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The Mechanism of Water Transport in Membranes [and Discussion]

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The mechanism of water transport in membranes

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Biological membranes, lipid membranes and organic polymer membranes have some chemical similarities. All can transport water and it is likely that the molecular transport processes have some common features in the three types of system. Polymer membranes, being stable and strong, can be subjected to more varied and intensive study than can either lipid or biological membranes. Furthermore, the structures, dimensions and molecular organization of polymer membranes, which are very simple compared with their biological counterparts, can be characterized in some detail. It is already a reasonable objective to analyse transport phenomena in polymer membranes in terms of the molecular processes likely to occur in media of known structure. If the level of understanding in this area could be improved it might become possible to make some confident deductions about structure in lipid and biological membranes from observations on their transport properties. This review is intended to assess the current situation regarding the interpretation of flux data in structural terms and, perhaps, to encourage further developments.

Although observations on transport processes are made by observing changes in the bulk phases in contact with the membrane, it is the processes within the membrane that are under study. The mathematical formulation of membrane transport must be designed to emphasize the rôle of the membrane as a phase in which irreversible processes are occurring. This requires the determination of diffusion coefficients, concentrations, activity coefficients and their profiles within the membrane. The concentration and activity of water in a membrane are studied through equilibrium absorption isotherms. These vary widely in form with the chemical structure and organization in polymer membranes. Almost nothing detailed is known about sorption by lipid membranes.

Most flow processes are studied between pairs of aqueous solutions that contain solutes which may also be able to permeate the membrane. The possibility of several irreversible processes occurring simultaneously in the membrane can best be handled through the formalism of non-equilibrium thermodynamics. This formulation provides also a convenient method of introducing pressure and osmotic pressure as driving forces in addition to that of simple Fickian diffusion flow.

A bridge between the phenomenological coefficients of thermodynamics and molecular processes can be built only on a model system. By regarding the membrane as intrinsically homogeneous and isotropic the frictional model can be applied in which steady flow is represented by a balance between thermodynamic driving forces and frictional retarding forces among the various flowing components and the membrane.

A case of particular interest arises where the solute is an isotopically labelled species of water. The number of independent frictional coefficients in the two-component system is reduced from three to two. They can be determined from measurements of the tracer diffusion coefficient and the hydrodynamic or osmotic permeability of the membrane.

This has been done for two sets of polymer membranes: highly hydrated hydrogels and moderately hydrated cellulose acetates. The membranes were prepared so as to be as nearly homogeneous as possible and it was found that the frictional coefficients observed were consistent with the homogeneous model theory.

By applying a form of argument which has several times been used to diagnose the existence of pores in biological and lipid membranes, it was possible to deduce from

the data on the synthetic membranes that they too transport water in, sometimes quite large, pores. This conclusion would be at variance with their known structures and shows that the argument is unsound when it is used as a criterion for the presence or absence of pores. It is clear also that the argument is valid only when applied to a homogeneous system and so would not be valid if applied to a system that were truly porous but also transported water through the matrix material supporting the pores.

Principal symbols

a	thermodynamic activity
C	concentration
D	Fick diffusion coefficient
D_T	'thermodynamic' diffusion coefficient
f_{ik}	friction coefficient of i with k
f_w	fugacity of water vapour
F_{ik}	frictional force of k on i
g	flow factor (equation 59)
G_{ww}	Zimm cluster integral
J	flux density
J_D	exchange flux density
J_v	volume flux density
k	partition coefficient
l	membrane thickness
L_D, L_p, L_{pD}	coefficients of practical flux equations
L	with two subscripts (except pD), phenomenological conductance coefficients
p	pressure
P	permeability
r	equivalent cylindrical pore radius
R	universal gas constant
R	with two subscripts, phenomenological resistance coefficients
S	entropy
T	absolute temperature
u	absolute mobility
v_i	mean velocity of component i
$\bar{V}$	partial molar volume (molecular in equation 1)
x	coordinate perpendicular to membrane face
X_i	total force on component i
γ	activity coefficient
η_w^0	viscosity of bulk water
θ	tortuosity factor
μ	chemical potential
π	osmotic pressure
σ	reflection coefficient
ϕ	volume fraction
Φ	dissipation function
ω	solute permeability

subscripts

i, k	components i, k, etc. in general
m	membrane matrix
n	non-electrolyte solute
S	pertaining to entropy flow
T	THO (except D_T)
w	water, H_2O
1,2	sides 1 and 2 of the membrane

superscript

—	mean value in membrane
o	in water or aqueous solution

1. Introduction

For as long as natural and synthetic membranes have been used as selective barriers to material transport, the study of water transport has occupied a central place. Probably the first widespread practical use of membranes was as semi-permeable barriers in the determination of the osmotic pressures of aqueous solutions. Although early workers devised semi-permeable membranes of inorganic materials, today the use or organic polymers is general. In order to obtain a quick response such materials require to be highly permeable to water while, at the same time,

remaining impermeable to solutes. The search for membranes satisfying these demands has been recently stimulated by research into the desalination of water by reverse osmosis (Merten 1966; Lonsdale & Podall 1972).

Polymeric films that are used as protective coatings and packaging must have a very low permeability to water. The polymers used for these purposes are usually hydrophobic and a good deal of work has been done on measuring their water transmission properties and in relating them to kinetic and thermodynamic factors.

Independently of the research on water transport in artificially made polymer membranes, biologists have been constantly engaged in the study of water transport across tissues and cell membranes. Originally these studies were promoted by the desire to observe and understand the physiology of water transport but it has progressively come to be realized that the exact study of water transport may throw light also on the structure of biological membranes (Dick 1971; Solomon 1968).

To extract structural information from flux data requires first a quantitative understanding of the mechanisms whereby water is transported in systems of known structure similar in at least some respects to the unknown structures under examination. All too often this quantitative understanding has been lacking. In the study of biological membranes recent advances have been aided by the parallel study of the structures and properties of bilayers formed from well-characterized lipids (Cass & Finkelstein 1967; Redwood & Haydon 1969).

Chemically, although not physically, lipid membranes have some similarities to organic polymers. This fact is bringing together the polymer scientists and the biologists interested in water transport, to their mutual benefit.

Very recently efforts have been made to model the water transporting function of biological systems quite closely with synthetic polymers and so to develop devices such as the artificial kidney and lung and to permit micro-corneal lenses to be worn continuously (Turbak 1970; Gardner 1976).

At the present time one finds physical chemists, polymer chemists and physiologists actively collaborating under the umbrella of biophysics and it would not be possible or prudent to cover such a wide spectrum of interests in a single review. Attention is restricted here to describing some important findings and theories in each area. Some attempt is made to trace common threads which help to unify the study of water transport in membranes.

2. The general consideration of membrane transport

The fundamental requirement of a membrane is that it shall be a system which, when interposed between two bulk phases, exerts a selective control of the transfer of matter and energy between them. Thus a membrane is defined through its function and not through its composition or form. It is usually convenient however to adopt the more specialized model of a membrane as being a phase bounded by parallel surfaces and of lateral dimensions large compared with its thickness so that edge effects may be neglected. With the help of such a model it becomes possible to formulate membrane phenomena so as to take into account explicitly the processes occurring in the membrane as well as to interrelate fluxes between the bulk phases.

In a simple treatment it may be assumed that each bulk phase is uniform and in thermodynamic equilibrium, i.e. it is kept well mixed so as to dissipate all gradients arising from the

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selective flows of heat and materials through the interface with the membrane. Furthermore, it is usually assumed that thermodynamic equilibrium with respect to temperature and partitioning of solutes exists between the bulk phase and the membrane at each interface. Hence all gradients in chemical potentials are confined within the membrane phase.

In practice the conditions required for these assumptions to hold cannot always be realized. The difficulty is particularly acute in studies of lipid bilayers which, being very thin, may carry large flux densities but which are too fragile to withstand vigorous stirring of the adjacent phases. Furthermore, inside some biological cells the fluid may be viscous due to the high concentration of plasma proteins. Concentration gradients in this intracellular solution may influence the fluxes across the cell membranes (Ling, Ochsenfeld & Karreman 1967).

In such circumstances one may either define the membrane boundaries to be the planes at which the chemical potentials start to vary and so include indeterminate regions of the bulk phases as belonging to the membrane, or one can try to describe and allow for the incomplete mixing of components in the boundary regions. The former procedure is adopted in the discontinuous treatment of membranes (de Groot & Mazur 1962). It is a formalistic device which precludes an analysis of the mechanism of the membrane function in molecular terms. The latter procedure, although involving further assumptions, has been much used and with good effect in the study of biological transport (Dainty 1963) and in the study of artificial membranes (Everitt, Redwood & Haydon 1969; Helfferich 1962).

We shall discuss first the formulation of membrane processes in the simplest case of uniform bulk phases. Because this article is concerned with water transport attention will be limited, apart from a brief mention of thermo-osmosis, to isothermal material flows. In principle the local (i.e. within a small volume element) flux density of any substance can be expressed as the product of three factors:

$$\text{flux density} = \text{concentration} \times \text{mobility} \times \text{force}.$$

The concentration is concerned with the thermodynamic factors that determine the sorption of substances from the two bulk phases by the membrane regarded as a third phase. The mobility is mainly influenced by the structural and molecular-kinetic properties of the membrane material. The forces which drive the flows are the result of differences between the intensive properties of the bulk phases. These intensive properties may be of several kinds but concentration (or, more strictly, activity) and pressure are of especial importance here.

3. Sorption and solubility of water in membranes

3.1 *Vapour sorption and molecular clustering*

It is important to examine the permeation of water between vapour phases at different pressures as well as between liquid water and aqueous solutions. Hence the sorption of water by polymers and membrane-forming substances from the vapour must be considered. Sorption isotherms have been measured in many polymers over virtually the whole range of relative vapour pressures from zero to unity. The isotherms can be grouped into three classes (Brunauer 1945): linear or Henry's law isotherms for hydrophobic and crystalline polymers, B.E.T. type III isotherms, found in relatively hydrophobic non-crystalline polymers, and B.E.T. type II isotherms, applicable to hydrophilic polymers particularly those with hydrogen bonding groups (Barrie 1968). Some examples appear in figure 1. Despite this use of the B.E.T. classification

of isotherms, one must not press too far the analogy of monolayer and multilayer adsorption to sorption into a polymer.

A more appropriate analysis of sorption data, based on the concept of clustering of the water molecules, has been developed by Zimm (1953) and Zimm & Lundberg (1956) from the statistical mechanics of fluctuations (McMillan & Mayer 1945). In a mixture of two components 1 and 2 the tendency of molecules of type 1 to cluster is related to a quantity $G_{ww}/\bar{V}_w$, where G_{ww} is called the cluster integral and $\bar{V}_w$ is the partial molecular volume of water. $G_{ww}/\bar{V}_w$ can be evaluated by graphical differentiation of the equilibrium sorption isotherm through the expression

$$\frac{G_{ww}}{\bar{V}_w} = -\phi_m \left[\frac{\partial(f_w/\phi_w)}{\partial f_w}\right]_T - 1, \qquad (1)$$

where ϕ_w and ϕ_m are the volume fractions of water and membrane respectively and f_w is the fugacity of water in the vapour phase. Water is defined to be component 1.

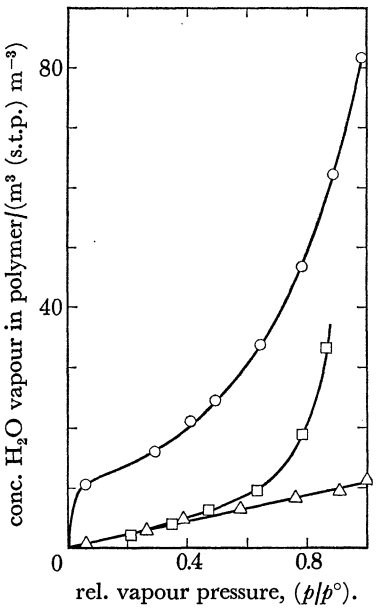

FIGURE 1. Sorption isotherms at 25 °C for water vapour in: △, polyethylene terephthalate (type I isotherm), ○, ethyl cellulose (type II isotherm), □, rubber hydrochloride (type III isotherm). (Reconstructed from Yasuda & Stannett 1962.)

$\phi_w G_{ww}/\bar{V}_w$ is the mean number of molecules of water in excess of the average concentration in the neighbourhood of any chosen water molecule. For a Henry's law isotherm the term in square brackets in equation (1) is zero and $G_{ww}/\bar{V}_w$ is -1, i.e. the chosen molecule excludes its own volume but does not affect the random distribution of molecules in its surroundings.

When $G_{ww}/\bar{V}_w < -1$, i.e. over the upward curved portions of the types II and III isotherms, the molecules of water tend to cluster together. Unfortunately, being a thermodynamic quantity, the clustering function does not give, without further assumption of a model, the average cluster sizes in terms of numbers of molecules (Lundberg 1969). Table 1 gives values of the clustering function for water in several polymers. These indicate that in many polymers at high humidities the water is extensively clustered and certainly not randomly distributed. By inference, the same must be true also in such polymers when saturated by contact with liquid

water. Clustering is less marked with sorbates less strongly hydrogen-bonded than water and it is believed that the clusters are groups of hydrogen-bonded water molecules distributed probably almost randomly in the non-polar polymer matrix (Barrie & Machin 1971).

