for their exclusion.

(1) These curves lie well outside the common locus of the other cells. If they were included the common locus would be so enlarged that it would be of no practical significance.

(2) The conventional permeability coefficients of these cells have been shown statistically (Dick, 1959*b*) to be significantly higher than those of other cells in relation to their surface/volume ratio.

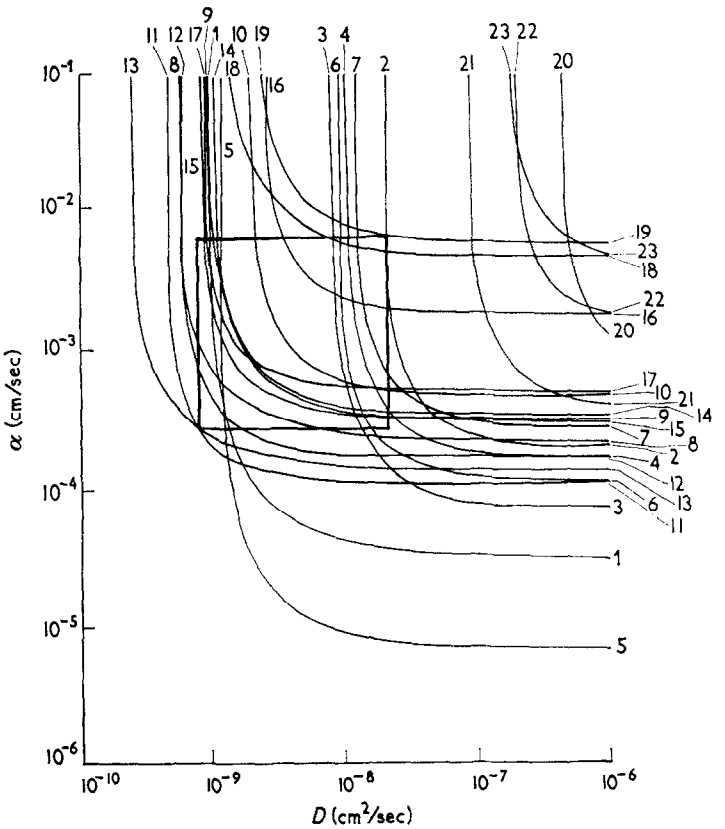

FIG. 2. Each curve represents the corresponding pairs of values of α and D for each single cell. The curves are numbered as in Table 1. The heavy square is the smallest common locus of all curves except those of cells 20 to 23 (see text).

(3) There are two independent points of distinction between these cells and the others.

(a) Unlike the others, they are not free living cells, but are isolated by dissection.

(b) In three of the four cells there is evidence that solute transfer occurred across the cell membrane during osmotic swelling or shrinkage (i.e. the membrane was not in a normal physiological condition) (see Hill (1950*a*); Prescott & Zeuthen, (1953)). This does not apply, however, to the single muscle fibre of Hodgkin & Horowicz (1959) in which osmotic shrinkage was reversible.

Hill (1950*b*) found that the water permeability of axons of *Loligo* and *Sepia* tended to increase with time and concluded that axons which had deteriorated

showed increased water permeability. It has also been noted that chick heart fibroblasts in tissue culture, which show morphological abnormalities, also show increased water permeability (Dick, 1959b).

Prescott & Zeuthen (1953) found that the water permeability coefficients, measured by osmotic swelling, of dissected frog oocytes were 68 times higher than those of undissected oocytes, while the permeabilities of the same cells measured by diffusion of D_2O differed by a factor of only 1·7. They concluded that there were "pores" in the membrane of the dissected cells, which were very much larger than those of corresponding undissected cells.

It has therefore seemed reasonable to conclude that the water permeability of dissected cells is increased and that, whether or not their permeability properties are to be regarded as actually abnormal, they are at any rate not directly comparable with those of free-living cells. It must, however, be noted that the accuracy of the dimensions of the common locus, which have been obtained above, is dependent on the truth of this conclusion.

7. Check on Accuracy of Approximations Used

It is possible to check the accuracy of the calculations in Section 3, 4 and 5 by comparing values of α calculated from equations (25) and (40) when $D \to \infty$ (i.e. $L \to 0$), with values calculated by conventional equations for calculating the permeability coefficient, assuming that the internal solute concentration is always uniform, i.e. that diffusion is infinitely rapid.

