Biochimica et Biophysica Acta, 1000 (1989) 411–412
Elsevier

Commentary by

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on 'Thermodynamic analysis of the permeability of biological membranes to non-electrolytes'
by O. Kedem and A. Katchalsky
Biochim. Biophys. Acta 27 (1958) 229–246

Ora Kedem (left) and Aharon Katchalsky

In 1960, a membrane meeting, embracing all topics from collodion to the nature of active transport, was held in Prague. In Professor Ussing's words, it was "the founding meeting of the Transport Workers' Union". Our paper on the thermodynamics of permeability was thus published in a period of increasing awareness of the topic.

Under the leadership of the late Aharon Katchalsky, we were studying as a group the developing formalism and background of the thermodynamics of irreversible processes. I was assigned the work of Staverman on membranes and had the great good luck to read at the same time a textbook on plant physiology. It was immediately clear that the inconsistency in the interpretation of plasmolysis would disappear, as it were, by itself, if the two pieces were united. This started an exciting and rewarding tour through data on water flow in the living organism. Review of customary equations and analysis of experimental methods led to the necessary formulation.

It had been clearly accepted for a long time that osmotic water flow needs not only a driving force, the difference in solute concentration, but also a certain mode of transfer, a semi-permeable membrane. Beautiful insight was shown in Pfeffer's work published in 1885. If at one point in an annular tube a porous diaphragm separates water and a solution, and at a point on the other side of the ring a semipermeable membrane separates the same two solutions, a circulating motion will be set up, enhancing the mixing of the two solutions.

The difficulty was in the gradual transition from free

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unselective diffusion to 'colloid' osmotic flow. The Onsager relation led to an a priori connection between the effective osmotic pressure and 'solvent drag'. The role of solvent drag as a possible additional force acting on solutes had been recognized. The necessary new concept was the fact that processes are moved not only by their own driving forces but also by coupling to other processes. Solvent drag modifies solute flow without influencing the thermodynamic potential of the solute. Further, a quantitative relation, independent of the specific mechanism, exists between osmotic flow and solvent drag.

For the design of experiments the most important conclusion was in the measurement of process parameters. Restrictions, i.e., the exact conditions of the experiment, have to be chosen and watched carefully. The role of exactly planned restrictions is clearly seen in an example outside the paper discussed here, the relation between active transport and water flow. If one measures water flow across an epithelium separating identical short-circuited solutions, the driving force for water and for all other solutes vanishes; nevertheless, one may observe water flow. If, however, the driving force for water and all other flows are zero, no water flow is seen: water flow is coupled to solute flows and not directly to metabolism. The machinery used to move around large quantities of material has an energy-converting core, the sodium pump. It is surrounded by auxiliary structures, on a molecular and on a larger scale, to hitch other wagons to the same locomotive.

The broader aspect of a quantitative definition of coupling, instead of a yes/no definition, is in the understanding of a dynamic stoichiometry in primary and secondary chemical energy conversion. Constant stoichiometry in 'uphill' and 'downhill' conditions reflects tight coupling.

We felt that a major message from irreversible thermodynamics to biology should be the lucid distinction between equilibrium states and stationary states, the maintenance of time-independent states far from equilibrium at the expense of continuously dissipated free energy. In the quantitative analysis of this dissipation the definition of coupling, matched for the purpose, is essential.

In the sixties and seventies, transport properties of various organs, intestine, kidney and its elements have been measured with great care and ingenuity, defining components and conditions in clear detail. The basis for an integrated picture now exists and computer-simulated function of whole organs can help to resynthesize the beautiful analytical experimental work.

When we prepared this paper for publication, all those years ago, one of my colleagues objected to its structure: people may want to refer to some equations and they are all mixed up in fish eggs. I believe this mix-up was more useful than we realized then.