TABLE 1. MEAN SIZE OF WATER MOLECULE CLUSTERS $(1+\phi_w G_{ww}/\bar{V}_w)$ IN SEVERAL POLYMERS AT 25 °C AND THREE ACTIVITIES OF WATER a_w
(Williams, Hopfenberg & Stannett 1969)

polymer	a_w		
	0.2	0.6	0.8
rubber hydrochloride	1.20	2.00	6.37
polyethyl methacrylate	1.18	1.50	2.21
polyvinyl chloride	1.40	1.30	2.45
polyoxymethylene	1.06	1.30	1.50
ethyl cellulose	1.04	1.58	2.37
cellulose acetate	1.13	1.08	1.71

3.2 *Formation and nature of gels*

In the hydrophilic polymers that exhibit type II sorption isotherms, including proteins, polyurethanes, cellulose derivatives and hydroxyl-containing polymers, the initial step in the isotherm frequently corresponds with the adsorption of one water molecule per hydrogen bonding group in the polymer (Barrie 1968). At the higher relative pressures substantial clustering of water is observed and it is believed that in such polymers the hydrogen-bonded clusters tend to be located preferentially in the regions of the hydrated polar groups.

When the concentration of polar groups is large, i.e. the groups are close together, the water clusters begin to overlap at a certain relative humidity so as to form an aqueous phase interpenetrating the polymeric network. The swollen polymer then constitutes a gel. In extreme cases, for example polyacrylamide, the polymer would dissolve completely at high humidity if it were not restrained by network crosslinks. The transition from polymer containing dissolved water molecules and isolated clusters of water molecules to a gel is accompanied by an apparent change in transport behaviour with which we shall be concerned later. It is necessary therefore to consider more fully the state of water in gel-forming polymers.

By co-polymerizing methyl methacrylate, hydroxyethyl methacrylate, hydroxypropyl methacrylate and glycerol monomethacrylate in varying proportions, together with tetraethylene glycol dimethacrylate as cross-linking agent when needed to prevent total dissolution, it is possible to prepare a series of substances of varying hydrophilicity but closely related chemically (Yasuda, Lamaze & Peterlin 1971). The sorption isotherms of these co-polymers do not appear to have been recorded but samples in the form of membranes swollen to equilibrium in water have been examined by n.m.r. and by differential scanning calorimetry (Yasuda *et al.* 1972).

The n.m.r. wide-line proton spectra were examined at temperatures from room temperature down to −100 °C. The spectrum does not differentiate between protons attached to the polymer chains and water protons. Typically the absorption spectra consist of a narrow line superimposed on a broad line. The former is due to mobile protons and the latter to immobile ones. The relative importances of these two components was found to vary systematically with the degree of hydration and with temperature.

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The first derivative spectra were decomposed into the narrow line, under which the area was measured, and the broad line. The proportion of the total area covered by the narrow line was taken as a measure of the fraction of protons in a mobile state. The variation of this fraction with temperature in a number of samples of different degrees of hydration is shown in figure 2. It should be noted first that, although neither water nor the dry polymer contains a significant fraction of mobile protons below 0 °C, mobile protons persisted to very low temperatures in the gels. Further, the fraction of the protons which were mobile increased with increasing hydration at any temperature up to a hydration of 30 % and then decreased again at higher hydration. The temperature down to which some mobility persisted was also lowest at 30 % hydration. In the gels of relatively low hydration, less than 30 %, the proportion of mobile protons often exceeded the total fraction of water protons. Hence some at least of the polymer segments must have been mobile even at temperatures far below the glass temperature of the dry polymer.

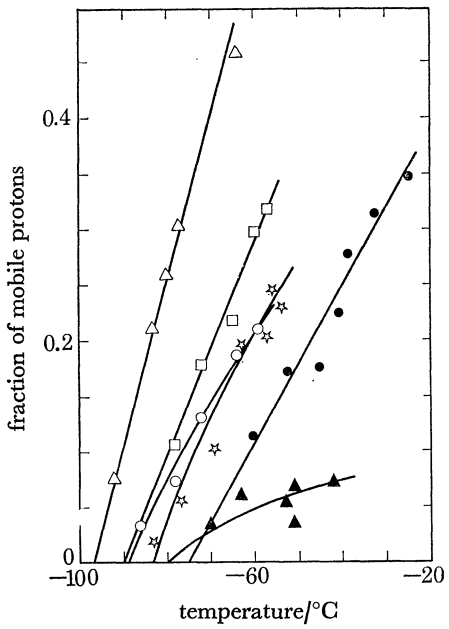

FIGURE 2. Fraction of protons which are mobile in poly(glycerol methacrylate) gels swollen with water to various volume fractions ϕ_w as a function of temperature. ●, $\phi_w = 0.1$; □, $\phi_w = 0.2$; △, $\phi_w = 0.3$; ☆, $\phi_w = 0.4$; ○, $\phi_w = 0.5$; ▲, $\phi_w = 0.9$. (Redrawn from Yasuda *et al.* 1972.)

It appeared that the decrease in the fraction of mobile protons at hydrations greater than 30 % was due to the formation of ice in the gel. Ice immobilizes protons directly and also acts as a filler inhibiting the motions of the polymer chains.

The most important conclusion for our purposes is that the enhancement of mobility with increasing hydration is a cooperative phenomenon involving water and polymer together and the polymer chains in gels must be regarded as osmotically active components of the system. Physically this is quite unlike the situation in a rigid porous matrix in which the pores, however narrow, are permanently filled only with water.

Only at hydrations greater than 30 % is there any water in contiguous molecular groups sufficiently large for normal crystallization to take place. The calorimetric data confirmed the

impression that at low hydrations no ice was formed but at hydrations above 30 % the additional water was able to freeze with the normal heat of crystallization of ice.

These n.m.r. and thermodynamic data show that the swelling equilibrium in a system of water + a hydrophilic polymer is similar to that in any solvent + lyophilic polymer. The equilibrium situation is determined by the interaction energy and configurational entropy. The entangled or cross-linked polymeric matrix is an osmotically active solute dispersed at high concentration in the swelling solvent. Only at very high degrees of swelling do the elastic constraints on the matrix molecules so restrict the ranges of their segmental Brownian motions that some regions of the swollen system may only rarely contain polymer. Such regions must then be occupied permanently by solvent and so have some characteristics of pores. Of course these regions may not run continuously through a swollen membrane from face to face. They will be of irregular sizes, shapes and spatial distribution and will be almost randomly interconnected, like the network they inhabit.

3.3 *Swelling of ionizable polymers*

In view of the substantial evidence that biological membranes include ionizable groups (Chapman 1970), the swelling behaviour of ionogenic polymers is relevant to this symposium. The equilibrium swelling of a cross-linked ion-exchange polymer in water is greater than can be accounted for by the entropy of mixing of polymer and solvent. Glueckauf & Kitt (1955) have shown from sorption isotherms that the first water molecules to enter hydrate the ionizable groups. Hydration loosens the exchangeable counterions from the oppositely charged ions which remain chemically bound to the polymer. The hydrated counter-ions are able to migrate more or less independently within the swollen polymer but cannot leave it except in exchange for an ion of equivalent charge. The driving force for the uptake of more water is the 'osmotic pressure' of these mobile ions in the polymer. It is usually far larger than the effective 'osmotic pressure' of the flexible polymer chains. The limit of water uptake is controlled mainly by the concentration of ionizable groups and by the degree of cross-linking of the polymer which determines the network restraints. Secondarily, the equilibrium is influenced by the nature of the counter-ions. It is greater when these are hydrated, e.g. Mg^{2+}, than when they are relatively non-hydrated, e.g. Cs^{+}. Most systematic studies have been carried out on cross-linked ion-exchangers with high concentrations of ionizable groups. It would be very interesting as a biological analogue to have more water sorption data for non-cross-linked non-polar polymers containing only a small proportion of an ionizable co-monomer, perhaps with weakly ionized groups.

3.4 *Absorption of water by lipids and biomembranes*

Direct measurements do not appear to have been made on the concentrations of the water in biological membranes. Such measurements would be technically difficult to perform. Instead one may look for information obtained on membrane components and related materials studied in bulk. Even these comparatively straightforward measurements have not been made in many cases.

A good deal of work has been done on the phase diagrams of lipids + water (Luzzati 1968). Although the precise relation of the liquid crystals and phase structures commonly observed to the organization of lipids in biological membranes is unknown, it is believed that the liquid-like type-α conformation is probably the most significant biologically. The water content of this α-phase is rather high; certainly greater than 10 % in many cases, e.g. dimyristoyl lecithin (Luzzati *et al.* 1972).

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On the other hand, the solubilities of water in normal alkanes of chain lengths C_{10} to C_{16} are approximately 0.01 % (Schatzberg 1963). The interiors of lipid bilayer membranes are thought to be mainly hydrocarbon chains in a liquid-like condition (Metcalfe, Birdsall & Lee 1972). Thus one is led to the conclusion that, if the overall water content of the bilayer membrane is at all comparable with that of the type-α bulk phase of lipid + water, most of that water must be associated with the polar groups at the membrane surfaces and very little will be found in the hydrophobic interior. The solubility of water in pure *n*-paraffin hydrocarbons decreases progressively as the chain length is increased and approaches an asymptotic value of 0.0054 % at 25 °C and 0.0104 % at 40 °C in *n*-$C_{16}H_{34}$ and higher paraffins (Schatzberg 1963, 1965). The solubilities of water in polyethylene and polypropylene are significantly greater than this (by about 20 %) and the sorption isotherms give no indication that water molecules cluster in these polymers (Yasuda & Stannett 1962). Clustering probably does not occur in the liquid hydrocarbons either.

In the case of a biological membrane, it is becoming increasingly evident that the lipid molecules are undergoing rapid reorientation. At any instant a small number of head groups may lie transiently in the membrane interior and may carry with them adhering water molecules. They would be sufficient to make a substantial contribution to the statistical average concentration of water in the membrane interior.

A further complication with biological membranes is the function and distribution of the membrane proteins. Some protein appears to be situated close to the internal interface between the two layers of lipid molecules (Branton, Elgsaeter & James 1972). Proteins will generally be associated with water even when in the membrane interior if the exterior is in contact with aqueous phases. The extent to which this water is either able to participate in transport or is immobilized on specific protein sites cannot be gauged at present.

Although no attempts have been made to model a system such as a lipid–protein complex with purely synthetic polymers, a possible approach can be inferred from recent work on graft co-polymers. In co-polymers of styrene grafted on to cellulose it has been shown that the water absorption per unit mass of cellulose is decreased as the percentage of hydrophobic graft chains is increased and that, at fixed overall co-polymer composition, the results are sensitive to the length and distribution of the graft chains on the backbone (Le Poutre, Hopfenberg & Stannett 1972). Hydrocarbon chains grafted on to a polypeptide might well give a co-polymer with water sorption properties of interest to biologists.

4. The formulation of water transport in membranes

4.1 *Ideal Fickian diffusion flow*

Discussions of material transport in membranes frequently start from Fick's first law of diffusion which can hold only for steady flow. It is

$$J = -D\,\mathrm{d}C/\mathrm{d}x, \tag{2}$$

where J is the flux density, i.e. flux per unit area, D the diffusion coefficient and C the local concentration in the membrane. C is presumed to vary with only a single linear coordinate x. This equation is then integrated across the membrane to give

$$J = D(C_1 - C_2)/l, \tag{3}$$

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where l is the membrane thickness and C_1 and C_2 are the concentrations of the permeant just inside the membrane faces. Only rarely is any serious consideration given to the idealizations inherent in equation (2), still less to the restrictions on integrating it and, occasionally, the definition of the concentrations is less lucid than is desirable.

It is safer to start from the purely phenomenological relation

$$J = P(C_1^\circ - C_2^\circ)/l, \tag{4}$$

where C_1° and C_2° are the concentrations in the external phases adjacent to the membrane and equation (4) defines the permeability coefficient P. (If the membrane area is not precisely known, J may be the total flux and P the permeability of the whole membrane.)

P may be a function *inter alia* of C_1° and C_2°; in special cases it is independent of these concentrations. To be independent of concentration not only must the assumptions made earlier of uniform bulk phases and thermodynamic equilibrium across the interfaces hold, but the partition coefficient k of permeant between the bulk and membrane phases must be independent of C°, i.e. Henry's law must hold and the diffusion coefficient D must be independent of the local concentration C and of position in the membrane.

Since the constant k is given by

$$k = C_1/C_1^\circ = C_2/C_2^\circ, \tag{5}$$

comparison of equations (3) and (4) shows that, in the idealized system governed by equation (2),

$$P = Dk. \tag{6}$$

In fact the phenomenological relation equation (4) can hold quite widely when the assumptions required to validate equation (3) are not applicable. For example equation (4) can hold for a set of ideal membranes of different properties in series, although D is then dependent on position, and also for a set of ideal parallel pathways, i.e. a mosaic membrane or pores in a homogeneous matrix, in which C varies with y and z at constant x.