When $L \to 0$, it may be shown that the solutions of equations (19), (50) and (60) are respectively

$$\beta_1^2 = 3L \tag{64}$$

$$\beta_1^2 = L \tag{65}$$

and

$$\beta_1^2 = 2L. \tag{66}$$

When (64), (65) and (66) are substituted in (22), (52) and (62) (neglecting terms after the first, since $Dt/a^2 \gg 0{\cdot}1$), there results for the spherical, plane and cylindrical cases respectively

$$\frac{V_e - V_t}{V_e - V_0} \cdot \frac{V_0}{V_t} = \exp(-3\alpha' t/a) \tag{67}$$

$$\frac{V_e - V_t}{V_e - V_0} \cdot \frac{V_0}{V_t} = \exp(-\alpha' t/a) \tag{68}$$

$$\frac{V_e - V_t}{V_e - V_0} \cdot \frac{V_0}{V_t} = \exp(-2\alpha' t/a). \tag{69}$$

TABLE 1

No.	Name of cell	a ($\times 10^{-4}$ cm)	t (sec)	$C_{2(0)}$ (mol-fraction)	V_0	V_t	V_e	b	Reference
				SECTION A					
1.	Frog egg (free)	806	14400	0·0036	1·00	1·18	∞	0·5‡	Prescott & Zeuthen (1953)
2.	Egg of *Chaetopterus pergamentaceus*	55	240	0·0180	1·00	1·33	1·39	0·42	Lucké, Harline & Ricca (1939)
3.	Egg of *Arbacia punctulata*	38·5	177	0·0180	1·00	1·23	1·43	0·36	Lucké, Harline & Ricca (1939)
4.	Egg of *Arbacia punctulata*	39·6	90	0·0180	1·00	1·23	1·41	0·38	Lucké, Ricca & Parpart (1951)
5.	Egg of *Arbacia punctulata*	39·9	840	0·0180	1·00	1·33	1·59	0·12	Lucké, Hartline & McCutcheon (1931)
6.	Egg of *Cumingia tellenoides*	34·1	130	0·0180	1·00	1·34	1·49	0·27	Lucké *et al.* (1939)
7.	Egg of *Ostrea virginica*	28·2	67	0·0180	1·00	1·41	1·59	0·41	Lucké & Ricca (1941)
8.	Gardner mouse ascites tumour†	5·23	5	0·0054	1·00	0·87	0·66	0·32	Lucké, Hempling & Makler (1956)
9.	Murphy-Sturm lymphoma†	4·6	5	0·0054	1·00	0·83	0·69	0·37	Lucké, Hempling & Makler (1956)
10.	Lewis rat lymphoma†	4·37	5	0·0054	1·00	0·80	0·71	0·41	Lucké, Hempling & Makler (1956)
11.	Rabbit leucocyte	4·7	53	0·0054	1·00	1·56	1·80	0·20	Shapiro & Parpart (1937)
12.	Human leucocyte	4·2	27	0·0054	1·00	1·51	1·80	0·21	Shapiro & Parpart (1937)
13.	C3H mouse lymphocyte†	3·38	5	0·0054	1·00	0·83	0·61	0·22	Lucké *et al.* (1956)
14.	Lewis rat lymphocyte†	3·28	5	0·0054	1·00	0·77	0·67	0·36	Lucké *et al.* (1956)
15.	Wistar rat lymphocyte†	3·39	5	0·0054	1·00	0·80	0·69	0·37	Lucké *et al.* (1956)

16.	Erythrocyte (ox)	2·6	2·15	0·0054	1·0	1·7	2·4	0·3‡	Jacobs (1932)
17.	Chick heart fibroblast	1·02(1)	8·5	0·00568	1·00	1·79	2·04	0·18	Dick (1959a)
18.	Erythrocyte (human)	0·7(1)	0·2	0·0054	1·00	1·09	1·46	0·54	Sidel & Solomon (1957)
19.	Erythrocyte (ox)	0·53(1)	0·2	0·0054	1·00	1·06	1·14	0·65	Villegas *et al.* (1958)
				SECTION B					
20.	Axon of *Loligo*	200	66	0·0180	1·00	1·07	1·14	0·2	Hill (1950a, b)
21.	Axon of *Sepia*	100	84	0·0180	1·00	1·06	1·12	0·3	
22.	Frog egg (ovarian)	769	300	0·0036	1·00	1·37	∞	0·26	Prescott & Zeuthen (1953)
23.	Frog muscle fibre	60	9	0·0036	1·00	0·83	0·66	0·2‡	Hodgkin & Horowicz (1959)

† Calculated from additional data kindly supplied by Dr. H. G. Hempling, Department of Physiology, Cornell University Medical College, New York.