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THERMODYNAMIC ANALYSIS OF THE PERMEABILITY OF BIOLOGICAL MEMBRANES TO NON-ELECTROLYTES

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I. INTRODUCTION

In spite of the large amount of information which has accumulated on permeability phenomena, the conventional equations of volume and solute flow (equations (1) to (6)) do not completely describe the physical behaviour of membranes, and the permeability data obtained by different methods are not quantitatively comparable. The insufficiency of the permeability equations was felt by previous authors and several attempts have been made to supplement them. Thus FREY-WYSSLING[1] and LAIDLER AND SHULER[2] took into account the contribution of the solute to volume flow; USSING[3] claimed that a force exerted by solvent flow "enters into the escaping tendency of all substances present in the solutions in contact with the membrane"; PAPPENHEIMER[4] treated the flow of solute through membranes as two flows—a flow by filtration and a flow by diffusion. These attempts did not however develop into a self-consistent and general set of equations able to cover the whole range of phenomena. A solution to this problem can be obtained through the methods of irreversible thermodynamics. STAVERMAN[5,6] has recently given a complete treatment of osmotic pressure measurements applying these methods and KIRKWOOD[7] has similarly treated the transport of ions through membranes. The expressions obtained by these authors are however not directly applicable to the physiological measurements described in the literature. The present work is devoted to a suitable modification and extension of the equations derived by the methods of the thermodynamics of irreversible processes in order to apply them to biological permeability data. It follows the approach of STAVERMAN.

The equations obtained are applied to the analysis of several commonly used experimental methods and it is shown how the coefficients defined by the thermodynamic equations can be evaluated from the data. Only these coefficients are of significance in the analysis of membrane structure and the mechanism of permeation.

Moreover, an examination, with the aid of the thermodynamic equations, of the results reported in the literature, reveals that in spite of numerous measurements carried out, additional data are needed in many cases to obtain the pertinent coefficients.

II. THE INSUFFICIENCY OF THE CONVENTIONAL PERMEABILITY EQUATIONS

The conventional description of transport through membranes makes use of two equations, one for solute flow and one for volume flow. Consider an isothermal system

References p. 246.

consisting of two compartments, an inner one designated by the superscript i and an outer one designated by the superscript o, the two compartments being separated by a membrane. Only non-electrolyte solutes are considered.

The equation for solute flow is written analogously to Fick's equation

$$\frac{dN_s^i}{dt} = k_s A (c_s^o - c_s^i) \tag{1}$$

where N_s^i denotes the number of moles of permeable solute in the inner compartment, k_s is the permeability coefficient of the solute which includes the thickness of the membrane, A is the membrane area, and c_s is the concentration of solute s in moles per unit volume. If the volume of the inner compartment is denoted by V^i, $c_s^i = N_s^i/V^i$ and eqn. (1) may be written in the form[8]

$$\frac{dN_s^i}{dt} = k_s A \left(c_s^o - \frac{N_s^i}{Vi}\right) \tag{2}$$

The equation for volume flow when no hydrostatic pressure difference exists between the inner and outer compartments is based on the proportionality between the flow dV^i/dt of volume (usually identified with the flow of water), and the difference $\pi^i - \pi^o$ of osmotic pressure between the inner and outer solutions, *i.e.*

$$\frac{dV^i}{dt} = k_w' A (\pi^i - \pi^o) \tag{3}$$

where k_w' denotes the permeability coefficient of water. Putting $\pi = RTc$ (c is the osmotic concentration) and absorbing RT into the proportionality coefficient, equation (3) can also be written

$$\frac{dV^i}{dt} = k_w A (c^i - c^o) \tag{4}$$

In (4), c denotes the sum of the concentrations of all the solutes whether the membrane is permeable for them or not.

If the system contains only one permeable solute and if the total amount of the non-permeable solutes in the inner compartment is denoted by N_m^i, c^i becomes

$$c^i = \frac{N_m^i + N_s^i}{Vi}$$

Denoting the concentration of non-permeable solutes in the outer compartment by c_m^o and that of permeable solute by c_s^o, c^o can be written

$$c^o = c_s^o + c_m^o$$

Introducing into (4) we obtain the equation used by JACOBS[9], namely

$$\frac{dV^i}{dt} = k_w A \left\{\frac{N_m^i + N_s^i}{Vi} - (c_s^o + c_m^o)\right\} \tag{5}^*$$

When a hydrostatic pressure difference $\Delta p = p^o - p^i$ exists between the compartments in addition to the osmotic pressure difference $\Delta\pi = \pi^o - \pi^i$ considered above,

* JACOBS writes $c_o V_o$ for N_m^i where c_o and V_o are the initial values of non-permeable solute concentration and cell volume.