In an ideal homogeneous membrane diffusion is by random Brownian motion and the Nernst–Einstein relation holds. Thus

$$D = uRT, \tag{7}$$

where u is the absolute mobility of the permeant, water, in the membrane material. Substituting equation (7) into equation (2) gives

$$J = -uRT\,\mathrm{d}C/\mathrm{d}x = -uC(RT\,\mathrm{d}\ln C/\mathrm{d}x). \tag{8}$$

From the relation between flux, concentration, mobility and force, the force in this ideal system is seen to be $-RT\,\mathrm{d}\ln C/\mathrm{d}x$. At constant temperature and pressure this force may be written as $-\mathrm{d}\mu/\mathrm{d}x$ where $\mu(x)$ is the chemical potential of the permeant in the membrane at the plane x. In steady Fickian diffusion the force per mole increases in proportion with the reciprocal of the concentration from one side of the membrane to the other.

4.2 *Deviations from Fickian diffusion*

The simple ideal situation described by the foregoing equations breaks down when D varies with C, when k is not independent of C° and if the membrane is not intrinsically homogeneous so that D and k vary with position. Some or all of these conditions apply to nearly every case of practical interest among polymer membranes and in biological systems. Attention is restricted

for the moment to homogeneous membranes that can be prepared, to a good approximation, from synthetic polymers, although they are not commonly met with in biology. There is ample evidence that in a physical transport process under most circumstances in such membranes the appropriate driving force is the chemical potential gradient (de Groot & Mazur 1962). In a thermodynamically non-ideal system, i.e. one with non-constant k at constant temperature and pressure, the chemical potential gradient can be represented by $RT\mathrm{d}\ln a/\mathrm{d}x$. Here a is the activity of the substance in the membrane. With this correction equation (2) may be replaced by

$$J = -D_T C \mathrm{d}\ln a/\mathrm{d}x. \tag{9}$$

D_T is called the thermodynamic diffusion coefficient and is related to D, now taken as defined by equation (2), by

$$D = D_T[1 + (\mathrm{d}\ln\gamma/\mathrm{d}\ln C] \tag{10}$$

with γ the activity coefficient of the permeant in its mixture with the membrane material at concentration C. γ may be either measured from sorption data or calculated from a statistical thermodynamic treatment of the mixture.

D_T or the related absolute mobility D_T/RT is, in general, a function of C, i.e. of local composition, but the value of D_T is determined by the local frequencies of molecular motions and frictional interactions. It should be possible to find a theoretical model to explain and express the connection between D_T and C. In the case of polymer + permeant systems the free volume model has filled this rôle very successfully (Fujita 1968).

4.3 *General thermodynamic formulation of transport processes*

In addition to the virtual force arising from a concentration gradient, other forces can give rise to material transport. Important in transport between liquid phases is a pressure difference across the membrane. In the case of diffusion between vapour phases one cannot separately vary the fugacity and the pressure. The total flow may be connected with the applied pressure either by including it in the general framework of a thermodynamic treatment or by applying hydrodynamic flow theory and using the Navier–Stokes equation. Here the thermodynamic approach is pursued.

The chemical potential of water in a solution may be written

$$\mu(p,T) = \mu^\circ(p^\circ,T) + RT\ln a + \bar{V}(p-p^\circ), \tag{11}$$

where μ° is the chemical potential in a standard state at pressure p°. p and T are the actual pressure and temperature. The partial molar volume $\bar{V}$ and activity a are assumed in equation (11) to be independent of pressure. Differentiating equation (11) gives for the driving force, at constant T,

$$-\left(\frac{\partial\mu}{\partial x}\right)_T = -RT\left(\frac{\partial\ln a}{\partial x}\right)_T + \bar{V}\left(\frac{\partial p}{\partial x}\right)_T. \tag{12}$$

Equation (12) shows that the activity or concentration gradient and the pressure gradient are superimposed in the total thermodynamic driving force. A simple calculation shows that pressure is an important driving force only for components present at high concentration, i.e. solvents such as water, and when the concentration difference across the membrane is small.

An important feature of the thermodynamic treatment of transport processes is that the flux of any substance is made up of contributions driven by the chemical potential gradient of the

substance itself and by any other non-equilibrium processes in the system (de Groot & Mazur 1962). This includes the generation of fluxes by the coupling of flows with chemical reactions, i.e. so-called active transport. Water has not yet been proved to be actively transported although some water may be indirectly transported as a result of the active transport of solutes. Hence coupling with chemical reactions will not be considered further in this article.

In order to be brief, we consider a discontinuous representation of a membrane system in mechanical equilibrium in which a steady state has been reached. The rate of dissipation of free energy in the system may be expressed by

$$\Phi = J_S \Delta T + \sum_{i=1}^{n} J_i \Delta \mu_i, \tag{13}$$

where J_S is the entropy flow and J_i is the material flow relative to the membrane of each of the n components under a temperature difference ΔT and chemical potential differences $\Delta \mu_i$ of the components between the reservoirs on either side of the membrane.

At sufficiently small values of ΔT and $\Delta \mu_i$, i.e. close to equilibrium, the fluxes can be written in the linear forms

$$J_S = L_{SS} \Delta T + \sum_{i=1}^{n} L_{Si} \Delta \mu_i \tag{14}$$

$$J_k = L_{kS} \Delta T + \sum_{i=1}^{n} L_{ki} \Delta \mu_i. \tag{15}$$

Furthermore, over the valid range of these linear equations the Onsager reciprocal relations

$$L_{Sk} = L_{kS}; \; L_{ik} = L_{ki} \tag{16}$$

also hold.

The right hand side of equation (15) includes the term $L_{kk} \Delta \mu_k$. L_{kk} is often called the straight coefficient concerning the flow of k in the membrane. If equation (9) is rewritten in the form

$$J_k = -\frac{D_T C_k}{RT} \left(\frac{\partial \mu_k}{\partial x} \right) \tag{17}$$

and is integrated across the membrane, it gives

$$J_k = \frac{D_T \bar{C}_k}{lRT} \Delta \mu_k. \tag{18}$$

(The negative sign in equation (17) may be eliminated by defining fluxes as positive in the direction opposite from that chosen to express increases in potentials.) Provided all forces other than $\Delta \mu_k$ are zero, equations (15) and (18) give

$$L_{kk} = D_T \bar{C}_k / lRT \tag{19}$$

where $\bar{C}_k$ is the average concentration of k in the membrane. As pointed out by Manning (1974), the exact nature of this average is not significant in the region where linear theory can be expected to hold.

The cross coefficients L_{ik} etc. represent contributions to flow connected with the existence of other forces. They have no counterpart in the ideal approach of Fick's equation but they are of real importance in multicomponent membrane transport.

Material flow generated by a temperature gradient is included via the term $L_{kS} \Delta T$ in equation (15). Relatively few measurements have been made on the thermo-osmotic flow of water

(Lorimer & Chan 1972). It is thought unlikely to be important in biological systems (Spanner 1954). Thermo-osmosis is dealt with very briefly here; a fuller treatment is due soon (Lorimer 1976).

Consider a single transportable component, water. Its chemical potential difference $\Delta\mu_w$ across a membrane can be written

$$\Delta\mu_w = -\bar{S}\Delta T + \bar{V}\Delta p \tag{20}$$

where $\bar{S}$ and $\bar{V}$ are mean values of the molar entropy and volume respectively. In this case equations (14) and (15) become

$$J_S = L_{SS}\Delta T + L_{Sw}\Delta\mu_w = (L_{SS} - \bar{S}L_{Sw})\Delta T + L_{Sw}\bar{V}\Delta p \tag{21}$$

and

$$J_w = L_{wS}\Delta T + L_{ww}\Delta\mu_w = (L_{wS} - \bar{S}L_{ww})\Delta T + L_{ww}\bar{V}\Delta p. \tag{22}$$

In an isothermal situation

$$J_w(\Delta T = 0) = L_{ww}\bar{V}\Delta p = L_p\Delta p \tag{23}$$

where L_p is called the filtration coefficient. When Δp is zero, equation (22) becomes

$$J_w(\Delta p = 0) = (L_{wS} - L_p\bar{S}/\bar{V})\Delta T. \tag{24}$$

Thus even in the absence of coupling between water and heat fluxes ($L_{wS} = 0$), a thermo-osmotic flow of water is anticipated in the direction of decreasing chemical potential (i.e. from cold to hot side) but the effect is small under practical circumstances.

4.4 *Coupling of material flows, including electro- and anomalous osmosis*

In an isothermal system in which the membrane separates two aqueous solutions of a single non-dissociating solute, denoted by subscript n, the flux equations (15) become

$$J_w = L_{ww}\Delta\mu_w + L_{wn}\Delta\mu_n, \tag{25}$$

$$J_n = L_{nw}\Delta\mu_w + L_{nn}\Delta\mu_n, \tag{26}$$

where the reciprocal relation

$$L_{wn} = L_{nw} \tag{27}$$

holds. The second term on the right of equation (26) is the quasi-Fickian diffusion flux of n. The first term represents an additional flux of n due to coupling with the flow of water. By analogy, the first term on the right of equation (25) might be interpreted as a diffusional flow of water, but in highly swollen membranes this interpretation can lead to difficulties discussed later. The second term corresponds with an additional flux of water generated by coupling with the flux of solute.

The Gibbs–Duhem equation shows that $\Delta\mu_S$ and $\Delta\mu_n$ have opposite signs. L_{ww} and L_{nn} must both be positive but L_{nw} can have either sign. It is subject to the restriction (Katchalsky & Curran 1965)

$$L_{nw}^2 \leqslant L_{nn}L_{ww}. \tag{28}$$

Thus the coupled flow may either augment or oppose the normal down-potential or congruent flow of water. In principle, coupling could reverse the normal flow but, with non-electrolyte solutes, this has not been observed. Usually coupling diminishes slightly the expected flow (Schlögl 1964).

When the solute is an electrolyte and the membrane can acquire a charge, either by dissociation of its ionizable groups or by the adsorption of solute ions, coupling between ion and solvent

flows can be very important. It gives rise to anomalous osmosis (Schlögl 1955) and to electro-osmosis (Schmid 1951; Mackay & Meares 1959*a*). These processes can be important in biology as well as in flow across artificial membranes. The need for brevity limits us to a qualitative discussion.

Both anomalous and electro-osmosis have the same underlying cause. When a membrane containing free mobile ions and water is subject to a gradient of electric potential the ions experience forces which induce movement, up or down field depending upon whether they are anions or cations. In a steady current the electric forces are exactly balanced by retarding forces created by the motions of the ions relative to their environment. The generation of these forces transfers the electrical force from the ions to their environment which consists of other ions, the fixed membrane material and water absorbed by the membrane. Depending on the concentrations, distributions and force fields of these components the amounts of force transferred to each will differ. For example, the concentration of water in the neighbourhood of an ion is greater than the volume average concentration and the amount of force transferred from the ions to the water is greater than its volume fraction would lead one to expect. Ions which tend to be highly hydrated interact relatively strongly with the water molecules.

TABLE 2. ELECTRO-OSMOTIC PERMEABILITIES OF BIOLOGICAL CELLS AND SYNTHETIC MEMBRANES

cell or membrane	electro-osmotic water transference (mol F^{-1})
† Chara australis	38
‡ Nitella translucens	100
§ Zeo-Karb 315 Na^+ form	58
‖ ACI (DK-1) Na^+ form	7

† (Barry & Hope 1969).
‡ (Fensom & Dainty 1963).
§ Phenol sulphonate cation membrane (McHardy, Meares, Sutton & Thain 1969).
‖ Asahi Chemical polystyrene sulphonate cation membrane (Breslau & Miller 1971).

Ions of charge opposite from the membrane matrix (counter-ions) are attracted to it and tend to transfer proportionately more force to it (i.e. less to the water) than do ions of the same charge as the matrix (co-ions). However, there are more counter-ions than co-ions and in a highly charged membrane there is usually a net coupled force on the water in the same direction as the electric current of counter-ions.

When there is no pressure or concentration difference across the membrane the flow of water induced by an electric current is due only to coupling with the ions and is called electro-osmosis. Its importance should not be underrated; transferences of more than 1 l/F are not uncommon. The electro-osmotic transfer of water is of considerable significance in electrochemical operations with synthetic membranes. In these, conductances and currents are large while osmotic permeabilities are small. In biological membranes the reverse holds. Thus, although electro-osmotic transference numbers are similar in synthetic and in bio-membranes, the latter, being very thin, have large osmotic permeabilities and usually osmotic flow outweighs the electro-osmotic flow. Some typical data on both classes of membranes are in table 2.

When there is a concentration difference of electrolyte across the membrane an osmotic flow from dilute to concentrated is expected. In addition, due to the different mobilities and

concentrations of anions and cations, a diffusion potential difference is set up inside the membrane (*N.B.* this must be distinguished from the observable p.d. between the solutions which includes also the Donnan potentials at the membrane/solution interfaces). The internal diffusion potential exerts forces on the ions and through them generates an electro-osmotic contribution to the osmotic flux. Frequently this contribution is so directed as to augment the normal osmotic flux but occasionally it is oppositely directed and large enough to reverse the sign of the net water flux which is then in the non-congruent direction and is called negative anomalous osmosis (Dawson, Dorst & Meares 1969; Pusch & Woermann 1968).

