THE EFFECTS PRODUCED BY DIFFUSION IN AQUEOUS SYSTEMS CONTAINING MEMBRANES

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CONTENTS

I. INTRODUCTION

The movement of molecules and ions through membranes is of fundamental biological importance, inasmuch as the entire content of all living cells has entered these cells through membranes permeable only to molecules and ions. In spite of the importance of this movement, the mechanism by which cells obtain a vital fraction of their contents at the expense of osmotic work is still unknown. Simple diffusion accounts for a large part of the movements of substances in and out of living systems but is of itself insufficient to account for all the observed facts. It is the purpose of this review to point out the various effects that can be produced by diffusion through membranes, so that effects that cannot be explained by simple diffusion will be more clearly apparent.

There have been an almost embarrassing number of reviews of various aspects of diffusion and permeability. In fact, it would hardly be an exaggeration to say that there are more reviews of diffusion than accurate measurements of diffusion coefficients. The most recent contributions are found in a symposium held by the New York Academy of Sciences (7, 35, 65, 81). The basic theory of molecular diffusion has been treated by Williams and Cady (107) and by Neurath (79) in this Journal and by Duclaux (27). Kincaid, Eyring, and Sterns (60) have contributed a treatment of the kinetics of diffusion, which is especially applicable to diffusion through dense structures such as membranes. Barrer has treated the general subject of diffusion in solids; his book (3) contains two chapters on the diffusion of gases and vapors through organic membranes. The permeability

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of natural and artificial membranes has been discussed in a symposium of the Faraday Society in 1937 (12, 13, 16, 61, 73, 94, 105) and in the Cold Spring Harbor Symposium of 1940 (49, 58, 66, 68, 82, 83). The literature on permeability has been periodically reviewed in the *Annual Reviews of Biochemistry* through 1937 (14, 51, 52, 57) and since then in the *Annual Reviews of Physiology* (8, 10, 59). Krogh (62) has reviewed numerous cases of the active transport of water and salts between aquatic animals and their environment.

II. DIFFUSION IN BULK SYSTEMS

A. Thermodynamic considerations

Diffusion is fundamentally a process that equalizes the activities of substances which are free to move in solution. In simple systems substances always diffuse from the higher to the lower activity. While it is possible to set up models in which some components move in the reverse direction from a lower to a higher activity, this can only be done at the expense of energy coming from heat, pressure, an external electrical or gravitational field, or *the diffusion of some other substance* (84). In the absence of activity differences, molecules will still move about but there will be no net movement, since equal quantities will move in opposite directions. It is often possible to represent diffusion between any two concentrations by the algebraic sum of the diffusion from each concentration into pure solvent. Whereas it was at one time popular to talk of membranes with irreciprocal permeability (28, 36, 102, 103) such that a solute could move faster across the membrane in one direction than in the other, the above considerations at once show that diffusion through such a membrane would result in the movement of material from a lower to a higher concentration, a change which requires work. This work in a non-reacting membrane could only come from the heat content of the system, and the system would thus violate the Second Law of Thermodynamics. While it is possible to visualize a membrane containing trap doors of colloidal dimensions which would thus violate the Second Law, no evidence has yet been presented to indicate that such a membrane actually exists.

B. The diffusion coefficient

The diffusion of molecules in solution is described mathematically by Fick's law:

$$\mathrm{d}Q/\mathrm{d}t = DA\ \mathrm{d}c/\mathrm{d}x \tag{1}$$

where Q is the quantity of material which crosses a plane of constant concentration c and area A per unit of time t. D is a proportionality factor, called the diffusion coefficient. As given, Fick's law is just a definition which is useful only because the proportionality factor D is, in many cases, nearly independent of concentration. Since the mathematical treatment of diffusion is greatly simplified by the assumption that D is a constant at a given temperature, D is often called the diffusion constant. Figure 1 shows how D varies with concentration for a number of salts which are of physiological interest. The variations are of the order of 5–30 per cent from infinite dilution up to 1 molar.

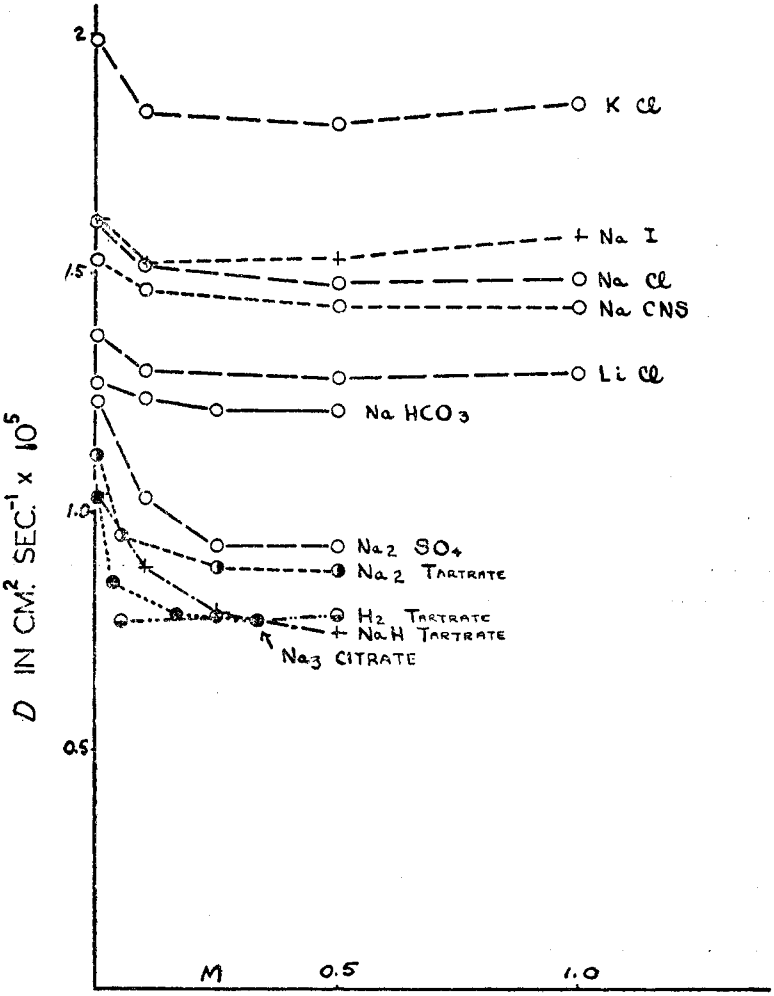

FIG. 1. Diffusion coefficients of some physiologically important electrolytes, recalculated from the data of Vinograd and McBain (99) according to Gordon (35).