References p. 246.

equation (3) according to Starling's hypothesis (*cf.* PAPPENHEIMER[4]) assumes the form

$$\frac{dV^i}{dt} = k'_w A\,(\Delta p - \Delta \pi) \tag{6}$$

Permeability to water

The inadequacy of the equations presented above for the consistent description of the behaviour of biological systems will be first demonstrated on the basis of the measurements of ZEUTHEN AND PRESCOTT[10] on the permeability of fish and frog eggs.

ZEUTHEN AND PRESCOTT suspended the eggs in a frog Ringer solution. After osmotic equilibrium was reached, the volume flow dV^i/dt became zero. Introducing into equation (5)*, this leads to the condition

$$\frac{N^i_m}{V^i} = c^o_m \tag{7}$$

as in this part of the experiment no permeable solute was present.

The equilibrated cells were then transferred into a Ringer solution of corresponding solute composition, in which however 10–15% of the water was substituted by heavy water (D_2O). It was found that the heavy water penetrates the cells following equation (2) exactly, thus proving that it behaves as any solute. At the same time it was observed that the cell volume remains *constant*, so that $dV^i/dt = 0$.

Further experiments were carried out on water flow in hyper- or hypotonic solutions of non-penetrating solutes and it was found that equation (5) accurately describes the volume changes of the eggs. The values of k_w obtained should thus enable us to calculate the volume flow in any solution. In particular, we should find that the flow in the experiments carried out in isotonic Ringer solutions containing heavy water is given by

$$\frac{dV^i}{dt} = k_w A\left(\frac{N^i_s}{V^i} - c^o_s\right) \tag{8}$$

as

$$\frac{N^i_m}{V^i} = c^o_m \tag{9}$$

Now, as demonstrated above, heavy water behaves as an ordinary solute which in this case has in the outer solution a very high molar concentration compared with the other solutes of the Ringer solution. It would be expected therefore that dV^i/dt would assume large negative values at the beginning of the experiment and would approach zero when N^i_s/V^i approaches c^o_s. The observation that $dV^i/dt = 0$ throughout the experiment proves therefore that equation (5) is incomplete. As shown in the following, this is due to the fact that no distinction is made between permeable and non-permeable solutes. ZEUTHEN AND PRESCOTT found moreover, that the penetration of hevay water in non-equilibrated solutions is not represented adequately by equation (2). It is more rapid in hypotonic solution and slower in hypertonic, thus proving that cross relations exist between diffusion and filtration flows which are not expressed in the conventional equations (1) to (6).

Plasmolytic measurements

The insufficiency of the equations is revealed also when less permeable solutes than heavy water are used. Especially instructive are the observations made on the

* It is generally assumed that between such cells and the surroundings, no pressure difference can be maintained.

References p. 246.

threshold concentration for plasmolysis of plant cells. In order to understand what underlies the concept of threshold concentration, let us consider the behaviour of a plant cell in solutions of different concentrations. Fig. 1 represents schematically the dependence of the cell volume V^i on time t in solutions of a single permeable solute of various concentrations c_s^o. Consider first a cell immersed in pure water. The cell swells to its maximal volume V^i_{max} and a hydrostatic pressure difference Δp is set up between the cell sap and the surrounding medium. Δp is maintained by the rigidity of the cell wall and is equal to the osmotic pressure difference $\Delta\pi$. V^i_{max} is independent of time and corresponds to the upper horizontal straight line.

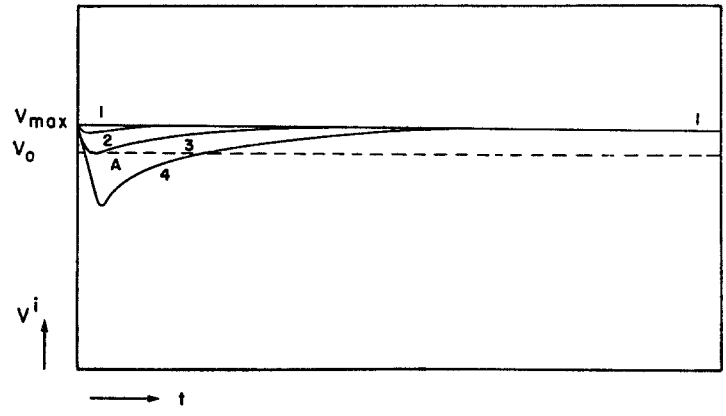

Fig. 1. Schematic representation of volume changes of a plant cell immersed in solutions of one permeable solute at different concentrations. Curve 1 in pure water; curves 2, 3, and 4 in increasing concentrations of permeable solute. The difference between V_{max} (the volume in pure water) and V_o (the equilibrium volume of the cellulose walls) is exaggerated. A is the plasmolytic point.