Streaming potentials and currents are also due to coupling of ion and water flows. They have been studied in the squid giant axon membrane by Vargas (1968*a*).

The mathematical treatments of these electrokinetic phenomena are different for swollen gel membranes and for membranes with small pores. Interested readers should consult the appropriate references (Schlögl 1955, 1964; Schmid 1951; Breslau & Miller 1971).

4.5 *Resistance coefficients and the frictional model*

The equality of L_{wn} and L_{nw} expresses the mutual character of the coupling process. The interactions between components are often quantified in terms of molecular friction coefficients although the concept of macroscopic friction cannot readily be extended to molecular encounters. The idea of describing steady transport in a membrane by a balance between thermodynamic forces and frictional interactions of components has received a good deal of attention (Onsager 1945; Klemm 1953; Spiegler 1958; Staverman 1972).

The relation of coupled flows to friction coefficients follows easily from an inverted form of the flux equations (25) and (26). Instead of these equations one may write

$$\Delta\mu_w = R_{ww}J_w + R_{wn}J_n, \tag{29}$$

$$\Delta\mu_n = R_{nw}J_w + R_{nn}J_n, \tag{30}$$

also

$$R_{nw} = R_{wn} \tag{31}$$

holds. The coefficients R_{ww}, R_{nn} and R_{wn} are called resistance coefficients. They help make the mechanism of coupling clear. If J_w were 0 in the presence of solute flux J_n, a counterforce $\Delta\mu_w$, equal to $R_{wn}J_n$, would have to be applied to the water to overcome the drag exerted on it by the solute.

On comparing equations (9) and (30) it is seen that, provided J_w is 0,

$$R_{nn} = lRT/\bar{C}_n D_T. \tag{32}$$

On comparing equations (19) and (32) it can be argued that L_{nn} measures the permeability of the membrane to n when all other components are unrestrained and may be dragged by n whereas $1/R_{nn}$ measures the permeability to n when all other components are held stationary. Only when the coupling coefficients are all zero are these two measures of permeability identical.

According to the frictional model, in steady flow the thermodynamic force X_i acting on 1 mol of species i is exactly balanced by interactive forces F_{ik} between 1 mol of i and all other substances present. This force balance is expressed by

$$X_i = -\sum_{k=1}^{m} F_{ik}, \quad k \neq i. \tag{33}$$

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If the interactive forces are regarded as frictional, each force F_{ik} is the product of a friction coefficient f_{ik} and the relative velocity $(v_i - v_k)$ of the two species. Thus

$$F_{ik} = -f_{ik}(v_i - v_k). \tag{34}$$

The summation in equation (33) includes a term for the membrane matrix m. Hence f_{im} is the friction coefficient of i with the membrane matrix which is usually taken as the velocity reference so that v_m is 0. The mean velocity of species i is taken to be

$$v_i = J_i/C_i. \tag{35}$$

Already we are on dangerous ground. The thermodynamic formulation used so far has been discontinuous, because measurements have to be made in the outer phases, but the forces X_i consistent with the frictional model are the chemical potential *gradients* $\partial\mu_i/\partial x$ in the membrane. Likewise v_i and C_i are respectively the velocity and concentration of i in the membrane phase. We are trying therefore to obtain the insight available from a continuous treatment while retaining the simplicity of a discontinuous treatment.

In a diffusion experiment under a concentration gradient, C_i decreases and v_i increases from the concentrated to the dilute side. If a mean membrane-phase concentration $\bar{C}_i$ is used, the friction coefficients will also be mean values. This may detract from their usefulness. This difficulty can be overcome but it involves much additional experimental work (Kramer & Meares 1969; Meares 1976).

If mean or integral friction coefficients in the membrane $\bar{f}_{ik}$ are defined by $\int_0^l f_{ik}\,\mathrm{d}x/l$, where l is the membrane thickness, one can retain the discontinuous form. Then, on substituting equations (34) and (35) into (33), one has

$$\Delta\mu_i/l = (J_i/\bar{C}_i)\sum_{k=1}^{m}\bar{f}_{ik} - \sum_{k=1}^{m}(J_k\bar{f}_{ik}/\bar{C}_k), \quad k \neq i. \tag{36}$$

Analogous equations exist for each component but only $m-1$ equations are independent. Usually the $m-1$ mobile components are considered relative to the membrane.

In mechanical equilibrium the sum of the forces on any volume element must vanish. Hence

$$C_i f_{ik} = C_k f_{ki} \tag{37}$$

must hold. To evaluate the friction coefficients in the case of two mobile components it is necessary to connect equation (36) with (29) and (30). Equations (36) and (29) become identical when i is replaced by w, k by n and the following relations hold

$$R_{ww} = l(\bar{f}_{wn} + \bar{f}_{wm})/\bar{C}_w, \tag{38}$$

$$R_{nn} = l(\bar{f}_{nw} + \bar{f}_{nm})/\bar{C}_n, \tag{39}$$

$$R_{wn} = -l\bar{f}_{wn}/\bar{C}_n = -l\bar{f}_{nw}/\bar{C}_w. \tag{40}$$

The concentrations in artificial membranes can usually be determined with a fair degree of accuracy. Thus it appears possible to evaluate the friction coefficients characterizing pair-wise molecular interactions in a membrane. The application of the frictional model to biological membranes is restricted by the difficulty of measuring or estimating the average or local concentrations of the permeating species. The treatment can be generalized to multicomponent systems but the number of f_{ik} coefficients to be determined increases rapidly. It is $\frac{1}{2}m(m-1)$.

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4.6 *Practical equations and coefficients*

In the system under discussion the pressure difference Δp and osmotic pressure difference $\Delta\pi$ are more suitable forces for experimental study than $\Delta\mu_w$ and $\Delta\mu_n$, which cannot be varied independently. Transformation to these forces, made with attention to the thermodynamic principles, yields (Katchalsky & Curran 1965)

$$J_v = L_p \Delta p + L_{pD} \Delta\pi, \tag{41}$$

$$J_D = L_{Dp} \Delta p + L_D \Delta\pi. \tag{42}$$

Here J_v is the total volume flow given by

$$J_v = J_w \bar{V}_w + J_n \bar{V}_n \tag{43}$$

and J_D is the exchange flow given by

$$J_D = J_n / \bar{C}_n^\circ - J_w \bar{V}_w \tag{44}$$

and $\bar{C}_n^\circ$ is the mean concentration of the solute in the solutions. The statement that J_D is the velocity of the solute relative to that of the solvent could be misleading, in fact J_D is simply related to the rate of change of concentration in the external solutions (Schlögl 1964).

A quantity σ, defined by $-L_{pD}/L_p$ and called the reflexion coefficient, was introduced (Staverman 1951) to measure the extent to which the membrane is impermeable to the solute. For ideal semi-permeability σ has its maximum value, unity. It may take any lower value, including negative values when the membrane is more permeable to solute than to solvent. When it is equally permeable to both, e.g. when the solute is an isotopically distinguished variant of the solvent, σ is 0.

In terms of σ equation (41) may be rewritten as

$$J_v = L_p \Delta p - \sigma L_p \Delta\pi. \tag{45}$$

With a little algebra it may be shown that J_n may be reintroduced in place of J_D by writing

$$J_n = \bar{C}_n^\circ (1-\sigma) J_v + \omega \Delta\pi, \tag{46}$$

where ω is often called the membrane permeability to the solute.

The coefficients of the practical equations (41), (42), (45) and (46) can be measured in relatively straightforward experiments and it is possible to test whether the linear equations hold and whether $L_{pD} = L_{Dp}$. It is necessary also to demonstrate that the values of these coefficients do not vary with the concentrations of the solutions as a result of changes in the uptake of solvent and solute by the membrane.

5. Determination of transport coefficients

5.1 *Water and a single solute*

Several experimental programmes have been devised to determine L_p, ω, σ and the other coefficients (Meares 1974; Pusch 1975). The fluxes and forces are recorded under conditions in which known concentration differences and pressure differences are set up across the membrane.

Pressure differences are inconvenient to work with. Either the membrane is strained, which alters its properties, or it is supported, which introduces uncertainties regarding the area

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available for transport and the concentration adjacent to the supported surface. With most cell membranes, though not with tissues (Page, Abramovich & Smith 1974*b*) work under a controlled hydrostatic pressure difference is extremely difficult although the pressures inside plant cells with intact walls may be large.

The problem has been attacked by using instead of a hydrostatic pressure an osmotic pressure π_i created by an impermeant solute added to the solution on the 'low pressure' side of the membrane.

Controlled studies of this kind have been carried out on lipid bilayers also. They are too fragile to withstand an applied pressure (Andreoli & Troutman 1971; Andreoli, Schafer & Troutman 1971).

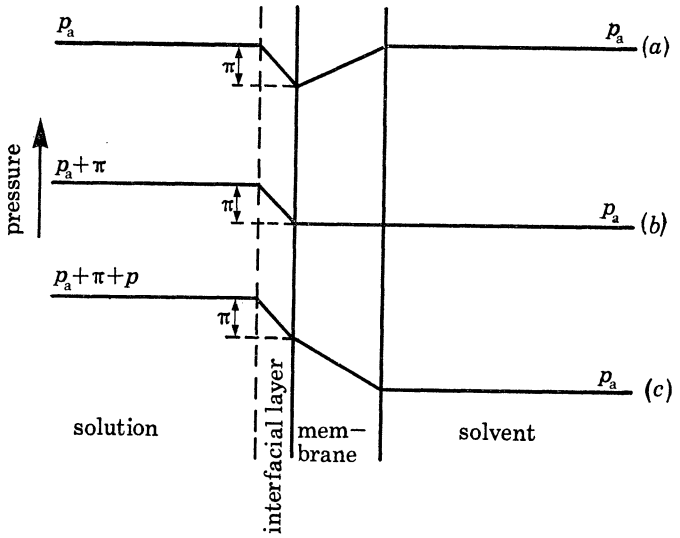

FIGURE 3. Pressure profiles in a membrane system under various circumstances: (*a*) osmotic flow, (*b*) osmotic equilibrium, (*c*) osmotic flow reversed by applied pressure. p_a, atmospheric pressure, p, applied pressure, π, osmotic pressure.

In the case of a truly impermeant solute σ is unity and $L_p = -L_{pD}$. In a simple binary solution when σ is unity, zero volume flow is secured when $\Delta p = \Delta\pi$. It is not immediately obvious however that the flow induced by an unbalanced osmotic pressure at $\Delta p = 0$ is equal to that induced by an equal hydrostatic pressure at $\Delta\pi = 0$, although the linear theory postulates that this is so.

The mechanism of osmotic flow is as follows (Mauro 1957, 1960; Longsworth 1960). The total reflexion of the solute and the partial transmission of the solvent at the membrane/solution interface causes an assymetry in the transfer of momentum by the heat motions of the molecules. This produces a drop in pressure across a layer a few molecules thick in the solution adjacent to the membrane. When the hydrostatic pressures in the bulk phases are equal a real pressure difference is set up between the faces of the membrane which contains some solvent but no solute. Thus the osmotically created pressure produces just the same solvent flux inside the membrane as would be created by an equal hydrostatic pressure applied to the solvent outside. The pressure profiles in the system in the osmotic and hydrostatic cases are illustrated in figure 3. The argument holds whether the membrane is porous or homogeneous. The thickness of the layer across which the pressure drop occurs is determined by the dimensions and mean free paths of the molecules; it has nothing to do with unstirred films.

In a ternary system where the impermeant solute is added to a binary solution containing a permeating solute, the situation is less straightforward. Even if the solutions were ideal, creating an osmotic pressure π_i by adding an impermeant solute affects the chemical potentials of the solvent and solute differently. μ_w is affected by π_i as by an equal hydrostatic pressure but μ_n is affected as if by a pressure $\pi_i \bar{V}_w/\bar{V}_n$. Depending on $\bar{V}_w$, $\bar{V}_n$ and the importance of the pressure term in $\Delta\mu_n$, this osmotic effect may or may not be important. It is necessary also that there shall be no specific or adsorptive interaction between the permeant and impermeant solutes otherwise much larger effects on $\Delta\mu_n$ may be produced.

These thermodynamic matters have not been thoroughly studied in the work published so far. In aqueous solutions, when a polymer is chosen as the impermeant solute and the permeant solute is small organic molecules the interaction between them could well be significant.

Up-to-date reviews of this subject in biological systems (Sha'afi & Gary-Bobo 1973) and in lipid bilayers (Haydon 1970*a*) may be consulted for more information.