When D varies with concentration, the integral diffusion coefficient that is measured is a weighted average of all the diffusion coefficients at all the concentrations through which diffusion is taking place. Nearly all the published values of diffusion coefficients refer to average or integral diffusion coefficients for which the symbol $\bar{D}$ will be used. The relationship between D and $\bar{D}$ is given by

$$D = \bar{D} + c\, \mathrm{d}\bar{D}/\mathrm{d}c \qquad (2a)$$

or

$$\frac{\bar{D}}{c_1 - c_2} = \int_{c_2}^{c_1} D \, \mathrm{d}c \tag{2b}$$

(65), where c_1 and c_2 are the concentrations between which diffusion is taking place. c_2 is frequently zero. Actual diffusion experiments always produce a change in the concentration of the diffusing substance. The errors introduced by assuming that $\bar{D}$ is actually being measured are negligible unless D is changing rapidly with concentration. Gordon (35) gives a method of successive approximations to determine D from $\bar{D}$ measured in diaphragm cells. When D falls rapidly as the concentration is increased, it can be shown that the integral diffusion coefficient measured in a diaphragm cell will fall more rapidly than the true value of $\bar{D}$. In the case of colloidal electrolytes this can result in negative values for D calculated from $\bar{D}$, if correction is not made for the finite change in concentration during the experiment (23).

Molecules move from one concentration to another because there is a difference of chemical potential μ ($\bar{F}$ of Lewis and Randall) between the two concentrations (44). For ideal dilute solutions of molecules

$$\mathrm{d}Q/\mathrm{d}t = -uRTA \, \mathrm{d}c/\mathrm{d}x \tag{3}$$

where u is the mobility of the molecule, R is the gas constant, and T is the absolute temperature. Therefore,

$$D = uRT \tag{4}$$

For ions the Nernst (78) equation for ion movement is

$$\mathrm{d}Q/\mathrm{d}t = -\frac{uA}{z}(RT \, \mathrm{d}c/\mathrm{d}x \pm cxF \, \mathrm{d}E/\mathrm{d}x) \tag{5}$$

where z is the valence of the ion without regard for sign, u is the equivalent mobility of the ion, c is the equivalent concentration of the ion, F is Faraday's constant, and E is the electric field in the direction of diffusion (99). $R = 6.66$ if u is measured in customary electrical units and D in cm.^{2} per second.

In non-ideal solutions, neglecting volume changes,

$$D = -uRT(1 + c \, \mathrm{d} \ln y/\mathrm{d}c) \tag{6}$$

where y is the activity coefficient (44, 65). The mobility, u, may vary with concentration. As a first approximation

$$u = u_0\eta_0/\eta \tag{7}$$

where u_0 is the mobility at infinite dilution, η_0 is the viscosity of the solvent; and η is the viscosity of the solution (but see Onsager (81)).

Variations in u may occur for other reasons besides viscosity; one of these is a change in hydration with concentration, which alters the size of the diffusing molecules. For example, discrepancies between calculated and observed diffusion coefficients for sucrose in concentrated solution may perhaps be explained by considering that the water of hydration is greater in dilute solutions than in concentrated solutions (63, 79). Sucrose is, however, an extreme case, and in general the viscosity factor can be trusted to give a slight overcorrection both for diffusion and for conductivity, except in the case of large molecules such as proteins and high polymers (7).

Sutherland (95) and also Einstein (30) equated the osmotic force acting on a diffusing molecule with the resistance to movement of a sphere in a continuous liquid given by Stokes's law. As usually quoted, the so-called Stokes-Einstein equation is

$$D = RT/6\pi r\eta N \tag{8}$$

where r is the radius of the molecule and N is Avogadro's number (79). For molecules which do not obey the ideal gas laws the diffusion coefficient should be corrected by the factor $(1 + c\ \mathrm{d} \ln y/\mathrm{d}c)$.

In practice for large molecules where the Stokes-Einstein equation applies, the gradient of the activity coefficient is rarely known except for some recent accurate work on carefully purified high polymers (71) and a few measurements on proteins. For small molecules such as sugars, the errors inherent in the derivation of the Stokes-Einstein equation are such that minor corrections for deviations from ideality are insignificant. From a practical point of view, a knowledge of the activity coefficient implies a knowledge of the molecular weight more accurate than can ever be obtained from diffusion data by use of the Stokes-Einstein equation.

It has not been generally noted that the Stokes-Einstein equation applies only to the differential diffusion coefficient D and can be applied to the integral diffusion coefficient $\bar{D}$ only when the diffusion coefficient is a true constant independent of concentration. If the diffusion coefficient varies with concentration, it is best where possible to extrapolate the data to infinite dilution, obtaining D_0, since

$$D_0 = \lim_{c \to 0} D = \lim_{c \to 0} \bar{D} \tag{9}$$

This extrapolation avoids all complications caused by changes in activity and viscosity with concentration, as well as secondary steric effects which may be serious for many high-molecular-weight compounds (7).