The maximally swollen cell is now introduced into a solution of low concentration (Curve 2). The stress in the cell wall will be slightly relieved as a small amount of quickly permeating solvent immediately leaves the cell. Later, as both solute and solvent penetrate into the cell, the stress recovers. As long as c_s^o is sufficiently small, the volume changes are insignificant and not observed under the microscope. However, above a definite concentration, the plasmolytic concentration c_s^{o*}, the rapid initial escape of solvent will become sufficiently large so as to cause the plasma to shrink away from the cell wall and thereby relieve the stress completely, making $\Delta p = 0$ (curve 3, Fig. 1). After the cell volume goes through a minimum, at which $dV^i/dt = 0$, the penetration of the solute will cause deplasmolysis and bring the volume slowly to its initial state at which dV^i/dt again equals zero. The phenomenon becomes even more pronounced as the concentration of the external solution is still further increased.

At the plasmolytic point both Δp and dV^i/dt equal zero (see Fig. 1). Thus one should expect eqn. (5) to be valid and, as $c_m^o = 0$ in these experiments, we find:

$$0 = k_w A \left(\frac{N_m^i + N_s^i}{V^i} - c_s^{o*}\right) \qquad \text{or} \qquad c_s^{o*} = \frac{N_m^i + N_s^i}{V^i}$$

As in this case the permeation of water is much faster than that of solute, the amount of solute already penetrated at the point of minimum volume is rather small. We find therefore that N_s^i/V^i is much smaller than c_s^{o*} and may be neglected.

References p. 246.

Moreover, as pointed out, the change in volume at the plasmolytic point is very small so that N_m^i/V^i equals the initial cell concentration c_{int}^i, hence

$$c_s^{o*} = c_{int}^i \tag{10}$$

Equation (10) shows that the plasmolytic threshold concentration might be expected to be independent of the nature of the external solute. Experience, however, has shown that the ratio c_s^{o*}/c_{int}^i differs from unity within a range of more than a thousand and the quantity $\mu = c_s^{o*}/c_{int}^i - 1$ has even been defined as a coefficient characterizing permeability—the Lepeschkin constant[11].

III. PERMEABILITY EQUATIONS DERIVED ON THE BASIS OF IRREVERSIBLE THERMODYNAMICS

The thermodynamic approach to the problem of permeability of membranes leads to the conclusion that the incompleteness of the equations (1)–(6) is due to the fact that they involve only two of the three coefficients, required to characterize permeability for a solute–solvent system. The necessity for three coefficients in the case of membranes permeable to solvent and only one of the solutes, may be understood in a qualitative way as follows:

In the case of free diffusion, solvent and solute migrate only relatively to each other. Hence the hydrodynamic resistance of diffusion flow is due to the friction between solute and solvent alone, so that diffusion in a solution of a single solute is determined by a single diffusion coefficient. The passage through a membrane, however, involves two additional factors, namely, the friction between solute and membrane and the friction between solvent and membrane. A full description thus has to take account of three coefficients whose values will depend on the nature of the three processes involved. Not in all cases, however, will all three processes be equally important. For example, in coarse membranes with large pore dimensions, the solvent–solute friction will contribute more than the other factors, and permeability will approach the behaviour in free diffusion (as is the case in the fritted disk method of NORTHROP AND ANSON[12]). In dense membranes on the other hand, in which part of the solute penetration takes place say through dissolution in the membrane, the contribution of the friction between solute and the membrane becomes predominant.