‡ Value of b calculated from dry weight measurements other than those in reference cited.

(1) Thickness in case of plane cells.

The above are the data on which the calculations were based. All available data have been used and no attempt has been made to discriminate between values obtained by different techniques although the precision of the resulting data obviously varies very widely. Symbols are: a, cell radius; t, duration of osmotic swelling or shrinkage; $C_{2(0)}$ is the solute concentration of the external solution with which the cell was in equilibrium before the beginning of the experiment (it has been given as a mol-fraction but may be converted into a volume fraction in accordance with equation (9) by multiplying by $\overline{V}_2/\overline{V}_1$, shown on p. 508 to be 0·63); V_0, V_t, V_e and b are given in units of V_0. Section A gives data for free cells and Section B for cells which have been isolated by dissection.

Permeability coefficients calculated from equations (67) and (68) in combination with equation (40) for cells 1 to 19 are shown in column 3 of Table 2. Such values are the horizontal asymptotes of the curves shown in Fig. 2.

TABLE 2

1 Cell no. (see Table 1)	2 Permeability coefficient (from eqns (73), (74), (76)) cm/sec	3 Permeability coefficient (from eqns (67) & (68)) cm/sec	4 Ratio
1	$1{\cdot}09 \times 10^{-4}$	$3{\cdot}15 \times 10^{-5}$	0·35
2	$1{\cdot}03 \times 10^{-3}$	$1{\cdot}97 \times 10^{-4}$	0·19
3	$4{\cdot}49 \times 10^{-4}$	$7{\cdot}30 \times 10^{-5}$	0·16
4	$9{\cdot}55 \times 10^{-4}$	$1{\cdot}65 \times 10^{-4}$	0·17
5	$1{\cdot}36 \times 10^{-4}$	$7{\cdot}02 \times 10^{-6}$	0·05
6	$9{\cdot}15 \times 10^{-4}$	$1{\cdot}11 \times 10^{-4}$	0·12
7	$1{\cdot}62 \times 10^{-3}$	$2{\cdot}69 \times 10^{-4}$	0·17
8	$6{\cdot}40 \times 10^{-4}$	$2{\cdot}16 \times 10^{-4}$	0·34
9	$8{\cdot}27 \times 10^{-4}$	$3{\cdot}12 \times 10^{-4}$	0·38
10	$1{\cdot}15 \times 10^{-3}$	$4{\cdot}60 \times 10^{-4}$	0·40
11	$1{\cdot}40 \times 10^{-3}$	$1{\cdot}09 \times 10^{-4}$	0·08
12	$2{\cdot}14 \times 10^{-3}$	$1{\cdot}65 \times 10^{-4}$	0·08
13	$5{\cdot}79 \times 10^{-4}$	$1{\cdot}36 \times 10^{-4}$	0·24
14	$9{\cdot}25 \times 10^{-4}$	$3{\cdot}32 \times 10^{-4}$	0·36
15	$8{\cdot}05 \times 10^{-4}$	$2{\cdot}98 \times 10^{-4}$	0·37
16	$2{\cdot}19 \times 10^{-2}$	$1{\cdot}78 \times 10^{-3}$	0·08
17	$8{\cdot}95 \times 10^{-4}$	$4{\cdot}96 \times 10^{-4}$	0·55
18	$1{\cdot}43 \times 10^{-2}$	$4{\cdot}63 \times 10^{-3}$	0·32
19	$1{\cdot}42 \times 10^{-2}$	$5{\cdot}90 \times 10^{-3}$	0·42
		Geometrical mean =	0·21

The permeability coefficient in column 2 is that calculated in section 7 from the flux of water across the cell membrane assuming that the interior of the cell is always uniform in composition, i.e. that internal diffusion is infinitely rapid. The permeability coefficient in column 3 is that derived by approximate calculation in sections 3, 4 and 5 allowing for finite internal diffusion but substituting the value $D = \infty$ in the equation. Values in column 3 are generally lower than those in column 2 owing to the approximations used. Column 4 gives the ratio of values in column 3 to those in column 2.