In general, even in favorable cases, diffusion measurements alone cannot provide values of the molecular weight to within better than 5 per cent accuracy. They are, however, useful, inasmuch as they are often the only measurements available for substances with biological activity that have not been purified.

C. Measurement of the diffusion coefficient

Longsworth (65) has adequately summarized the various methods available for the measurement of diffusion coefficients in liquids. In the opinion of the author, insufficient consideration has been given to the necessity of eliminating convection currents in diffusion cells. This can be done by the use of thin cells or capillaries of known size for absolute methods. According to the calculations of Young (108), wall effects are not likely to be important at dimensions greater than 10^{-7} cm., so that a refinement of the method of Nakamura (77) or the modification used by Zuber (109) (see also Furth (34)) should give valid diffusion coefficients with a cell thickness of 0.1 mm., which is thin enough to eliminate convection.

The diffusion coefficient in a homogeneous membrane which is bathed on one side by a solution at constant composition can be determined from the time required to reach a steady state (2, 3). This is especially convenient if optical or electrical methods for continuously recording concentration are available. This method is unfortunately not applicable to sintered-glass membranes, because of the presence of cul-de-sacs. The sintered-glass cells introduced by Northrup and Anson (80) must be calibrated by reference to a known substance. McBain's students (99) have used 0.1 N potassium chloride and have calculated a value for $\bar{D}$ at 25°C. from the measurements of Cohen and Bruins (12) at 20°C. of 1.631 cm.^{2}/day, equivalent to 1.888×10^{-5} cm.^{2}/sec. Gordon (35) points out that the value should be 1.838×10^{-5} for the conditions used. In his opinion the method is capable of a relative accuracy of 0.1 per cent, though it must be admitted that deviations of 1 per cent or greater are present in most of the published work.

D. Units of the diffusion coefficient and the diffusion of water

Rashevsky and Landahl (83) have reviewed some of the mathematical aspects of membrane permeability. They point out that the diffusion coefficient should be expressed in cm.^{2} sec.$^{-1}$ instead of in any one of a large number of complicated expressions that have often been used. Diffusion coefficients expressed in cm.^{2}/day should be divided by 86,400 to reduce them to cm.^{2} sec^{-1}. Many people have difficulty at first understanding why the diffusion coefficient is centimeters *squared* per second instead of centimeters per second, since the diffusion coefficient is proportional to the ionic mobility, which is a velocity per unit force. Fick's law (equation 1) can be rewritten as:

$$-D = \mathrm{d}Q \cdot \mathrm{d}x / \mathrm{d}t \cdot \mathrm{d}c \cdot A \tag{1b}$$

Substituting for the quantities their units we obtain for the units of D:

$$D = Q \cdot \text{cm./sec.}\ (Q/\text{cm.}^3)\ \text{cm.}^2 = \text{cm.}^2\ \text{sec.}^{-1} \tag{1c}$$

The quantity transported, Q, may be expressed in any suitable units provided the concentration is expressed in the same units per cubic centimeter. Under these conditions the units of Q will cancel. Diffusion coefficients are usually calculated from the change in concentration of a given volume, and in such cases the units of concentration, whether moles per liter, milligrams per 100 cc., colorimeter readings, rat units per cubic centimeter, or any other, are immaterial so long as they are expressed on a volume basis. Weight normality or weight per cent concentrations are not suitable for use in diffusion calculations. Activity coefficients must also be converted to a volume concentration basis (43).

Water will move through a porous membrane at a rate proportional to the difference in hydrostatic pressure on the two sides of the membrane. If the membrane is semipermeable, water will also move at a rate proportional to the difference in osmotic pressure (or activity of the water) on the two sides of the membrane. The net rate of water movement across a rigid semipermeable membrane is proportional to the difference between the osmotic and the hydrostatic pressures. Eyster (31) has reviewed the definitions that have been used for osmosis and osmotic pressure.

The permeability of a membrane to water is expressed by Krogh (62) by the number of minutes necessary to pass 1 cm.^{3} through 1 cm.^{2} of membrane under a pressure of 1 atm. Lucké (66) expresses permeability in terms of the number of cubic micra crossing a membrane per minute per square micron per atmosphere of difference in osmotic pressure. Both of these units neglect the thickness of the membrane. Rashevsky (83) points out that the correct unit for water permeability is cm. sec. when the thickness is unknown; pressure must be expressed in dynes cm.$^{-3}$ and water movement in grams. If water movement is expressed in cm.^{3}, the units of membrane permeability are cm. dyne^{-1} sec.$^{-1}$ Although it is formally possible to express the osmotic pressure as a difference in molar concentration and determine a diffusion coefficient for water in units of cm.^{2} sec.$^{-1}$ by expressing the water movement in moles, the validity of this formalism is very doubtful, particularly since very few membranes are truly semipermeable.

E. The diffusion of electrolytes

When a salt diffuses, the separate ions formed in solution cannot diffuse independently, although their mobilities may be different. When one ion starts to move ahead of the other because of its higher mobility, the resultant separation of charge produces an electric field which retards the fast ion and accelerates the slow ion, so that both move at the same rate. This electric field appears as the liquid-junction potential whenever a potential is measured between two different solutions.

Vinograd and McBain (99) discuss the diffusion of idealized mixtures of salts. They derive from the classical Nernst equation (equation 5) an exact differential equation for the diffusion of any ion in a mixture of other ions in ideal solution. Their equation has a tractable, integrable form only when the diffusing salts all have linear concentration gradients. Since linear gradients, even if produced artificially, will not persist when more than one salt is diffusing, the integral form must be inexact for real cases. However, it does give a good first approximation that is surprisingly close to the experimental findings. The assumption of linear concentration gradients is incidentally the same assumption made by Henderson (47) to obtain his well-known equation for the diffusion potential.