Derivation of the equations of flow in two-component systems

The starting point of the thermodynamic description of non-equilibrium systems is a calculation of the entropy production during the process. In the case of a two component system in which two solutions of the same solvent and solute are separated by a membrane, the entropy production per unit time $\mathrm{d}_i S/\mathrm{d}t$ is given by the equation

$$\frac{\mathrm{d}_i S}{\mathrm{d}t} = \frac{1}{T}(\mu_w^o - \mu_w^i)\frac{\mathrm{d}N_w^i}{\mathrm{d}t} + \frac{1}{T}(\mu_s^o - \mu_s^i)\frac{\mathrm{d}N_s^i}{\mathrm{d}t} \tag{11}$$

where μ denotes the chemical potential (of the solvent, subscript w, and solute, subscript s), $\mathrm{d}N_w^i/\mathrm{d}t$ and $\mathrm{d}N_s^i/\mathrm{d}t$ represents the number of moles of solvent and solute respectively entering the inner compartment per unit time[13].

It is often convenient to use the "dissipation function" which is given by $T\,\mathrm{d}_i S/\mathrm{d}t$. We shall use the dissipation function per unit area *i.e.*

References p. 246.

$$\Phi = T.\frac{1}{A}\frac{\mathrm{d}_i S}{\mathrm{d}t} = (\mu_w^o - \mu_w^i)\,\dot{n}_w + (\mu_s^o - \mu_s^i)\,\dot{n}_s \tag{12}$$

where

$$\dot{n}_w = \frac{1}{A}\frac{\mathrm{d}N_w^t}{\mathrm{d}t} \text{ and } \dot{n}_s = \frac{1}{A}\frac{\mathrm{d}N_s^i}{\mathrm{d}t}$$

It will be observed that the dissipation function (12) is composed of the sum of products of flows per unit area ($\dot{n}_w$ and $\dot{n}_s$) and corresponding "forces"—the differences in chemical potential.

Equation (12) constitutes a special case of the general expression $\Phi = \Sigma_i J_i X_i$ where J_i denotes a flow and X_i the generalized conjugated force. The choice of flows and forces is arbitrary to a certain extent, so long as their products sum up to the same dissipation function.

In the following we shall make the approximation that the chemical potentials for ideal solutions may be used, so that

$$\mu^o - \mu^i = \bar{v}\,\Delta p + RT\,\Delta \ln \gamma \tag{13}$$

where $\bar{v}$ is the partial molar volume, Δp is the difference in pressure between the outer and inner compartment, and γ the molar fraction of the constituent. In the case of dilute solutions, where the volume fraction φ of the solute is small, $\varphi = c_s\bar{v}_s \ll 1$ and equation (13), written for the solute, becomes

$$\mu_s^o - \mu_s^i = \bar{v}_s\Delta p + RT\,\Delta \ln c_s = \bar{v}_s\,\Delta p + RT\,\frac{\Delta c_s}{c_s} \tag{14}$$

where $\Delta c_s = c_s^o - c_s^i$ and c_s is a mean of the concentrations of the solute in the two compartments given by

$$\frac{\Delta c_s}{c_s} = \ln \frac{c_s^o}{c_s^i}$$

If

$$\frac{\Delta c_s}{c_s^i} \ll 1,\ c_s = \frac{c_s^i + c_s^o}{2}$$

The corresponding equation for the solvent is

$$\mu_w^o - \mu_w^i = \bar{v}_w\,\Delta p - RT\,\frac{\Delta c_s}{c_w} \tag{15}$$

where $c_w = (1 - \varphi)/\bar{v}_w$ or, to a good approximation, $c_w = 1/\bar{v}_w$. Introducing equations (14) and (15) into equation (12) and rearranging we get

$$\Phi = (\dot{n}_w\bar{v}_w + \dot{n}_s\bar{v}_s)\,\Delta p + \left(\frac{\dot{n}_s}{c_s} - \frac{\dot{n}_w}{c_w}\right) RT\,\Delta c_s \tag{16}$$

It will be observed that in (16) the dissipation function is represented by a new set of forces and flows. The new forces $X_v = \Delta p$ and $X_D = RT\Delta c_s$ are the forces usually employed in permeability studies, Δp is the hydrostatic pressure while $RT\Delta c_s$ is the driving force in Fick's equation. The conjugate flows are the total volume flow per unit area:

$$J_v = \dot{n}_w\bar{v}_w + \dot{n}_s\bar{v}_s \tag{17}$$

and the relative velocity of solute versus solvent which is a measure for exchange flow:

$$J_D = \frac{\dot{n}_s}{c_s} - \frac{\dot{n}_w}{c_w} \tag{18}$$

References p. 246.