For spherical cells, the conventional permeability coefficient was calculated from the fundamental equation

$$\frac{dV}{dt} = \alpha A(\Pi - \Pi_e) \tag{70}$$

by substituting

$$\Pi = \frac{\Pi_0(V_0 - b)}{V - b} \tag{71}$$

and

$$A = (36\pi)^{\frac{1}{3}} V^{\frac{2}{3}} \tag{72}$$

so that after integration there results

$$\alpha = \frac{V_e - b}{V_0 - b} \cdot \frac{1}{(36\pi)^{\frac{1}{3}} \Pi_0 t} \cdot$$

$$\cdot \left[\left(1 - \frac{b}{V_e}\right) V_e^{\frac{2}{3}} \left(\tfrac{1}{2} \ln \frac{V_e^{\frac{2}{3}} + V_e^{\frac{1}{3}} V^{\frac{1}{3}} + V^{\frac{2}{3}}}{(V_e^{\frac{1}{3}} - V^{\frac{1}{3}})^2} + \sqrt{3} \arctan \frac{2V^{\frac{1}{3}} + V_e^{\frac{1}{3}}}{\sqrt{3}\, V_e^{\frac{1}{3}}} \right) - 3V_e^{\frac{2}{3}} \right]_{V_0}^{V_t} . \tag{73}$$

Values of α were thus calculated by the use of a Mercury computer for cells 2 to 16 and are shown in column 2 of Table 2. For cell 1 since $V_e = \infty$ the following modified equation was used

$$\alpha = \frac{\frac{1}{2}(V_t^{\frac{2}{3}} - V_0^{\frac{2}{3}}) - 2b(V_t^{\frac{1}{3}} - V_0^{\frac{1}{3}})}{(36\pi)^{\frac{1}{3}} \Pi_0 (V_0 - b) t} . \tag{74}$$

For the plane cells 17 to 19 the fundamental equation (70) was solved by substituting by means of (71) and

$$A = \frac{V_0}{x} \tag{75}$$

(where x = cell thickness), thus giving

$$\alpha = \frac{(V_e - b)x}{\Pi_0 (V_0 - b) V_0 t} \left[V_0 - V_t - (V_e - b) \ln \frac{V_t - V_e}{V_0 - V_e} \right]. \tag{76}$$

Such values of α are also shown in column 2 of Table 2.

It may be seen that due to the approximations used in the calculations values in column 3 are generally lower than those in column 2. The ratio of column 3 to column 2 values is shown in column 4. The geometrical mean ratio is 0·21 and all ratios except two lie within the range $\frac{0{\cdot}21}{\sqrt{10}}$ to $0{\cdot}21\sqrt{10}$ so that, provided the permeability coefficients in column 3 are corrected by dividing by the factor 0·21, they are then likely to lie within the correct order of magnitude.

Since the most inaccurate approximations occur in the derivation of equation (40) which is used only in the calculation of the permeability coefficient, no serious discrepancy is to be expected in the calculated values of the diffusion coefficient, D. In fact in the limiting case when $\alpha \to \infty$, i.e. $L \to \infty$, values of D can be calculated with only a small approximation. Such values are represented by the vertical asymptotes of the curves in Fig. 2; they do not deviate significantly from the rest of the curve.

8. Discussion

It would, of course, be preferable to make direct measurements of both the parameters α and D separately. This has not so far been found possible. Even if measurements were made, however, it is difficult to imagine techniques free from physiological or technical objections. The merit of the experimental data on which the present analysis is based is that the cells are studied under normal physiological conditions with no greater interference than a degree of swelling or shrinkage. Although the limits of the estimates of α and D are somewhat wide, it seems likely that they are of the correct order of magnitude and apply to the living cell under physiological conditions. The finding of Robertson (1959) that the surface membranes of a wide variety of cells have a similar structure may be considered to strengthen the assumption on which the calculation is based. The width of the limits may, of course, simply be due to the quite considerable errors in the difficult techniques of measuring water flow or to the approximations used in the above calculations. On the other hand, it may be due to a real scatter of the values of α and D, since, while the present calculation depends upon the assumption that the values of α and D are similar for all cells, it is not to be expected that they are actually identical.