5.2 *Transport of water and isotopically labelled water*

A special case of a binary solution arises when the solute is an isotopically labelled variant of the solvent. The commonest choice for labelled water is THO although other possibilities exist. If the differences in physical properties between H_2O and THO arising from mass, etc. are ignored an analysis of the mechanism of water transport in the membrane may be possible. The principle of comparing data on water transfer by osmosis and tracer diffusion was introduced by Pappenheimer, Renkin & Borrero (1951). It has been frequently used since in biological and non-biological systems. L_p for water in the membrane under either a hydrostatic or an impermeant-solute osmotic pressure may be determined together with the 'solute' permeability to THO. If the water concentration in the membrane and its thickness are measured, the diffusion coefficient of THO in the membrane at zero pressure difference can be evaluated.

An analysis of the data is developed here in terms of molecular friction coefficients (Thau, Bloch & Kedem 1966; Meares, Craig & Webster 1970). Subscript T represents THO, w represents water generally or H_2O and m represents the membrane. The system is treated as incompressible ($\bar{V}_w$ constant), the concentration of water is constant through the membrane so that the mean and true friction coefficients are identical.

In a flow experiment under pressure Δp the driving force on the water is $\bar{V}_w \Delta p/l$. The velocity of the water is J_w/C_w with J_w expressed in molar units. The only frictional interaction involved is between water and the membrane f_{wm}. Thus the balance of forces is expressed by

$$\bar{V}_w \Delta p/l = f_{wm} J_w/C_w. \tag{47}$$

The volume flux J_v is $J_w \bar{V}_w$; thus equation (41) becomes

$$J_v = J_w \bar{V}_w = L_p \Delta p. \tag{48}$$

f_{wm} is obtained by combining equations (47) and (48) to give

$$f_{wm} = C_w \bar{V}_w^2/(lL_p). \tag{49}$$

In THO diffusion under zero pressure difference one may use Fick's law

$$J_T = D_w(\mathrm{d}C_T/\mathrm{d}x). \tag{50}$$

The thermodynamic driving force on THO is $-RT(\mathrm{d}\ln C_T/\mathrm{d}x)$ and the velocity is J_T/C_T. D_w is the diffusion coefficient of water in the membrane measured with THO.

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Friction between THO and H_2O has to be considered as well as friction between THO and the membrane. The frictional forces may be evaluated through equation (34). They are

$$F_{Tm} = -f_{Tm}J_T/C_T \tag{51}$$

and

$$F_{Tw} = -f_{Tw}(J_T C_T - J_w/C_w). \tag{52}$$

In the diffusion experiment Δp and J_v are zero, hence J_w is equal and opposite to J_T. Also $C_T \ll C_w$, hence J_w/C_w may be neglected in equation (52). From the balance of forces

$$-RT(dC_T/dx) = J_T(f_{Tm} + f_{Tw}) \tag{53}$$

holds and, on comparing equation (50) with (53), one finds

$$RT/D_w = f_{Tm} + f_{Tw}. \tag{54}$$

Since THO and H_2O molecules are regarded as physically equivalent, $f_{Tm} = f_{wm}$ and f_{Tw} may be regarded as a measure of the strength of interaction of water molecules with one another in their diffusional or heat motion encounters. Combination of equations (49) and (54) then gives

$$f_{ww} = RT/D_w - C_w\bar{V}_w^2/(lL_p). \tag{55}$$

In homogeneous membranes the frictional coefficients f_{Tw} and f_{wm} are often regarded as useful measures of molecular events on which to base speculations about the state and transport mechanisms of water. The correlation of f_{Tw} in the membrane and in water alone gives information on the state and distribution of water in the membrane. The values of f_{wm}, its variations with the degree of swelling and temperature, and its correlation with the free volume theory of diffusion in polymers give further useful evidence.

6. Uses and abuses of friction coefficients

6.1 *Viscous flow*

A number of workers have tried, by comparing f_{Tw} and f_{wm}, to distinguish contributions to the total water flux from 'pure diffusion' and from 'bulk or viscous flow', the latter being presumed to take place in a porous network pervading the membrane. A fundamental objection to the procedure has been raised by Manning (1972). The original definition of the frictional model requires that the driving forces be exactly balanced at all points by the frictional interactions. In these circumstances local mechanical equilibrium would exist everywhere and there could be no velocity gradients such as exist across a pore in which viscous flow is taking place. Thus, it is argued, the foregoing analysis of the volume and diffusional fluxes is invalid where there is a viscous contribution to the flow.

This objection is undoubtedly sound in the case of a membrane with pores so large that the liquid flowing in them may be regarded as a structureless continuum. When the pores have dimensions comparable with the mean free paths of the molecules of the fluid the situation is less clear (Longuet-Higgins & Austin 1966). The problem has been discussed several times (Mikulecky 1967, 1972). One cannot find a readily operated solution to it because the precise expression of viscous flow as a mechanism of mass transport distinct from diffusion requires a detailed knowledge of the geometry of the pore system (the equations could only be solved for

simple cases) and of the law of force between the flowing substances and the pore walls (Mikulecky 1972).

It seems certain that nothing can be deduced about the presence and structure of pores in a membrane from diffusion and flow data alone on a single component such as H_2O or THO but evidence from these properties may be found to be consistent with other evidence regarding pores obtained from, for example, the reflexion coefficients of a series of solutes of graded molecular volume (Solomon 1973).

When a membrane of known pore structure is used and the solvent in the pores can be safely treated as a continuum, the problem of flow and diffusion of solvent and solutes can be treated through the equations of fluid dynamics. An excellent discussion of the use of the Navier–Stokes equation in this context and of the influence of the relative sizes of solute molecules and pores on the reflexion coefficient has been given by Bean (1972). Careful measurements on solvent flow and solute diffusion in membranes with uniform and almost cylindrical pores, made by etching damage tracks in mica, have confirmed that the hydrodynamic treatments hold well for pores 2.5 nm radius (Quinn, Anderson, Ho & Petzny 1972; Beck & Schultz 1972). This does not go far enough to validate deducing pore radii as small as 0.4 nm, as has been claimed, from the analysis of flux data on biological and modified bilayer membranes.

We shall proceed with the calculation of friction coefficients in an isotropic membrane of unknown structure. Even when pores are present the average flux densities can be related to the forces by linear equations with symmetrical coefficients (Mikulecky & Caplan 1966). It is wise always to test experimentally that $L_{pD} = L_{Dp}$ so as to be sure that one is working in the range over which the linear approximation holds.

6.2 *Membrane homogeneity*

Much of the foregoing discussion has been restricted to the consideration of homogeneous membranes. At the molecular level, homogeneous should be understood as meaning that the time-average composition and properties of all volume elements of the membrane at equilibrium are identical provided the volume elements chosen for study have dimensions at least comparable with the mean free paths of the diffusing particles and that time-averages are taken over intervals at least a few times the reciprocal of the mean molecular jump frequencies of the diffusing particles.

These criteria should hold in moderately swollen polymeric gels of randomly tangled and crosslinked chains which are flexible enough to take part as osmotically active components in the heat motion of the whole system. Such a membrane has no fixed pore structure although at any instant solvent molecules may find themselves in locations where they are mainly surrounded by other solvent molecules and exchange momentum directly with them. The continual local concentration and density fluctuations in such a system will prevent any stationary velocity gradients being established and it does not appear that flow could be described as viscous in the normal sense.

When the polymer chains are stiff and the gel highly swollen, the ranges of the diffusional translations accessible to the non-entangled chain segments may not everywhere be large enough to permit them to enter sufficiently frequently into all regions of the gel. In such cases regions permanently occupied by mobile components may exist. It could be argued that such regions constitute randomly directed and interconnected pore spaces even though they do not provide continuous pathways from one face of the membrane to the other. This degree of local

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inhomogeneity might reasonably throw doubt on the significance of friction coefficients calculated from flux data on such membranes.

In a membrane with a permanent pore structure and where all flow takes place in the pores and none in the intervening membrane matrix, frictional coefficients cannot be evaluated from flow data.

When flow in matrix and pores occurs in parallel pathways it is obvious that the membrane is not homogeneous. The same applies to a 'membrane of mosaic' structure, and overall frictional coefficients are meaningless in such cases.

Where a membrane consists of a number of layers in series, which have different properties, the criterion of homogeneity does not hold and linear behaviour is expected in only the simplest cases (Kedem & Katchalsky 1963). Friction coefficients for the whole membrane are, here again, meaningless. Examples of such membranes are the asymmetric cellulose acetate membranes used in reverse osmosis (Merten 1966; Pusch 1975) and in model studies to throw light on biological transport (Gary-Bobo & Solomon 1971).

In the case of lipid bilayers, on account of their extreme thinness, it is perhaps necessary to think of the outer layers, where the polar head groups are located, as having properties different from and in series with the hydrophobic interior in so far as overall permeability properties are concerned. It is possible that the permeability of the polar layers is large enough for them to constitute a negligible barrier to transport but this might not hold if, for example, the head groups had an immobilizing effect on the water structure at the interface, thereby creating a layer of low permeability to some solutes.

6.3 *Stagnant solution films*

A somewhat comparable case of membrane inhomogeneity due to layers of different properties in series arises where there is difficulty in agitating the solution layers adjacent to the membrane surfaces. In such circumstances a part of the barrier to transport is due to the gradients needed to induce mass transfer of the permeating components through the solution up to and away from the membrane faces. This leads to concentration polarization at the interfaces and means that fluxes and bulk concentrations cannot be directly related to the permeability of the membrane itself.

This mass transfer problem is frequently treated by an idealized model, due originally to Nernst, in which completely stagnant layers are imagined to exist on each side of the membrane and to constitute additional resistances in series with it. A major difficulty is that these layers do not interfere equally with all types of transport measurements.

Methods for determining and correcting for such layers have been used for a long time in studies on synthetic membranes (Mackay & Meares 1959*b*). More recently they have been taken into account in biological work (Dainty 1963). The effect of films has been particularly closely studied by investigators of lipid bilayers (Cass & Finkelstein 1967; Andreoli & Troutman 1971; Everitt & Haydon 1969). If the effects of inadequate mixing in the solution phases are allowed for in the appropriate ways, true membrane permeabilities can be evaluated from flux measurements.

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7. Experimental results and their interpretation

7.1 *Permeability and diffusion coefficients in polymeric membranes*

The transport property most frequently reported is the permeability. Being the product of a solubility factor and a mobility factor, its variations with concentration and temperature cannot be easily understood. Often the permeability is almost independent of concentration because the solubility and mobility are varying in opposite directions (Barrie 1968). The so-called activation energy for permeation, derived from its temperature dependence, is the unresolved sum of the heat of sorption and the heat of activation for the diffusion step and so resists simple interpretation.

The diffusion coefficient can be obtained from steady permeation rates if the concentration of permeant in the membrane is determined. In suitable cases it can be determined also from non-steady measurements either by analysing the kinetics of sorption or by making permeation time-lag measurements without requiring solubility data. The results of the different methods do not always agree exactly, especially when clustering of water molecules occurs in the membrane matrix (Barrie 1968) but usually agreement is good (Barrie & Machin 1969, 1971; Hopfenberg, Kimura, Rigny & Stannett 1969).

In order to examine the concentration dependence of the diffusion coefficient of water in the membrane it is usual to have water vapour as the bulk phases because the activity range available with solutions of convenient concentration is not large and the effect of the solute on the membrane may be unknown. Experimental work with water vapour poses several difficulties which have been discussed by Barrie (1968) and by Yasuda & Stannett (1969).

Many results on synthetic polymer membranes have been reviewed by Barrie (1968). Later work has often been on membranes of cellulose derivatives and other polymers of interest in reverse osmosis. Such membranes absorb considerable quantities of water. The usual experience is that the diffusion coefficient of any vapour in a polymer membrane increases almost exponentially with the concentration of vapour absorbed by the membrane. The steepness of the increase is greatest just above the glass temperature of the polymer but gets less as temperature is increased. Such behaviour has been satisfactorily explained by the free volume model of diffusion (Meares 1958).

It has been suggested that water molecules are so small that they do not diffuse by the place exchange mechanism and so the free volume theory may not be applicable to water diffusion. Recent work has shown however that even in the diffusion of simple gases in polymers the free volume principle holds and is needed to interpret the results obtained over a wide range of pressures (Stern 1976).

The free volume theory connects the rate of diffusion with the frequency of chain segmental motions in the polymer which require a 'free volume of activation'. The availability and distribution of the excess free volume required to librate the segments is evaluated from fluctuation theory. It is a function of the local free volume in the polymer + diffusate mixture and this increases with increasing diffusate concentration. The theoretical analysis, which is not developed here, leads to a concentration dependence of the thermodynamic diffusion coefficient D_T of the form (Fujita 1961; Meares 1965)

$$\frac{1}{\ln\left(D_T/D_T^{\circ}\right)} = \frac{A}{b}\left[1+\frac{A}{\phi_{\mathrm{d}}\Delta v_{\mathrm{f}}}\right]. \tag{56}$$

ϕ_d is the volume fraction of the diffusate and D_T° is D_T at $\phi_d = 0$. A and b are constants for the particular polymer and temperature, while Δv_f is the difference between the specific free volumes of the diffusate and the pure polymer. It is usually expressed by

$$\Delta v_f = T(\alpha_d - \alpha_p), \tag{57}$$

where α_d and α_p are the coefficients of thermal expansion of the liquid diffusate and the polymer above its glass transition respectively. Other versions of the free volume theory exist but there are no essential differences from that outlined above (Kumins & Kwei 1968).