The general theory of irreversible thermodynamics is based on the assumption that the flows J are functions of all the forces operative in the system and that, if the forces are sufficiently small, the dependence is linear. Thus in the case of two flows J_1 and J_2 dependent on two forces X_1 and X_2, the relation between the J's and X's is given by

$$J_1 = L_{11}X_1 + L_{12}X_2 \qquad (19)$$
$$J_2 = L_{21}X_1 + L_{22}X_2$$

where the L's are called the phenomenological coefficients.

The phenomenological coefficients are correlated by the law of Onsager which requires the equality of the cross-coefficients $L_{ik} = L_{ki}$ or in our case

$$L_{12} = L_{21} \qquad (20)$$

Writing (19) in the notation applying to our system, we obtain:

$$J_v = L_p \Delta p + L_{pD} RT \Delta c_s \qquad (21)$$
$$J_D = L_{Dp} \Delta p + L_D RT \Delta c_s$$

with
$$L_{Dp} = L_{pD}$$

The second law of thermodynamics requires that entropy production be always positive, from which we may conclude that the straight coefficients L_p and L_D must be positive, while L_{pD} may be either positive or negative. The magnitude of L_{pD} is restricted by the condition, $L_p \cdot L_D - L_{pD}^2 > 0$.

The physical meaning of (21) may be seen in the following way: in very coarse membranes, volume flow and exchange flow are independent. Each of the flows is determined only by its conjugate force: J_v by the pressure gradient Δp and J_D by the concentration gradient Δc_s. However in many less permeable membranes, the flows are interdependent and the gradient in solute concentration produces a volume flow, even when $\Delta p = 0$; this is known as osmotic flow. Similarly, a pressure difference causes not only a total volume flow but also a relative velocity in the solute–solvent flow—this is known as ultrafiltration. These interdependences are incorporated in the coefficient L_{pD}.

Consideration of some special cases

The volume flow at *zero concentration difference*,

$$J_v = L_p \Delta p \qquad (\Delta c_s = 0)$$

measures the mechanical permeability of the membrane for a given solution, and L_p is the filtration coefficient.

In an ideal semipermeable membrane $\dot{n}_s = 0$ so that $J_D = -\dot{n}_w/c_w$ and $J_v = \dot{n}_w \bar{v}_w$. However, as pointed out, for dilute solutions $c_w = 1/\bar{v}_w$ and hence

$$J_D = -J_v \text{ (ideal semipermeable membrane)} \qquad (22)$$

This is obvious since in this case both volume and exchange flows are due to solvent only. Introducing (21) into (22) and rearranging terms, we obtain

$$(L_p + L_{pD})\Delta p + (L_D + L_{pD}) RT \Delta c_s = 0 \qquad (\dot{n}_s = 0) \qquad (23)$$

As (23) must hold for all pressure and concentration differences, it can only be satisfied

References p. 246.

if both bracketed expressions equal zero. Hence

$$L_p = -L_{pD} = L_D \text{ (ideal semipermeable membrane)} \tag{24}$$

The system is then fully characterized by the filtration coefficient L_p alone. It will be shown below (V) that in the case of permeable membranes, L_{pD} should be smaller than L_p. In the case of completely non-selective membranes, L_{pD} is zero, as is readily seen: with these latter membranes a pressure difference alone ($\Delta c_s = 0$) does not produce an exchange flow, so that

$$J_D = 0 = L_{pD}\Delta p \text{ or } L_{pD} = 0 \text{ (non-selective membrane)} \tag{25}$$

The same conclusion may also be derived from the fact that in non-selective membrane no volume flow is produced by a concentration difference alone *i.e.*

$$J_v = 0 = L_{pD} RT \Delta c \text{ or again } L_{pD} = 0 \text{ (non-selective membrane)}$$

In the intermediate cases L_{pD} is negative and lies between 0 and $-L_p$. When however the membrane is more permeable to solute than to solvent L_{pD} is positive.