The integral form of the diffusion equation for one ion in a mixture of salts all diffusing into pure water is,

$$\bar{D}_+ = u_+RT/z\left(1 - z\,\frac{\Sigma m_+c_+/z_+ - \Sigma u_-c_-z_-}{\Sigma u_\pm c_\pm}\right) \tag{10}$$

For anions the minus sign is changed to plus. The symbols have the same meaning as in equation 5. For the purposes of calculation, u and v can as a first approximation be taken from the values at infinite dilution. A better value is obtained from the product of the mean transport numbers and the observed diffusion coefficient of the salts in question. The second term inside the parentheses is the contribution of the electric field set up by the diffusion to the movement of the ion in question. For the diffusion of a single salt, equation 10 reduces to the Haskell equation (46):

$$D = \left(\frac{1}{z_+} + \frac{1}{z_-}\right)RT \Big/ \left(\frac{1}{u_+} + \frac{1}{u_-}\right) \tag{11}$$

Vinograd and McBain (99) apply their equation to the diffusion of mixtures of two salts and compare the experimental and theoretical results. The agreement is very good. The qualitative conclusions are that a cation present in relatively small amount will be accelerated by the accompanying diffusion of a salt with a slower cation than anion and will be retarded by a salt with a faster cation than anion. Conversely for an anion present in small amount. For example they reported that when a mixture of 0.01 N hydrochloric acid and 0.466 N barium chloride diffused into pure water the hydrogen ion had a diffusion coefficient of 9.50 cm.^{2}/day, a value which is 18 per cent greater than the calculated value for the free diffusion of hydrogen ions (8.05 cm.^{2}/day at 25°C.).

When one or more salts are initially at the same concentration throughout the system, the diffusion of other ions is not given by equation 10. If it is assumed that the concentration gradients of the diffusing ions are linear and that the supporting electrolyte (those ions which have no concentration gradient) does not move, it is possible to integrate the differential equation given by Vinograd and McBain (99). Dean (22) obtains the equation:

$$\bar{D} = D_0\left(1 \mp \frac{z\Delta}{\Lambda_s}\left(1 - \frac{\Lambda_0}{\Lambda_s}\ln\left(1 + \frac{\Lambda_0}{\Lambda_s}\right)\right)\right) \tag{12}$$

where $\Delta = \Sigma u_+c_+/z_+ - \Sigma u_+c_-/z_-$ for the diffusing salt,
$\Lambda_s = \Sigma u_+c_+ =$ the conductivity of the diffusing salt, and
$\Lambda_0 = \Sigma u_+c_+ =$ the conductivity of the supporting electrolyte.

The experimental results are quite close to those predicted by equation 12, in spite of the fact that it is based on approximations. Equation 12 predicts that the effect of a supporting electrolyte will be determined only by its conductivity relative to that of the diffusing salt and will be independent of the relative ionic mobilities of the non-diffusing salt. The supporting electrolyte does actually move in amounts which are of the same order of magnitude as the movement of the diffusing ions. Nevertheless, this movement has a relatively small influence on the movement of the diffusing ions when the supporting electrolyte is present in excess.

When an electric current is applied to a diffusing system, the ions are moved by a summation of electrical and diffusive forces. The current can accelerate or retard the motion of the ions and can even cause a reversal of the direction of movement of some of the ions. In the absence of diffusion the effects are those of Hittorf transport.

Substances normally insoluble in water may be solubilized in detergent solutions to form homogeneous solutions. Solubilization appears to be the result of an association between one or more molecules of the water-insoluble material and colloidal micelles which may contain several hundred detergent molecules. The movement of solubilized materials in solution therefore depends almost entirely on the movement of the corresponding detergent micelles (26). If the detergent is diffusing it will carry the solubilized material with it. When there is no net movement of the detergent, there will still be a slow exchange of micelles full of solubilized material. A membrane such as cellophane, impermeable to micelles but permeable to molecules, may also be permeable to molecules of the solubilized material, which leave their micelles on one side of the membrane and enter new micelles on the other side.

The drag effect of a diffusion stream on other molecules in the medium has been observed by McBain and Dawson (69) and likened to the effect produced by a Langmuir diffusion pump. Young (108) has considered some of the mathematical theory, and Brooks (10) has reviewed recent contributions. In general, the drag effect appears to be a very inefficient mechanism for producing movement of molecules against an activity gradient.

III. DIFFUSION IN MEMBRANES

A. Diffusion of non-electrolytes through membranes

Diffusion takes place in the relatively wide-pored membranes of sintered-glass filters at exactly the same rate as if glass were not there, with the advantage that the diffusion is not disturbed by convection currents (70, 80).

Membranes with small pores, such as collodion, cellophane, parchment, and many biological membranes, greatly slow down or stop the diffusion of large molecules and ions, while offering relatively little resistance to the movement of small particles.[3] This is the so-called sieve effect, which is of practical use in dialysis. For example, if a protein solution containing a salt is placed in a cellophane or Visking sausage skin sack and suspended in running water the small salt ions will diffuse out, leaving all the large protein molecules behind in the sack.

Another important aspect of the permeability of biological membranes is their so-called lipoid solubility. Experimentally it has been found that a large number of cell membranes are more permeable to substances that are soluble in oils than to substances insoluble in oils. The explanation of this phenomenon visualizes the membrane as a separate phase having lipoidal characteristics.

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[3] There has been considerable discussion in the literature with regard to whether highly dried collodion contains pores. There is now agreement that partially dried collodion is a typical porous membrane (88), and since highly dried collodion is practically impermeable to dissolved solutes, the distinction is not of great interest here.