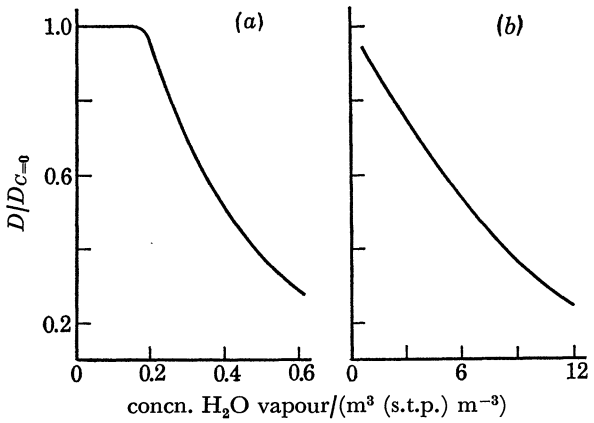

FIGURE 4. Variation of diffusion coefficient of water with concentration in two polymers at 30 °C: (*a*) poly-(fluoromethyl siloxane), (*b*) poly(ethylmethacrylate). (Adapted from Barrie 1968 and Barrie & Machin 1971.)

In the hydrophobic polymers, polyethylene and polypropylene, the diffusion coefficient of water is independent of concentration which is low (Yasuda & Stannett 1962). Energies of activation are 59 and 68 kJ mol^{-1} respectively. In several silicone rubbers D was constant up to moderate pressures of water vapour and decreased at higher pressures (Barrie & Machin 1969). At low pressures the activation energy was also low, about 13–16 kJ mol^{-1}, which is typical of diffusion in silicones, but at high pressures it increased greatly, reaching as high as 70 kJ mol^{-1}. The decreasing D and increasing activation energy are indications that water molecules cluster in this polymer at higher concentrations. Similar effects can be induced in polyethylene and in rubber when polar impurities are present that act as nuclei for the formation of water clusters. The behaviour of silicone rubbers and methacrylates is depicted in figure 4. A decrease in diffusion coefficient with increase in concentration of water has been observed quite frequently in vinyl polymers such as polymethacrylates and polyacrylates. It has been correlated with cluster formation (Barrie 1968; Barrie & Machin 1971). When the non-ideality of sorption is taken into account and D_T is calculated in place of D, the diffusion behaviour appears more nearly normal.

Even where considerable swelling of the polymer by water occurs D may be independent of concentration (Long & Thompson 1955). This is true in polyvinyl acetate. When however the immobilization of part of the sorbed water in clusters is taken into account, it is probable that the mobility of the molecularly dispersed water increases with increasing concentration as predicted by the free volume theory.

In cellulose acetate, D decreased as the concentration of water increased as though there were molecular clustering. When polystyrene chains were grafted to cellulose acetate to give a co-

polymer with polar and non-polar regions, which may be a little more like a biological material than is a homo-polymer, D, as seen in figure 5, was almost independent of concentration at low levels of grafting but at high levels the behaviour resembled that of the backbone (Hopfenberg *et al.* 1969). When graft co-polymers of different monomers on cellulose acetate were examined at the same water concentration, D was found to decrease in the order polystyrene > polyacrylonitrile > polyethyl acrylate > polyacrylic acid (Le Poutre, *et al.* 1972). Thus the more polar the graft chains the lower the mobility of water in the graft co-polymer.

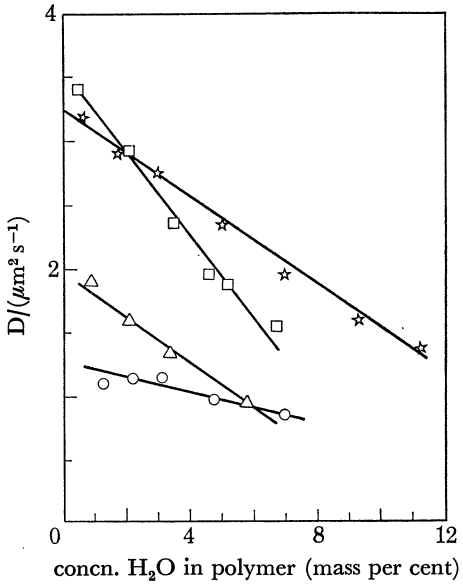

FIGURE 5. Variation of integral diffusion coefficient of H_2O with concentration in cellulose acetate grafted with polystyrene from kinetic sorption data at 25 °C: ☆, 0% polystyrene, ○, 27.2% polystyrene, △, 44% polystyrene, □, 48.5% polystyrene. (Reconstructed from Le Poutre *et al.* 1972.)

A rather contrary conclusion might be drawn by examining some data on the permeation of water through secondary cellulose acetate in which the residual hydroxyl groups had been substituted in various ways. Broadly speaking the rule that the lower the water content the lower the permeability held. Three materials had about the same permeability but their water contents decreased in order of the increasing hydrogen bonding power of the substituents: methoxyl > dimethyl sulphamate > hydrogen phthalate, i.e. the less the hydrogen bonding power the more water is required to provide a given permeability. This observation is consistent with the mechanism proposed by Reid & Breton (1959) for water movement along chains of hydrogen bonding sites.

A resolution of this apparent contradiction may be found in the measurements of Vieth, Douglas & Bloch (1969) on several polymers and co-polymers by the sorption-kinetic and pressure-driven permeability methods. The two types of measurements gave results that agreed when comparison was made through D_T, rather than through D, and the kinetics of cluster formation were taken into account. They found that where clustering occurred due to interactions of water molecules among hydrophobic polymer segments the clusters remained isolated, the water was immobilized and low diffusivity resulted. When however the clusters formed around polar groups in a polymer containing many such groups, communication

between the clusters by single water molecules was retained. Diffusivities were then high and, if swelling were extensive, clusters could overlap and provide continuous, if short-lived, aqueous pathways through some regions of the membrane.

Finally it may be mentioned that in polymers having type III water sorption isotherms, where the first molecules to enter are tightly bound and immobilized at specific sites, D shows a sharp increase with total C_w once these binding requirements have been satisfied (Barrie 1968). This is not a consequence of the free volume mechanism but of the relative unavailability of mobile water molecules below a certain overall concentration. Similar effects might be expected in the presence of proteins combined with lipids.

Apparently no measurements have been reported on the diffusion coefficients of water in lipid bilayers or in biological membranes. This is not surprising in view of the difficulty of determining their water contents and the virtual impossibility of determining diffusion time-lags of 10 ns (Everitt *et al.* 1969). Information on the mobility of water in such systems would be very interesting, particularly if the effects of composition, additives and temperature were studied. Measurements made with thick lipid layers or with lipids supported in inert porous matrixes could be helpful here. Many permeability measurements on lipid bilayers have been made and are referred to in §7.4.

7.2 *Evidence from molecular friction coefficients*

Equations (49) and (54) show that friction coefficients in homogeneous membranes can be evaluated from hydrostatic or osmotic and tracer fluxes of water provided the membrane thickness and the concentration of water in the membrane have been determined also. These requirements have been complied with in a few investigations all carried out on relatively thick (in comparison with biological systems) membranes.

Meares *et al.* (1970) made measurements on several homogeneous cellulose acetate membranes cast from different solvents and concentrations. Thau *et al.* (1966) studied several membranes, three of which may have been homogeneous. These were cellulose acetate, polyvinyl alcohol and cellophane but the two latter were commercial membranes and may have been produced by a method intended to create a porous structure. The required measurements have been made (Yasuda *et al.* 1971; Peterlin, Yasuda & Olf 1972) on a series of 15 membranes of widely differing water contents. Meares (1976) and Mackay (1960) have examined a highly swollen ion-exchanging gel.

In every case the measurements were made at only a single temperature. This is most unfortunate and it is hoped that some data on the effect of temperature will soon be forthcoming.

The various groups cited have presented their results in different ways. The author has attempted to reduce all data to a common format for the purposes of this discussion. This has required some guesses about the density and isotropy of swelling of the membranes but the uncertainty introduced in this way is less than many of the experimental errors. The results are assembled in table 3.

By examining the friction coefficients f_{ww} (i.e. f_{Tw}) and f_{wm} in table 3, we may deduce how far the membranes can be regarded as isotropic swollen polymeric gels. If the water were randomly distributed in each membrane (i.e. neither clustered nor concentrated into permanent pores in the polymeric matrix) each water molecule would make contact with other water molecules on average in proportion to their volume fraction ϕ_w in the membrane. Thus

f_{ww}/ϕ_w may be thought of as a molecular resistance coefficient characteristic of the interaction between pairs of water molecules in each particular membrane.

In a permeation experiment the mean of the velocity components of the permeant molecules in the direction perpendicular to the membrane faces is observed. However, in moving the net distance l in this direction the molecules actually have to travel a distance θl inside the membrane. Here θ is the mean tortuosity of the transport pathways. As a result of tortuosity the observed diffusion coefficients are reduced by the factor θ^2 relative to their absolute local mobilities along the transport pathways, i.e. the mobilities dictated by the force fields operating in pair-wise molecular encounters. Thus the apparent friction coefficients are increased by the factor θ^2.

Unfortunately the estimation of tortuosity at the molecular level is far from straightforward and some authors have chosen to ignore tortuosity altogether. A less unsatisfactory alternative is to attempt an estimate of θ on the basis of an idealized model of the system. Several models and expressions for θ have been used (Mackie & Meares 1955; Prager 1960). The results differ somewhat in composition ranges where the tortuosity is large but in the range of most interest here the differences are insufficient to affect seriously any qualitative conclusions drawn from the data.

It seems probable that the thermal kinetic motions of water molecules in the membrane are considerably more frequent than those of the polymer segments. Thus, where the local concentration of water is sufficiently high, diffusion of THO will occur preferentially by place exchange with other water molecules. In such circumstances the length of an average diffusion path may be estimated from the lattice model of a polymer + liquid mixture by making a simple random walk calculation (Mackie & Meares 1955). The result is

$$\theta = (2-\phi_w)/\phi_w. \tag{58}$$

Thus it is believed that a quantity truly characteristic of the local frictional interaction between pairs of water molecules in the membrane would be given by $f_{ww}/\phi_w\theta^2$. This is listed in column 9 of table 3.

In liquid water the friction coefficient f°_{ww} is given by RT/D°, where D° is the tracer diffusion coefficient of THO in water. At 25 °C one finds $f^\circ_{ww} = 1.16 \times 10^{12}$ J s m^{-2} mol^{-1}. This figure may now be compared with those in column 9 of table 3. In view of the inaccuracies in f_{ww} and of the uncertainty in k, any value of $f_{ww}/\phi_w\theta^2$ lying between about 0.8 and 1.5×10^{12} should be regarded as being in agreement with f°_{ww}.

The first eleven lines in table 3 concern cellulose acetate membranes of various acetyl contents and cast from several solvents. In six cases $f_{ww}/\phi_w\theta^2$ is less than 0.8×10^{12} although never to a great extent. These discrepancies are not correlated with the water content of the membrane, the acetyl content or the casting conditions. In lines 8–11 ϕ_w is so small that diffusion cannot be entirely by place exchange of water molecules and the random walk estimation of θ may be invalid in these cases. Thus one hesitates to speculate on the meaning of these discrepancies between the reduced friction coefficients and f°_{ww}.

Lines 12–18 in table 3 concern a series of hydrogels of various types and compositions which, from their method of preparation, should certainly be homogeneous. In every case there is agreement between $f_{ww}/\phi_w\theta^2$ and f°_{ww}.