The above range of values of D may be compared with mutual diffusion coefficients for protein-water systems which lie for the most part between 10^{-7} and 10^{-6} cm^2/sec (Edsall, 1953). However, it must be noted that these values have been extrapolated to infinite dilution. In protein concentrations comparable to those inside living cells (*c.* 20%) the mutual diffusion coefficient must be much lower, but unfortunately data at such concentrations are not available. However, it has already been shown in section 2 that the mutual diffusion coefficient approximates closely to the self-diffusion coefficient of the slow component. Thus the figure of $8{\cdot}7 \times 10^{-8}$ cm^2/sec given by Wang *et al.* (1954) for the self-diffusion coefficient of ovalbumin in 24·4% solution makes a more realistic comparison with the value of D obtained in the present calculations. Since protein is not necessarily the slowest-moving component in the cell cytoplasm, the mutual diffusion coefficient for nucleic acid given by Jordan (1960), from the average of values from several sources, as 2 to 3×10^{-8} cm^2/sec may also be a relevant comparison (values at high nucleic acid concentrations would probably be even lower). Thus the range of values of D found, i.e. from 8×10^{-10} to 2×10^{-8} cm^2/sec is not unreasonable in comparison with *in vitro* measurements on various cell components. It must further be remembered that intracellular membranes such as possibly the endoplasmic reticulum or the nuclear membrane might also tend to impede water diffusion in the cell cytoplasm.

In order to avoid confusion, it may be useful to note three measurements with which, as discussed in section 2, the present value of D cannot properly be compared (1) the value of $1{\cdot}5\times10^{-5}$ cm^2/sec found for the diffusion coefficient of KCl in the giant axon of *Sepia* by Hodgkin & Keynes (1953), (2) the value of $1{\cdot}6\times10^{-6}$ cm^2/sec, which may be calculated from the data of Nevis (1958) for the diffusion coefficient of ^3H_2O in the giant axon of *Loligo* (assuming the resistance of the cell membrane to water to be negligible), and (3) the value of $4{\cdot}76\times10^{-6}$ cm^2/sec obtained by Løvtrup (1963) for the diffusion coefficient of ^2H_2O in frog oocytes. In none of these measurements does any cell swelling occur so that what is measured is the rate of diffusion of ions or tracer water in the aqueous component of the macromolecular solution which makes up the cytoplasm: the macromolecules are not themselves displaced. The appropriate *in vitro* comparisons for the findings of Hodgkin & Keynes, Nevis and Løvtrup respectively are the value of $1{\cdot}52\times10^{-5}$ cm^2/sec obtained by Ogston (1949) for the diffusion coefficient of KCl in lactoglobulin solution and the value of $0{\cdot}978\times10^{-5}$ cm^2/sec obtained by Wang *et al.* (1954) for the diffusion of H_2O^{18} in a 24·5% solution of ovalbumin.

Fenichel & Horowicz (1963) have recently concluded, somewhat in contradiction to the results of Hodgkin & Keynes, Nevis & Løvtrup, that the efflux of n-butanol, propionamide, and thiourea from frog muscle is limited by cytoplasmic-diffusion rather than by surface resistance and have calculated diffusion coefficients ranging from $4{\cdot}6\times10^{-10}$cm^2/sec for thiourea at 5°C to $2{\cdot}7\times10^{-8}$cm^2/sec for n-butanol at 20°C. Since there was presumably little or no shrinkage or swelling of the cells, protein–water mutual diffusion coefficients naturally do not make appropriate comparisons in these experiments in spite of the fact that the magnitude of the diffusion coefficients obtained agree well with the present calculations. The appropriate *in vitro* comparison would be with the diffusion coefficient of the non-electrolytes in a concentrated protein solution and since the molecules are small this would in general be expected to be high. Fenichel & Horowicz have attributed the low diffusion coefficients to an ordered ice-like structure in the cytoplasmic water presumably of greater degree than that present in a protein solution *in vitro*. This is quite a reasonable explanation, but it must be noted that the explanation and indeed the physical conditions of their experiments, i.e. lack of change in cell volume, are quite different from those of the present data although the values of the diffusion coefficients obtained are remarkably similar. It may be suggested that the possible retarding effect of intracellular membranes, mentioned above, may lower the diffusion coefficients both in the present case and also in that described by Fenichel & Horowicz, and is therefore a tentative but feasible explanation in both cases.