The concentration of the diffusing substance inside the membrane at either boundary will be l times the concentration in the water outside. The partition fraction, l, is defined by the equation:

$$l = \frac{\text{concentration in membrane phase}}{\text{concentration in water}}$$

The rate of diffusion across the membrane is, of course, dependent on the absolute difference in concentration of the diffusing substance inside the two boundaries. Therefore substances which have a high partition fraction, l, will diffuse more rapidly across the membrane. A classical example of this phenomenon is given by a membrane of vulcanized rubber, which is freely permeable to benzene and almost impermeable to water. Benzene is soluble in rubber, whereas water is insoluble in the rubber and can penetrate only through pores or traces of impurities in the rubber.

For many years the sieve theory and the lipoid solubility theory were held by different schools as rival theories explaining the permeability of living membranes. In 1935 Wilbrandt (104) showed that the two theories were not incompatible, and in 1937 Collander (16) summarized a remarkable series of careful diffusion measurements which showed that both molecular volume and lipoid solubility controlled the diffusion of non-electrolytes through a large number of plant-cell membranes.

At about the same time it became apparent from electrical measurements (13) that most vital membranes are extremely thin, perhaps only one or two molecules thick. In such thin membranes the distinction between pores and solubility breaks down and the rival theories appear as different aspects of the same phenomenon.

Fortunately for the mathematical treatment of membrane permeability it makes no difference which theory is used. In a thin membrane diffusion very rapidly reaches a steady state with a linear concentration gradient within the membrane. (The treatment of cases in which the rate of diffusion and mixing in the fluids on the two sides is slow, is outside the scope of this paper. These cases have been discussed by Rashevsky and Landahl (83) and by Barrer (3).) When a steady state has been reached, the amount crossing the membrane in unit time, $\mathrm{d}Q/\mathrm{d}t$, is proportional to the difference in concentration on the two sides.

For diffusion in a collection of pores such as are present in a sintered-glass disk full of water,

$$\mathrm{d}Q/\mathrm{d}t = \bar{D}A'\Delta c/x' \tag{13}$$

where A' is the effective total cross-sectional area of the pores and x' is the mean length of the pores. Neither A' nor x' is directly measurable, but each may be assumed to be a constant fraction of the corresponding over-all area, A, and the thickness of the membrane, x. Equation 13 can be rewritten

$$\mathrm{d}Q/\mathrm{d}t = DAK'\Delta c/x \tag{14}$$

where K' is a conductivity factor for the membrane. Since A and x are constant for a given membrane they can be included in the proportionality factor as K, which is then called the membrane constant.

$$dQ/dt = DK\Delta c \tag{15}$$

K is determined from the rate of diffusion of a known reference substance, such as 0.1 N potassium chloride (35).

Sieve membranes may consist of pores small enough to reduce the rate of diffusion of large molecules either by having fewer pores large enough to pass the larger molecules or by a wall friction effect. This changes $\bar{D}$ to $\bar{D}'$, a mean diffusion coefficient within the membrane. The actual length of the pores is also unknown, so that the measured diffusion of a given substance is given by

$$dQ/dt = \bar{D}'A'\Delta c/x' = hA\Delta c \tag{16}$$

where h is called the permeability of the membrane for the substance in question. Collander (14) compared the permeability coefficients of a series of non-electrolytes in a copper ferrocyanide membrane with the molecular diameter (which is very nearly inversely proportional to the diffusion coefficient in water). He found that the molecular diameters and the permeability coefficients in the membrane were in the inverse order, but that a thirteenfold range of molecular diameters corresponded to a 62-fold range in permeability coefficients. If the pores were all of the same diameter, there would be a sharp cut-off at some definite molecular diameter. The observed range of permeabilities is evidence that there is a range of diameters, so that while relatively few pores can pass large molecules, more pores are available to smaller molecules (32).

In thin lipoidal membranes when diffusion reaches a steady state:

$$dQ/dt = \bar{D}Al\Delta c/x \tag{17}$$

Nearly all biological membranes are of unknown thickness and composition, so that l/x must be replaced by another constant K''. Equation 17 can be rewritten:

$$dQ/dt = \bar{D}AK''\Delta c/x = hA\Delta c \tag{18}$$

where h is again the permeability of the membrane for the substance in question (83).

All the equations considered in this section reduce to one of the two forms:

$$dQ/dt = \bar{D}'A\Delta c/x \tag{19}$$

or

$$dQ/dt = hA\Delta c \tag{20}$$

$\bar{D}'$ is the integral diffusion coefficient of the diffusing substance in the membrane and h is the permeability coefficient. The permeability coefficient h equals $\bar{D}'/x$ and is especially useful when the thickness of the membrane cannot be measured.

When membranes have appreciable thickness and the solutions on both sides are well stirred, the time, L, for diffusion to reach a steady state is given by equation 21 (2, 3):

$$L = x/6h = x^2/6\bar{D}' \tag{21}$$

B. Diffusion of electrolytes through membranes

All the conditions controlling the diffusion of non-electrolytes through membranes, as well as the conditions controlling the diffusion of electrolytes in bulk solution, apply to the diffusion of electrolytes in membranes that contain no indigenous ions. In general, either or both ions may suffer changes in their relative diffusion mobility, owing either to the different medium or to pores small enough to hinder the ions. Neutral oil layers are good examples of homogeneous uncharged non-reacting membranes, while cellophane or very pure collodion is a fairly good approximation to uncharged pore membranes. Very little work has been done on these systems, because absolutely isoelectric solid membranes have not been available (see, however, 75) and also because of the greater interest in the large and striking effects which are obtained when electrolytes diffuse through ionized membranes or oils which contain ionizing molecules.

Beutner (5, 6) carried out a long series of experiments in which he measured the electrical potentials set up when electrolytes diffuse through oils containing acidic or basic dissolved substances (37). Osterhout (82) followed up these oil-membrane experiments, doing most of his work with guaiacol, an acidic phenol. He measured actual transport in some cases as well as the potentials produced.