Lines 19–21 concern co-polymers of a hydrophilic and a hydrophobic monomer. In lines 19 and 21 f_{ww} appears to be negative. This is physically meaningless and one concludes that,

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TABLE 3. COLLECTED DATA ON PERMEABILITY AND TRACER DIFFUSION OF WATER IN VARIOUS MEMBRANES TOGETHER WITH FRICTIONAL COEFFICIENTS AND 'EQUIVALENT PORE' RADII

number	membrane	remarks	${}_{w}\phi$	$l L_p$ ($m^4 N^{-1} s^{-1}$)	D_w ($10^{-11} m^2 s^{-1}$)	f_{wm}	f_{ww}	$\frac{f_{ww}\phi_w}{(2-\phi_w)^2}$	$\frac{f_{wm}}{\phi_m}$	g	r (nm)
						($10^{12} J s m^{-2} mol^{-1}$)					
1	CA(31–33% acetyl)	cast from 14% in mixed solvents	0.24	1020×10^{-21}	16.5	4.2	10.8	0.84	5.6	3.6	0.58
2	CA(31–33% acetyl)	cast from 14% in mixed solvents	0.20	940×10^{-21}	15.7	3.8	11.9	0.73	4.8	4.1	0.63
3	CA(39.8% acetyl)	cast from 30% in acetone	0.19	301×10^{-21}	8.4	11.2	18.4	1.05	13.8	2.6	0.46
4	CA(39.8% acetyl)	cast from 3% in dioxan	0.18	589×10^{-21}	15.5	5.6	10.4	0.58	6.9	2.9	0.48
5	CA(39.8% acetyl)	cast from 3% in acetone	0.17	112×10^{-21}	4.5	28.0	26.7	1.39	33.9	2.0	0.36
6	CA(39.8% acetyl)	no. 5 annealed in H_2O at 80°	0.15	56×10^{-21}	3.5	49.4	22.4	1.00	58.3	1.8	0.32
7	CA(39.8% acetyl)	no. 4 annealed in H_2O at 80°	0.15	135×10^{-21}	5.4	19.3	26.3	1.10	22.6	2.4	0.43
8	CA(39.8% acetyl)	no. 3 annealed in H_2O at 80°	0.14	102×10^{-21}	6.2	24.2	16.0	0.63	28.0	1.7	0.30
9	CA(39.8% acetyl)	cast from 20% in acetone	0.12	144×10^{-21}	8.0	15.0	16.0	0.54	17.0	2.1	0.38
10	CA(39.5% acetyl)	cast from 10% in $CHCl_4$	0.12	154×10^{-21}	6.9	14.3	21.8	0.75	16.2	2.5	0.44
11	CA(43.8% acetyl)	cast from 6% in $C_2H_2Cl_4$	0.10	85×10^{-21}	5.6	21.3	23.0	0.64	23.7	2.1	0.38
12	Zeo-Karb 315	phenol sulphonate resin	0.75	3×10^{-18}	107	0.016	2.27	1.09	0.06	143	4.3
13	GMA not crosslinked	hydrogels polymerized in water and formic acid or ethylene glycol, crosslinked with TEGDMA where noted	0.86	1230×10^{-18}	112	0.013	2.20	1.46	0.09	170	4.7
14	GMA slightly crosslinked		0.78	1130×10^{-18}	107	0.012	2.29	1.20	0.06	192	5.0
15	GMA 10% crosslinked		0.60	248×10^{-18}	91	0.043	2.69	0.82	0.11	64	2.9
16	GMA 12% crosslinked		0.58	148×10^{-18}	60	0.070	4.06	1.17	0.17	59	2.7
17	HEMA not crosslinked		0.40	19×10^{-18}	38	0.38	6.06	0.95	0.63	17	1.4
18	HEMA not crosslinked		0.38	28×10^{-18}	47	0.24	5.06	0.73	0.39	22	1.6
19	HPMA 95: MMA5	co-polymers prepared in dioxan and acetone solution	0.21	141×10^{-21}	9.5	26.9	(−1.0)	—	34.1	—	—
20	GMA 30 : MMA70		0.15	165×10^{-21}	9.9	16.4	8.50	0.37	19.3	1.5	0.25
21	GMA 15 : MMA85		0.01	14×10^{-21}	52.2	12.9	(−8.1)	—	13.0	—	—
22	Avisco	commercial film	0.58	99×10^{-18}	56	0.11	4.32	1.24	0.25	40	2.2
23	Cuprophane	commercial film	0.47	61×10^{-18}	47	0.14	5.09	1.02	0.26	38	2.2
24	Cellophane	commercial film	0.44	59×10^{-18}	23	0.14	10.7	1.97	0.24	77	3.1
25	polyvinyl alcohol	commercial film	0.36	4.3×10^{-18}	13	1.51	17.5	2.34	0.97	13	1.2

Membranes 1, 2, 9, 11: Yasuda *et al.* 1971 films 12, 13, 14, 15; membranes 3–8: Meares *et al.* 1970 films IIa, IId, III; membranes 10, 24, 25: Thau *et al.* 1966 polymeric membranes; membrane 12: Meares 1976 Na^+-form cation membrane; membranes 13–18: Yasuda *et al.* 1971 hydrogels 1–6; membranes 19–21: Yasuda *et al.* 1971 co-polymers 9–11; membranes 22, 23: Yasuda *et al.* 1971 commercial films 7, 8.

in the swollen state at least, these membranes are not homogeneous and the analysis of data into frictional coefficients is invalid.

Of the four commercial membranes at the foot of the table, Avisco and Cuprophane appear to be consistent with the homogeneous-gel analysis but in Cellophane and polyvinyl alcohol $f_{ww}/\phi_w\theta^2$ is larger than f°_{ww}. A possible explanation here is that these membranes are asymmetric and are 'tighter' on one surface than on the other. Such an asymmetry would increase L_p relative to D_w and hence lead to a spuriously large value of f_{ww} when the membrane is treated as homogeneous.

The overall impression to be gained from the values of $f_{ww}/\phi_w\theta^2$ is that most of the swollen membranes are not far from homogeneous and uniform in the distribution of water and polymer and that the strengths of water–water interactions in the membranes are similar to that in pure water. Although a wide range of ϕ_w is covered by the data in table 3, nevertheless the membranes are all reasonably hydrophilic and well-swollen; thus the effects of isolated clusters of water molecules would not be observed.

An interpretation of f_{wm} is harder to give because such a wide variety of polymeric matrixes is covered. By estimating the diffusion coefficient of water at zero concentration in the polymer an upper limiting value f°_{wm} can be found. For cellulose acetate (Hopfenberg *et al.* 1969) this is 730×10^{12}, for polymethyl, polyethyl and polypropyl methacrylates it is 1300, 146 and 55×10^{12} respectively (Barrie & Machin 1971). These values might be compared with f_{wm}/ϕ_m (table 3, column 10) where ϕ_m is the volume fraction of polymer in the membrane (i.e. $1-\phi_w$).

Except for the co-polymers in lines 19–21, whose anomalous behaviour has already been mentioned, f°_{wm} is much greater than f_{wm}/ϕ_m. This trend is anticipated from the free volume theory of diffusion in polymers. The greater free volume available in the swollen material facilitates the oscillations and rotations of polymer chains and so eases the relative motions of polymer and water molecules. A quantitative application of free volume theory cannot be attempted because insufficient information on the other coefficients of equation (56) is available.

Yasuda *et al.* (1971) have made a number of simplifying assumptions in order to apply the free volume theory to their membranes. Thence they have inferred that the variation of D_w for THO with ϕ_w is consistent with the free volume theory. Other investigators (Kishimoto, Maekawa & Fujita 1960) have claimed, from the experimental evidence of constant D_w with polyvinyl acetate and polymethyl acrylate, that the free volume theory does not hold for water in these polymers. This seems improbable because the theory has been found to hold for permeant molecules smaller than water (Stern, Fang & Frisch 1972). It is possible that, in the case of water, the increase in D_w caused by the extra free volume contributed by the absorbed water is sometimes cancelled by a decrease in D_w caused by water clustering.

7.3 *Convection and equivalent pore flow*

Equations (19) and (32) suggested that alternatives to D_w for expressing the mobility of water in the membrane might be $lRTL_{ww}/C_w$ or $lRT/R_{ww}C_w$. In defining the former any mobile solutes present are permitted to be dragged along by the water while in defining the latter they are regarded as stationary. Data are available on these quantities in the case of the ion-exchange gel (line 12 in table 3). $lRTL_{ww}/C_w$ is about 6×10^{-8} m^2 s^{-1}, $lRT/R_{ww}C_w$ about 4×10^{-8} m^2 s^{-1} while D_w is 0.1×10^{-8} m^2 s^{-1}. The difference of D_w from those quantities is very obvious. It arises because in tracer diffusion there is effectively zero momentum relative

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to the stationary membrane whereas when a force is applied to one or more components it induces a net mass flow and hence momentum in the flux direction. This momentum can be directly exchanged in collisions between flowing molecules at all concentrations of water but it will be important at values of ϕ_w above about 0.2. Then, at any instant, some continuous, though very tortuous, chains of contact between water molecules may extend from one membrane face to the other. Such chains of contact are continually being broken and reformed in other locations.

This momentum exchange does not differ fundamentally from that between molecules flowing in a permanent pore. Thus the diffusion and flow characteristics of any membrane can be represented as pore flow or a combination of matrix diffusion and pore flow in an unlimited number of ways depending only on the arbitrary choice of a physical model of the membrane.

A factor g has been defined through the relation (Thau *et al.* 1966)

$$g = 1 + f_{ww}/f_{wm} \tag{59}$$

and it is supposed that when $g \gg 1$ pore flow is indicated. By postulating that all flow occurs in uniform straight cylindrical pores extending perpendicularly from face to face in the membrane and that the water flows as a fluid continuum governed by Poiseuille's law, an equivalent pore radius may be defined by

$$r = 8\eta_w D_w^\circ \bar{V}_w (g-1)/RT^{\frac{1}{2}}, \tag{60}$$

where η_w is the viscosity of bulk water.

Columns 11 and 12 of table 3 list g and r. It may be seen that in the least swollen membranes g is less than 2 and the values of r are so small that the use of Poiseuille's law is obviously not justified. In the most swollen membranes g exceeds 100 and r reaches 5 nm. No real significance can be attached to such radii which depend entirely on the model chosen to describe the membrane. For example, one might suppose that a homogeneous swollen polymer gel would be better represented as a random mesh of non-circular, tortuous and interconnecting pores. In this case the mean pore radius of Carman (1948) is more appropriate. It is about 60 % greater than that given by equation (60).

More sophisticated combinations of diffusion and convection in relation to the interaction between water and pore walls have been discussed by Mikulecky (1972) but cannot be tested for lack of specific information. Briefly, when one has only two flow parameters, D_w and L_p, one can answer at most two questions about the membrane. If one wishes to specify a number and size of pores then their disposition, shape, uniformity and the absence of other transport paths, etc. must be postulated. Most authors who have used the equivalent pore concept appear to understand this limitation but there is a danger that some readers may believe that the transport data prove the real existence of uniform pores in membranes. As mentioned already, membranes deliberately prepared with known pore structures behave as expected from hydrodynamic laws by assuming the normal continuum behaviour of water to hold in pores as small as 4.5 nm radius. Data on smaller pores of reliably known size and distribution are not yet available.

It may be noted that an attempt has been made to explain the apparent difference in diffusive and hydrodynamic permeabilities of swollen membranes without invoking convection (Paul 1973, 1974) but this has not been widely accepted. The contrary case has been put by Peterlin (Peterlin 1974; Peterlin & Yasuda 1974).

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7.4 *Water transport in lipid bilayers*

Several references to studies of water transport in bilayers have been made already in this article (Cass & Finkelstein 1967; Redwood & Haydon 1969; Everitt *et al.* 1969; Everitt & Haydon 1969; Haydon 1969, 1970*b*). Precise measurements of film thicknesses and water content have not been possible and this has prevented the evaluation of friction coefficients. However, it was demonstrated that, when proper precautions were taken to eliminate the effects of unstirred layers in the solution phases, the tracer and osmotic permeabilities agreed well. This fits the expectation that the concentrations of water should be very low leading to negligible convective transfer of momentum between water molecules.

Andreoli & Troutman (1971) dealt with the boundary layer problem in a rather different and ingenious way and arrived at a value of 1.22 for g, the ratio of osmotic and tracer water permeabilities. This value may not differ from unity by more than the experimental errors but an alternative explanation exists. A lipid bilayer can scarcely be regarded as a homogeneous membrane because it consists of two layers of polar head groups with a lipid interior sandwiched between them. The whole bilayer is so thin that the head group layers occupy an appreciable fraction of the total thickness. It is likely that the barrier to osmotic flow is the lipid interior. The diffusional resistance will be made up of that due to the lipid interior and contributions from the head group layers in series with it. Thus a small excess of the osmotic over the diffusional permeability might be anticipated.

Parallel experiments have been carried out on bilayers modified by the introduction of nystatin and amphotericin B (Andreoli & Troutman 1971; Holz & Finkelstein 1970; Finkelstein & Holz 1973). A marked increase was observed in both permeabilities and their ratio g increased to 3–4. These observations have been interpreted as evidence for the creation of pores 0.4–0.5 nm in radius by the association and regular packing of the antibiotic molecules in the membrane. Of course, one can argue only that the water permeability data are consistent with the pore model among others. More compelling evidence for the existence of these pores comes from data on the permeabilities of the bilayers to other solutes and to data on their reflexion coefficients.

The observation of coupling of solute and water flows in these modified bilayers (Andreoli *et al.* 1971) could scarcely be explained if all components were uniformly distributed in the membranes. It is probable however that, in the hydrophobic interior of the bilayer, the hydrophilic solute molecules and water molecules would tend to be associated for thermodynamic reasons in a way that might be regarded as the inverse of the hydrophobic bonding which occurs in aqueous systems. Such association would lead to flow coupling without the need to postulate the existence of pores. The influence of amphotericin B or nystatin might then be to solubilize particular solutes and water into the lipid, thus altering the net partition coefficients. This alternative mechanism is set down not with the intention of contradicting the now carefully argued case for pores in these modified bilayers but to emphasize that some thermodynamic or solubility data could provide an important supplement to the permeabilities in confirming the existence of pores. Such data might include the solubility of water in lipids as a function of temperature and lipid composition and partition coefficients of the solutes most commonly studied between water and lipids both in the absence and in the presence of membrane modifiers.