It may be noted that Dainty (1963) has indicated that an unstirred layer on the surface of cells during experiments on the diffusion of tracer water may have a decisive effect on the apparent permeability coefficient obtained. However, Dainty has also calculated that the effect on the apparent permeability coefficients to osmotic flow, such as that calculated in the present experiments, will be very much less (it will be about 2% lower than the real value depending on the diffusion coefficient of the external solutes). Thus it is to be expected that external unstirred layers of water will not have a significant effect in the present interpretation of experiments based on osmotic flow of water.

If it is assumed that

$$\alpha = \frac{D'}{d} \tag{77}$$

where D' is the average diffusion coefficient of water in the cell membrane and d is the thickness of the membrane, then provided the value of d is known, D' may be calculated. If d is taken as 75 Å, the thickness of the unit membrane described at the surface of a variety of cells by Robertson (1959), then the range of values of D' is from $2{\cdot}3 \times 10^{-10}$ to $5{\cdot}3 \times 10^{-9}$ cm^2/sec. Since this is smaller than the diffusion coefficient of D calculated for the interior of the cell, the membrane might be regarded as a uniform layer having a somewhat greater degree of resistivity to water flow than the cytoplasm. On the other hand if there are channels or pores in the cell membrane in which the diffusion coefficient for water is of the same order as in free solution, i.e. $2{\cdot}4 \times 10^{-5}$ cm^2/sec, then it must be concluded that such channels occupy only a fraction between 10^{-4} and 10^{-5} of the surface area of the cell membrane. This figure may be compared with the fractional pore area of 10^{-4} calculated by Villegas, Barton & Solomon (1958) for the erythrocytes of ox, dog and man assuming a membrane thickness of 50 Å. It must further be remembered that the above range of values of α should probably be divided by the correction factor 0·21 to take account of the approximations inherent in the present calculations as discussed in section 7. The values of D' and of the apparent fractional pore area would also have to be correspondingly increased.

The values of both α and D calculated above are clearly highly significant cell parameters whose values can be interpreted in terms of the structure of the cell membrane and of the cytoplasm. Unfortunately, owing to the great complexity of the necessary mathematical model and to the approximations resulting from this used in the present calculations, the values of α and D given cannot be taken as indicating more than the order of magnitude of the true values. It can only be hoped that the present paper may serve to stimu-

late the production of more accurate data and also of a more exact mathematical formulation.

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Appendix I

It is desired to evaluate

$$\frac{\left(\frac{\partial C_1}{\partial r}\right)_{r=a} \Big/ \left(\frac{\partial C}{\partial r}\right)_{r=a}}{(C_{1(a)}-C_{1(e)})/(C_a-C_e)} = A \tag{1'}$$

$$\left(\frac{\partial C_1}{\partial r}\right)_{r=a} = \partial/\partial r \left(\frac{\mathrm{d}V_1}{\mathrm{d}(V_1+V_2)}\right)_{r=a}$$

$$\approx \partial/\partial r \left(1 - \frac{\mathrm{d}V_2}{\mathrm{d}V_1}\right)_{r=a} \qquad \text{(since } V_1 \gg V_2\text{)}$$

$$= \partial/\partial r \left(-\frac{\mathrm{d}V_2}{\mathrm{d}V_1}\right)_{r=a} \tag{2'}$$

$$\left(\frac{\partial C}{\partial r}\right)_{r=a} = \partial/\partial r \left(\frac{\mathrm{d}V_1}{\mathrm{d}(V_1+V_2-V_3)}\right)_{r=a}$$

$$\approx \partial/\partial r \left(1 - \frac{\mathrm{d}V_2}{\mathrm{d}V_1} - \frac{\mathrm{d}V_3}{\mathrm{d}V_1}\right)_{r=a} \qquad \text{(since } V_1 \gg V_2 \text{ or } V_3\text{)}$$

$$\approx \partial/\partial r \left(-\frac{\mathrm{d}V_3}{\mathrm{d}V_1}\right)_{r=a} \tag{3'}$$

(assuming that $V_3 \gg V_2$, i.e. the volume of macromolecular solute, V_3, is always much greater than the volume of salts, V_2, in spite of the fact that $n_2 \gg n_3$).