An oil membrane can be set up between two aqueous layers, for example, by placing a heavy oil at the bottom of a U-tube with different concentrations of potassium chloride solution in the two arms. If identical calomel electrodes are placed in the two potassium chloride solutions, there will be a potential difference between these two electrodes. The more dilute potassium chloride solution will be positive if an acidic oil such as guaiacol is used, and negative if a basic oil such as aniline is used. Beutner (5, 6) ascribed these results to phase-boundary potentials, while Bauer (4) and later Ehrensvärd and Sillén (29) ascribed the results in some cases to adsorption on the interface. Dean (19, 20, 24) pointed out that, at equilibrium, adsorption could not influence potentials and that the potential effects could all be explained by the diffusion of the solute, or one of its ions, into the oil phase, producing both phase-boundary and liquid-junction potentials. In the case of potassium chloride and guaiacol, the potassium ion forms potassium guaiacolate and can diffuse across the guaiacol as such, while the chloride ion and the hydrochloric acid formed by hydrolysis are practically insoluble and cannot diffuse in the oil layer. The guaiacol therefore behaves as a membrane permeable only to cations. Aniline, on the other hand, behaves as a membrane only to anions, since chloride ions are soluble in aniline as aniline hydrochloride, and potassium ions and potassium hydroxide are not soluble in aniline.

When a membrane separates two different concentrations of the same electrolyte, the potential difference produced is given to a fair degree of approximation by the well-known Nernst equation (78):

$$E_1 - E_2 = \frac{u - v}{u + v} \frac{2.3RT}{F} \log c_1/c_2 \tag{22}$$

where $E_1 - E_2$ is the difference in potential between the two solutions. $2.3RT/F$ has the value 0.059 volt at 25°C. u and v are the effective mobilities of the ions in the membrane. Equation 22 can be written in the more general form:

$$E_1 - E_2 = -\frac{RT}{F}\left(\int_{c_2}^{c_1} t_+/z_+ \,\mathrm{d} \ln c_+ - \int_{c_1}^{c_2} t_-/z_- \,\mathrm{d} \ln c_-\right) \tag{23}$$

where t_+ and t_- are the transport numbers of the cation and anion, respectively. This is a useful form when the concentrations of the two ions in the membrane are different but the activity coefficients are constant over the concentration range. Equation 23 is a special case of the general equation given by Guggenheim (41). When the concentration c_1 is ten times c_2, the maximum potential difference that can be obtained is 59 millivolts at 25°C. This can occur if the membrane is completely impermeable to one ion so that t_+ (or t_-) $= 0$ or u (or v) $= 0$.

Since potential measurements are relatively so easy to make, numerous workers (including the author (18)) have interpreted measurements on membranes as indicative of permeability to ions by means of equation 23 (for examples see 76, 82). It must be pointed out that this equation is only valid: (*a*) when the diffusing ions penetrate all the way through the membrane; (*b*) when no other ions, stored or being produced in the membrane, are diffusing out; and (*c*) when the membrane consists of one homogeneous medium. Conditions (*b*) and (*c*) are not often realized for biological membranes, and conditions (*a*) and (*b*) are often not satisfied for highly impermeable synthetic membranes.

When a membrane exhibits the maximum thermodynamic potential difference because only cations or only anions can penetrate, there can be no diffusion through the membrane in the absence of an external circuit to remove the charge carried by the diffusing ions. In general, the more closely the potential of a membrane approaches to the theoretical maximum the more nearly impermeable it is to the diffusion of salts.

Although a guaiacol membrane should not allow potassium chloride to diffuse from a higher to a lower concentration, it will allow potassium ions to exchange for sodium ions if such a membrane separated potassium chloride from sodium chloride. The guaiacol (symbol HG) dissociates as an acid to form G^- and H^+ ions. K^+ and Na^+ are soluble in guaiacol and can diffuse either as ions with G^- as the gegen ion, or they can diffuse as undissociated KG and NaG. Osterhout (82) assumes that potassium and sodium diffuse as undissociated molecules, but some diffusion of the free ions probably occurs also, since guaiacol saturated with water and potassium chloride is a reasonably good electrical conductor.

Solid membranes that have bound ions in their pores have been investigated potentiometrically by Meyer and his coworkers (73, 75) and later by Marshall and Bergman (72). A theoretical treatment of the potentials produced was developed independently by Meyer *et al.* and by Teorell (96). Fetcher (33) showed that the potentials actually observed by Meyer *et al.* do not agree quantitatively with those that would be predicted from their theoretical equations, and the discrepancies have been further explained by Sollner and Carr (92). Qualitatively, however, ionized porous membranes are of great interest and diffusion through them may produce striking results, as has been shown by Sollner and coworkers (89, 90).

The term "charged membrane" is a misnomer, as a membrane cannot maintain a static charge in a conducting solution. The term "charged membrane" (like the term "charged colloid") is applied to membranes that contain fixed ions of one charge combined with water-soluble ions of the opposite charge. "Charged" membranes might better be called ionic membranes. The sign of the charge on the membrane is the sign of the bound ions. The bound ions may be adsorbed on the surface of the pores, or they may be chemically combined with the substance of the membrane. An example of the first type is provided by the positively charged permselective membranes of Sollner (1, 39). These membranes are made by the adsorption of protamine cations on nitrocellulose membranes. The second type is exemplified by oxidized nitrocellulose membranes (38), where oxidation has produced carboxyl groups presumably on the sixth carbon atom of the glucose residues (89). These carboxyl ions will be accompanied by an equivalent number of sodium gegen ions. When such an oxidized membrane is set up between two solutions of sodium chloride of different concentration, the membrane will be more permeable to sodium than to chloride ions, because the concentration of sodium ions inside the pores is greater than the concentration of chloride ions. The concentration is greater because sodium ions must be present to balance the bound carboxyl ions as well as the diffusible chloride ions. When the membrane is bathed in sufficiently dilute solutions, nearly all the mobile ions in the membrane will be sodium ions and the membrane will behave electrically as if it were a connected pair of electrodes reversible to sodium ions. The membrane will also be almost impermeable to sodium chloride if the pores are sufficiently small. Obviously, the concentration of bound ions in the membrane and the pore size will control the ratio of sodium ions to chloride ions in the membrane for any given concentration of sodium ion outside. Membranes that have small pores and a high ratio of bound ions to pore volume will produce nearly the maximum thermodynamic electrical potentials and will likewise be nearly impermeable to the diffusion of salts.