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7.5 *The water permeabilities of biological membranes*

No attempt is made here to review in detail the extensive data on biological membranes. Two comprehensive and modern reviews (Dick 1971; House 1974) exist prepared by persons more competent than the present author to discuss biological systems. There are reviews also on more specific aspects of the subject by Solomon (1968, 1973), by Gary-Bobo (1975) and Sha'afi & Gary-Bobo (1973).

Investigations may be divided into two broad categories: transport into and out of single cells of plants and animals, and transport through more complex systems such as epithelia. Many studies have been directed towards the solution of physiological problems but in several cases the objective has been to gain an insight into membrane structure from permeability measurements. Such work has usually been conducted, as already discussed at length, by the comparison of osmotic and isotopic diffusional permeabilities. These measurements have often been supplemented by solute permeabilities and reflexion coefficients also. In the case of dog and human red cells measurements have been made over a range of temperatures.

The many experimental difficulties and uncertainties attending water transport measurements on biological cells have been thoroughly discussed by House (1974). They include particularly the effects of stagnant boundary layers outside the cell, where stirring can at least be attempted, and inside the cell. There stirring is not possible and the dispersal of permeating components can take place only by diffusion in the intracellular fluid. Boundary resistances are particularly serious in reducing the apparent permeability of the cell membrane in tracer diffusion work. Their effect on measurements of osmotic permeability is less pronounced. Since measurements frequently depend on observing the rate of cell shrinkage due to osmotic outflow of water when placed in a hypertonic solution, it is necessary to extrapolate the data in some way to give the rate of shrinkage at zero time when the osmotic pressure difference is accurately known. It appears to have been overlooked so far that such observations of cell shrinkage give transport data referred to a moving reference frame.

It should be possible also to determine the permeability to water under a hydrostatic pressure difference but relatively few cells are suitable for this type of measurement. Where it has been possible the comparison of osmotic and hydraulic permeabilities has given some disquieting results. Vargas (1968*b*) measured the hydraulic L_p and osmotic L_{pD} permeabilities of the squid giant axon in both perfused and intact states. He found that L_p exceeded L_{pD} by about 100-fold. Although the axon membrane was not ideally semi-permeable to the solutes, glycerol and sodium chloride, it was quite evident that their reflexion coefficients were very much greater than 0.01 if judged on the basis of solute permeability. In other words the overall behaviour of the axon membrane was not consistent with the linear non-equilibrium thermodynamic equations. Vargas quoted comparable findings with the blood–brain barrier and in an algal cell (*Valonia ventricosa*). He suggested the most likely explanation was the existence of a small number of large membrane pores that contributed greatly to L_p but which were freely permeable to solute and water and did not contribute to L_{pD}, i.e. the membrane was not homogeneous.

In view of the various and incompletely resolved difficulties surrounding work on such systems it is doubtful whether any clear significance could be attached to the ratio of permeabilities, g of equation (59). House (1974) has given a table of values of g which, in the most reliable cases, range from 1 up to about 7. Although it has been argued that factors of g in excess of 2

indicate some contribution from transport through pores, such arguments were not convincing when applied to data on the homogeneous membranes in table 3. The arguments cannot be made at all if the membranes are suspected of being inhomogeneous or the detailed arrangement of layers in series and elements in parallel cannot be specified.

It seems certain that biological membranes composed of lipid layers, associated protein and, perhaps, modified water bound to the polar head groups of the lipids and to the protein cannot be regarded as homogeneous. Where a membrane consists of layers with different permeabilities arranged in series it is clear that the normal interpretation of g is totally misleading (Hays 1968).

Attempts have been made to gain additional evidence by determining also the temperature variation of the permeabilities and evaluating activation energies for tracer permeation and osmosis (Vieira, Sha'afi & Solomon 1970). In the red cell membranes of dog and man low values of the activation energies were observed that were quite close to the values found for diffusion and viscous flow in liquid water. These findings were interpreted as supporting the theory that water was transported through small pores in these membranes.

The apparent activation energy for permeation is the sum of the heat of solution of water in the membrane and the activation energy of the diffusion process itself. Only the latter term should be compared with the activation energy for diffusion in the water and it cannot be evaluated without data on the variation of solubility of water in the membrane with temperature from which to obtain the heat of solution.

An even more significant quantity, if it could be evaluated, would be the entropy of activation for diffusion (Barrer 1942). From this an estimate might be made of the size of the zone of activation or the degree of cooperation among molecular motions of membrane components and water which were required for a unit diffusion step (Meares 1954). Until fundamental data of this kind become available the interpretation of permeabilities in cell membranes must remain largely speculative.

If the understanding of water transport in single cell membranes is complicated by ignorance regarding the molecular structures of such membranes and of the location of the most important permeation barriers, the situation with regard to multi-cellular systems is much worse. Nevertheless much work has been done on transport across epithelia, partly because of their vital physiological rôle in connection with the water balance of most organisms but also because epithelia are convenient for mounting in conventional permeability apparatus. Few fundamental deductions have been possible and most work has confirmed that in a structurally complex system a very rich variety of transport phenomena is possible. Chapter 9 of the book by House (1974) gives a comprehensive and excellent review.

It is clear that the simplest model of an epithelium must include several types of barrier in series and parallel and that any attempt to interpret data through a linear force–flux representation appropriate to a homogeneous membrane is doomed. To this author it appears likely that the region of linear behaviour will be restricted to such small forces and fluxes that it may not be possible even to make measurements in the linear régime.

Most workers have measured diffusion and osmotic flows of water. Coupling between water and solute fluxes has also been observed. Only few measurements have been made on permeation under a hydrostatic pressure (Hakim & Lifson 1969; Moody & Durbin 1969). It has been found that the hydraulic permeability greatly exceeded the osmotic permeability, just as was observed by Vargas (1968*b*) in the axon membrane.

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Page, Abramovich & Smith (1974*a*, *b*) have studied the diffusion of tracer water and flow under osmotic and hydrostatic pressures across human amnion and amnio-chorion. These highly swollen systems exhibit, as usual, a lower diffusional than flow permeability and, in the case of amnio-chorion, the hydrodynamic permeability L_p was greater than the osmotic permeability L_{pD}. The same appears to be true of amnion alone when these authors' hydrodynamic values are compared with Seeds' (1970) osmotic data.

The hydrodynamic permeability of amnio-chorion was found to fall as the applied pressure was increased. The decrease was about ten-fold over the range from 0.5 to 6.0 kN m^{-2} in supported tissue. When the tissue was unsupported and strained by the pressure, L_p still decreased with increasing pressure, though less markedly. The osmotic permeability also decreased with increasing osmotic pressure difference generated by dextran as solute. Although a polymeric solute was used there was evidence that it would penetrate the membrane to some extent so that its reflexion coefficient must have been less than unity. It was clear from this work that a complex multi-cellular system exhibits nonlinear behaviour under even small forces.

Work on complex tissues will undoubtedly continue because of their interest to physiologists. Explanations of the phenomena observed may be sought at the level of coupling between various transport processes in terms of the tissue structure. It is premature to look for fundamental molecular mechanisms. Progress might be helped if measurements of solute flux ratios and water fluxes under hydrostatic and osmotic pressures with truly impermeant solutes could be made simultaneously. It would be useful also to know whether nonlinearity in the force/flux relations could be related to observations on membrane deformation by pressure, swelling by solutes and osmotic shrinkage.

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Discussion

F. H. Stillinger. (*Bell Laboratories, Murray Hill, N.J., U.S.A.*). I would like to question Professor Meares concerning the proper molecular model to be applied to water transport and retention in pure lipid bilayer membranes. The ability of the experimental freeze-fracture technique to split such membranes literally in half suggests that their mid-plane region is a 'weak spot' in their structure. This region, where alkane chain ends reside, seems a likely 'resting station' for molecules in transit across the membrane. If water clusters are to form at high water activity, this mid-plane region is where they are probably to be found.

By contrast to this picture, the membrane molecules displayed by Professor Meares during his lecture possessed more uniform, indeed monotonic, charges in local properties in passage from one membrane face to the other.

For the lipid bilayers, isn't it relevant to use properties (partition coefficient, mobilities, etc.) which vary non-monotonically with normal distance?

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P. Meares. The theory of stationary and non-stationary diffusion across membranes composed of several layers with different properties has been extensively developed by Barrer, Petropoulis and others. The treatment of steady multicomponent transport under external forces and with coupled flows across two different membranes in series was described by Katchalsky and Kedem. There is no formal difficulty in extending their treatment to more layers but the number of parameters required would be formidable and a detailed quantitative knowledge about the structure and properties of the system considered would be required. It would not be feasible to perform the reverse operation and derive the structural information from flux data. The structural feature of bilayers referred to by Dr Stillinger may have a significant influence on overall permeability. Certainly one might expect that water clusters, if they were proved to exist in hydrophobic regions, would find the median plane of the bilayer to be a preferred location. Bearing in mind the effect of chain ends (i.e. molecular mass) on mobilities in polymer films, I would expect the mobility of molecules in the median plane to be higher than normal. However, the region is so thin that water molecules would cross it in a single jump and concepts such as mobility and diffusion coefficient hardly seem appropriate there.

Of particular interest is the discovery that in biological membranes some protein is to be found in the median plane. In such circumstances the accumulation of water, perhaps bound, in the mid-plane region seems to be highly probable. In my paper I have suggested a crude polymeric model of such a system that might be worthy of experimental study.

F. Franks. (*Botany Department, University of Cambridge, Downing Street, Cambridge*). Since knowledge of the adsorption isotherm of water on the membrane material enables the mean cluster size to be determined, and from scanning calorimetry and other techniques one can estimate the amount of unfreezable water, is it possible, by combination of these data to obtain a measure of the critical cluster size for the nucleation of ice in a membranous system?

P. Meares. It must always be borne in mind that a mean cluster size derived from an absorption isotherm does not mean necessarily that a group of water molecules of that size is in direct contact, e.g. through H-bonds. As with the determination of a surface excess from Gibbs' isotherm, the location of the excess particles is not specified by thermodynamic arguments. Nevertheless the concept of H-bonded molecular clusters does seem to provide a reasonable molecular model consistent with the thermodynamic data. Almost nothing is known however about the distribution of cluster sizes about the mean. If the largest clusters were to freeze first at a given temperature it is likely that a redistribution of sizes would take place among the unfrozen clusters to maintain the correct statistical situation. This would complicate the identification of the critical cluster size. Any attempt to act on Professor Franks' suggestion would require absorption isotherms to be recorded at a series of temperatures above, below and in the region where freezing occurs. This has not yet been done.

F. Franks. What is the real significance of a protein adsorption isotherm, since a protein cannot exist in the anhydrous state? The word 'protein' implies a polypeptide/water system with enough water to allow the polymer to maintain its native conformation. It is known that a number of proteins undergo transitions at low water activities in which case the adsorption isotherm has little to do with the native polymer.

P. Meares. Professor Franks has raised here an important question regarding protein/water systems. In general, protein derived materials, such as wool, which can be examined in the

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anhydrous state exhibit type II isotherms but native proteins must be associated with so much water that only the upper end of the isotherm could be explored. The relation between conformational transitions, water content and pH of collagen membranes has been studied and such transitions have a major effect on the membrane permeability.

F. Franks. Experiments on transport across single bilayer phospholipid liposomes shows that these are quite good barriers to ions but hardly any barriers to the flow of water. The lipid molecules do not tumble in a way that the head groups could carry water across the bilayer. Can the lecturer suggest the mechanism of rapid water flow across what is to all extents and purpose an oriented hydrocarbon film? I believe that this is an unsolved problem as far as membrane biologists are concerned.

P. Meares. It is hard to make any definitive comment on how far the water permeability of phospholipid liposomes is anomalously large until one has reliable information on the stationary concentration of water in the bilayer.

The combination of electrostatic and van der Waals force fields to which the bilayer is subject may lead to a relatively low density or large 'free volume'. Diffusion coefficients are extremely sensitive to free volume and it would not be difficult to imagine that the diffusion coefficient of water molecules in the lipid region was a good deal higher than the self-diffusion coefficient in liquid water.

D. A. T. Dick. (*Department of Anatomy, University of Dundee*). Conventionally biologists have defined the permeability coefficient as flux/Δ concentration thus including the membrane thickness as well as the partition coefficient as used by Professor Meares. What contribution do studies of water flux parameters on lipid bilayers treated with (apparently pore-forming) antibiotics make to the pore hypothesis of cellular membranes?

P. Meares. It would not be appropriate for a physical chemist to offer an opinion on how far any studies on the permeabilities of lipid bilayers, modified or unmodified, can be regarded as contributing to the understanding of cellular membranes. Professor Dick's remark in parentheses points to a more immediate problem to which I have paid a little attention in my paper. The effect of certain antibiotics on the permeabilities of bilayers to water is consistent with the theory that such antibiotics create pores in the bilayers. The coupling of solute and solvent fluxes in such modified bilayers provides more evidence for the pore hypothesis. Other mechanisms, perhaps less plausible or, certainly, less popular, can be devised, however, to explain the same data. More evidence, particularly on equilibrium partition coefficients, would be useful to confirm the pore hypothesis in bilayers.

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