Thus

$$\frac{\left(\frac{\partial C_1}{\partial r}\right)_{r=a}}{\left(\frac{\partial C}{\partial r}\right)_{r=a}} \approx \left(\frac{\mathrm{d}V_2}{\mathrm{d}V_3}\right)_{r=a} \tag{4'}$$

Also

$$C_{1(a)}-C_{1(e)}=\left(\frac{dV_1}{d(V_1+V_2)}\right)_{r=a}-\frac{V_{1(e)}}{V_{1(e)}+V_{2(e)}}$$

$$\approx 1-\left(\frac{dV_2}{dV_1}\right)_{r=a}-1+\frac{V_{2(e)}}{V_{1(e)}} \quad \text{(since } V_2 \ll V_1\text{)}$$

$$=-\left(\frac{dV_2}{dV_1}\right)_{r=a}+\frac{V_{2(e)}}{V_{1(e)}} \tag{5'}$$

and

$$C_a-C_e=\left(\frac{dV_1}{d(V_1+V_2+V_3)}\right)_{r=a}-\left(\frac{V_1}{V_1+V_2+V_3}\right)_{t=\infty}$$

$$\approx 1-\left(\frac{dV_2}{dV_1}\right)_{r=a}-\left(\frac{dV_3}{dV_1}\right)_{r=a}-1+\left(\frac{V_2}{V_1}\right)_{t=\infty}+\left(\frac{V_3}{V_1}\right)_{t=\infty}$$

(since $V_1 \gg V_2$ or V_3) so

$$C_a-C_e \approx -\left(\frac{dV_3}{dV_1}\right)_{r=a}+\left(\frac{V_3}{V_1}\right)_{t=\infty} \tag{6'}$$

(since $V_3 \gg V_2$).

Thus combining (1'), (4'), (5') and (6')

$$A \approx \frac{\left(\frac{dV_2}{dV_3}\right)_{r=a}\left[-\left(\frac{dV_3}{dV_1}\right)_{r=a}+\left(\frac{V_3}{V_1}\right)_{t=\infty}\right]}{-\left(\frac{dV_2}{dV_1}\right)_{r=a}+\left(\frac{V_{2(e)}}{V_{1(e)}}\right)} \tag{7'}$$

$$\approx \frac{-\left(\frac{dV_2}{dV_1}\right)_{r=a}+\left(\frac{dV_2}{dV_3}\right)_{r=a}\left(\frac{V_3}{V_1}\right)_{t=\infty}}{-\left(\frac{dV_2}{dV_1}\right)_{r=a}+\left(\frac{V_{2(e)}}{V_{1(e)}}\right)} \tag{8'}$$

But if it is assumed that $\left(\frac{dV_2}{dV_3}\right) r=a$ remains approximately constant during osmotic flow (and since the region near the cell membrane is almost unaffected by diffusional delays, this is probably quite a close approximation), then

$$\left(\frac{dV_2}{dV_3}\right)_{r=a}=\left(\frac{dV_2}{dV_3}\right)_{r=a,\,t=\infty}=\left(\frac{V_2}{V_3}\right)_{t=\infty} \tag{9'}$$

(since at equilibrium internal concentrations are uniform through the cell), so that

$$\left(\frac{V_3}{V_1}\right)_{t=\infty}\left(\frac{dV_2}{dV_3}\right)_{r=a}=\left(\frac{V_3}{V_1}\right)\left(\frac{V_2}{V_3}\right)_{t=\infty}=\left(\frac{V_2}{V_1}\right)_{t=\infty}. \tag{10'}$$

But

$$\frac{V_{2(e)}}{V_{1(e)}} = \left(\frac{V_2}{V_1}\right)_{t=\infty} \tag{11'}$$

Therefore the R.H.S. of (8) is equal to unity, i.e. $A \approx 1$.

The accuracy of this and the other approximations has been tested as described in section 7.

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