If a cell is completely enclosed by a uniform membrane and a potential difference exists across this membrane, the potential cannot of itself produce any movement of ions. Such movement can only occur when a current flows, and in order that current may flow the potential must have different values in different parts of the cell surface.

If a current flows through a membrane which bears ions on its surface, there will be a movement of the solution through the membrane. This flow is known as electroösmosis or electroendosmose (9) and will be in the same direction as the movement of those ions which balance the ions on the membrane. Electroosmosis can be considered to be the direct result of the drag of the gegen ions on the columns of solvent in the pores. In bulk solutions the drag produced by cations is exactly equal and opposite to the drag produced by the anions. In a pore, however, one type of ion cannot be moved, because it is attached to the wall of the pore and the drag of the gegen ions on the solution is unopposed. The magnitude of the effect is proportional to the fraction of the current in the pore which is carried by the gegen ions. Consequently, electroösmosis is decreased by increasing the electrolyte content of the system.

IV. ACTIVE TRANSPORT THROUGH MEMBRANES AGAINST CONCENTRATION GRADIENTS

Diffusion, so far, has been discussed only from the standpoint of ideal systems where diffusion transports material from a higher to a lower concentration. There are many other cases, particularly in living systems, where transport of material takes place from the lower to the higher concentration. Indeed, life as we know it would be impossible if living systems did not have the ability to do this "osmotic work." However, there are no proven cases where inanimate systems have been demonstrated as capable of causing *all* solutes to move against their activity gradients at constant temperature.

The minimum thermodynamic work ΔF required to move material in solution from one concentration to another at constant pressure and temperature is given by

$$\Delta F = \Delta N R T \ln (a_1/a_2) \qquad (24)$$

where ΔN is the number of moles of material moved, a_1 is the activity at the concentration from which the substance is moved, and a_2 is the activity at which the substance is delivered. When several constituents change in activity, the work term is the sum of the work terms for each substance.

Equation 24 shows that work will be done when a substance diffuses from a higher to a lower activity and that work is required to move a substance from a lower to a higher activity. For ordinary dilute systems the activity is closely proportional to the concentration. That is, $a = c\gamma$, where c is the concentration and γ is the activity coefficient, which often does not differ greatly from unity.

Although the activity usually follows the concentration, there are cases where diffusion is caused by changes in activity at constant concentration. An example of this is given by the *diffusion retrograde* of Thovert (45). In one experiment a solution of potassium chlorate in water was separated from a solution of potassium chlorate of equal concentration in a mixture of acetone and water. Acetone of course diffused into the aqueous solution, but in addition potassium chlorate diffused into the aqueous solution, although it was initially at the same concentration in the two systems. The movement of potassium chlorate took place because it had a higher activity in the acetone–water mixture than in pure water. This is demonstrated by the fact that its solubility is much less in acetone–water mixtures than in pure water. Conversely, if a substance proportionately more soluble in acetone, such as acetanilide or butyric acid, had been used, the movement would undoubtedly have been in the opposite direction. Thovert's *diffusion retrograde* therefore is a case of spontaneous diffusion occasioned by differences in the activity coefficient of the substance at the same concentration. Schreinemakers (84, 85) has discussed numerous examples of anomalous concentrations set up while diffusion is taking place.

The movement of ions initially at the same concentration throughout can easily be produced by setting up an electrical field. The electrical field can in turn be produced by the diffusion of a suitable salt, preferably though not necessarily through a membrane.

Among examples of this type of "up hill" diffusion are many experiments of Osterhout (64, 82), where the constant diffusion of carbon dioxide (as carbonic acid) produces accumulation of potassium ion or chloride ion in one or another chamber. Teorell (97) has presented a theory of this movement and has set up an experiment where the constant diffusion of perchloric acid, constantly added to a small chamber containing a solution of ammonium chloride in contact through a membrane with a large vessel of the same concentration of ammonium chloride, produced an increase in the ammonium-ion concentration and a decrease in the chloride-ion concentration inside the membrane. In this case, as in most cases of its type, there was no increase in the product of the concentrations of the two ions moved. The system is exactly analogous to that occurring when a Donnan equilibrium is set up between two systems one of which contains a non-diffusible ion. In Teorell's experiment the concentration of perchlorate ion was artificially maintained by the constant addition of perchloric acid. No simple system of this sort can concentrate both cations and anions simultaneously.

Although modifications of the Donnan equilibrium cannot increase the activity product of a cation and an anion at the same time, there is at least one result of diffusion through membranes which could lead to accumulation of salts. Ingraham, Peters, and Visscher (53) proposed a fluid circuit theory, in which a solution was pumped around a circuit containing a membrane impermeable to one of the solutes. The non-penetrating solute would be accumulated in the chamber behind the membrane. This mechanism in its original form merely shifted an uncertainty about the mechanism for concentrating the solute to an uncertainty about the mechanism for moving the solution as a whole. A mechanism capable of moving the solution was not postulated. Sollner and coworkers (40, 87, 89) have demonstrated and explained one mechanism whereby diffusion across a membrane can produce large movements of the solution, which may be in the direction of natural osmotic flow but more rapid than normal or may be opposed to this flow. This phenomenon is known as anomalous osmosis and is termed negative (anomalous) osmosis if the fluid movement is initially from the more concentrated to the more dilute solution. Negative osmosis is one mechanism which might produce the flow of fluid required by the fluid circuit theory if some electrolyte were supplied continuously to one chamber and removed as rapidly as it diffused into the other chamber. According to Grollman and Sollner (40), negative osmosis can occur when the electrical potential set up in one membrane (or one part of a membrane) is sufficient to produce electroosmotic flow through another membrane in circuit with it. The two membranes might be only pores of different character or dimensions in the same membrane. If, for example, one membrane consisted of small pores lined with negative ions and consequently permeable only to positive ions, there would be a potential set up by any cation which was more concentrated on one side of the membrane than the other. The side having the lower concentration would be positive. If the second membrane had large pores which were lined with positive ions, this potential would cause current to flow and some of the current would be carried as an electroösmotic stream from the more dilute to the more concentrated solution. Variations of this phenomenon permit negative osmosis in membranes bearing the same charges on all pores if the ions have very different mobilities (87). Since proteins are amphoteric it is not hard to imagine a membrane with some pores containing excess cations while other pores contain excess anions. If one set of pores is larger than the other, negative osmosis must accompany the diffusion of an electrolyte through the membrane. The identification of the diffusing electrolyte presents some difficulties, however. Ingraham and Visscher (55) suggested that the production of ammonia in the gut and its diffusion into the blood stream where its concentration is held at a very low level by the activity of the kidneys might provide the necessary electrolyte. Although electrokinetic phenomena are in general reduced by high salt concentrations, anomalous osmosis is shown to the greatest degree by solutions of the order of 0.02 N (89) and the effects are still strong at 0.16 N, a concentration which is in the physiological range. Although Sollner's work shows that anomalous osmosis might provide a mechanism for the active transport of electrolytes under rather special conditions, there is as yet no positive evidence that such a mechanism actually operates *in vivo.*

Other mechanisms for active transport which are of a somewhat similar nature postulate the formation of a compound between the solute being transported and some constituent of the membrane. The compound is then supposed to diffuse through the membrane and liberate the original solute on the other side. Specifically, Wilbrandt and others (61, 67, 98, 106) have proposed a mechanism to move glucose out of the kidney tubules or the intestine by the formation of glucose phosphates and their subsequent hydrolysis in or near the blood stream.

Brooks (11) has reviewed several theories of ion-accumulation mechanisms which depend on the production of an electrolyte inside the cells which by its outward diffusion causes ionic accumulation in the cell. He particularly favors a mosaic membrane having adjacent regions of cation and anion permeability and what appears to be a one-for-one ion exchange through these regions. For example, in a cation-permeable region each hydrogen ion coming out would do so by exchanging for one potassium ion going in, while in a nearby anion-permeable region each bicarbonate ion coming out would bring in one chloride ion· The carbonic acid which diffuses out would be supplied by the respiration of the cell. This mechanism cannot be effective in tissues with low respiratory rates, where the moles of carbon dioxide produced are less than the moles of salt accumulated. Another similar mechanism involves cation exchange for ammonium ions.

Wells (101) proposed a mechanism whereby the continuous formation of an osmotically active substance in a cell would produce fluid flow through a series of membranes.

Many examples of anomalous permeability occur in physiological literature. For example, dyes are often found to be much more concentrated in certain cells than in the surrounding fluid. This is often a case of sorption or precipitation of the dye inside the cell, which fixes its activity and permits more dye to come in (37). However, there are undoubtedly cases where secretory or excretory organs concentrate dyes (and other substances) without precipitating them or otherwise significantly altering their activity coefficients (54). Another experiment which has caused some confusion is the observation by Wertheimer (102, 103) that dyes penetrate frog skin more rapidly in one direction than the other. Eckstein (28) has shown that in the case of dyes such effects may occur in the early stages of an experiment without involving active transport. Differences in the dye uptake of the two sides of the membrane are adequate to explain the observations. Nevertheless, Krogh (61) has shown that frogs can actively transport sodium chloride from tap water into their plasma. Even after all the spurious cases of apparent active transport have been accounted for (and all cases of apparent active transport without a continuous supply of energy in non-living systems have been so accounted for), there still remains a vast collection of undoubted cases where living systems do perform osmotic work. These range from the uptake of salts from very dilute spring water by fresh water algae to the excretion of a concentrated urine by land animals (50, 62). Virtually all cells have contents which differ in concentration and activity from the surrounding fluid, and all such cells must obtain their contents at the expense of osmotic work. Only a minute fraction of the many types of active uptake have been adequately explained (for example, the concentration of oxygen in fish bladders (56)). We know only that the general respiration of the tissue responsible in every case that has been tested is many times greater than the osmotic work accomplished (21, 23, 93). A complete list of proven examples of active osmotic work is outside the scope of this review. The most that can be done is to point out the universality of the phenomenon and our general ignorance of its mechanism.

Mention should be made of the use of radioactive or other isotopes as a tool for investigating permeability (42, 48, 86). By the use of radioactive potassium ions it has been possible to show that potassium is continuously moving in and out of red blood cells at a rate much too slow for measurement by conventional methods (25). It has also been possible to trace the movement of water, sodium ions, and chloride ions both out of and into the gut during the active absorption of sodium chloride and water (100). The advantages of this method stem from the fact that radioactive isotopes do not alter either the ionic composition of the fluids or the metabolism of the organism when used in normal doses. It is therefore possible to find out the actual magnitude of ionic movements even under static conditions. For example, since the red blood cell maintains its potassium content in the face of a continuous leak to the blood plasma, it must be continuously expending energy to pump the potassium back in again.

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