Besides volume flow, the flow generally measured is not J_D but the solute flow $\dot{n}_s$. In terms of J_v and J_D, $\dot{n}_s$ is given by

$$\dot{n}_s = (J_v + J_D)\, c_s \qquad (\varphi = c_s \bar{v}_s \ll 1) \tag{26}$$

$\dot{n}_s$ is often measured at constant volume, *i.e.* at $J_v = 0$ and Δp in this case is given by

$$\Delta p = -\frac{L_{pD}}{L_p} RT \Delta c_s \qquad (J_v = 0) \tag{27}$$

(see (21)).

Introduction of (21) and (27) into (26) gives

$$\dot{n}_s = \frac{L_p L_D - L_{pD}^2}{L_p} c_s RT \Delta c_s \qquad (J_v = 0) \tag{28}$$

Thus at constant volume the solute flow is proportional to the concentration difference.

The equations for practical calculation

For comparison with experimental data it is convenient to pass from the system L_p, L_D and L_{pD} to another set of coefficients. STAVERMAN has introduced the reflection coefficient, σ, defined in our terms by

$$L_{pD} = -\sigma L_p \tag{29}$$

It is clear that $\sigma = 0$ applies to a non-selective membrane and $\sigma = 1$ to an ideally selective one, permeable to solvent only.

We further define the mobility of the solute, ω, as

$$\omega = \frac{L_p L_D - L_{pD}^2}{L_p} c_s = (L_D - L_p \sigma^2)\, c_s \tag{30}$$

It is seen from equation (28) that ωRT is the proportionality coefficient between the solute flow at constant volume and Δc_s. Therefore ωRT is the equivalent of the solute permeability constant k_s measured at *constant volume*. For an ideally semipermeable membrane $\omega = 0$ (see (24)). We shall now transcribe our equations in terms of L_p, σ and ω. Introducing (29) and (30) into (21) and (26) we obtain

References p. 246.

$$J_v = L_p (\Delta p - \sigma RT \Delta c_s) \tag{31}$$

$$\dot{n}_s = c_s L_p (1 - \sigma) \Delta p + [\omega - c_s L_p (1 - \sigma) \sigma] RT \Delta c_s \tag{32}$$

Another convenient formulation which permits us to correct the equation for solute flow directly for volume flow readily derives from (31) and (32):

$$\dot{n}_s = \omega RT \Delta c_s + (1 - \sigma) c_s J_v \tag{33}$$

Equations (31) and (32), derived on a thermodynamic basis, will now be compared with the conventional expressions (1)-(6).

The equation for $J_v = dV^i/dt \cdot 1/A$ corresponds to equation (6), and L_p is the equivalent of k'_w. It will be observed that, as $RT\Delta c_s = \Delta\pi$, equation (6) becomes identical with (31) only if $\sigma = 1$, *i.e.* if the membrane is impermeable to the given solute. However, as will be shown later, for many solutes σ is only a small fraction of unity.

The simultaneous use of (1) and (6) or (4) is therefore self-contradictory.

Comparison of (1) and (33) shows that the expressions become identical, and $k'_s = \omega RT$, only if $J_v = 0$. (The case $1 - \sigma = 0$ is not an exceptional one, as then also $\omega = 0$).

It is seen from equations (32) and (33) that the solute flow can be described as a function of Δc_s alone if either Δp or J_v are zero. It should however be emphasized that for the same Δc_s, the flow in these two cases may be quite different. In designing physiological measurements, it is thus of the utmost importance that the conditions under which the experiments are performed are fully known. For example for hydrodynamic calculations the significant coefficient is ω so that the measurements of $\dot{n}_s$ have to be carried out at constant volume.

Polycomponent systems with a single permeable solute

In biological studies, we are not dealing with simple two-component systems containing only one solute and solvent. On one side of the membrane at least, a large number of components is to be found even if only two—the solvent and one solute—are permeable. In the latter case, the number of streams and coefficients remains the same as described previously, but the presence of the non-permeable solutes modifies the chemical potentials and hence the driving forces.

We continue the derivation of the pertinent equations on the assumption that the solutions are ideal. The case of real solutions can be treated by introducing activity factors. Let the system contain solvent (w) a permeable solute (s) and several non-penetrating solutes (i) with molar fractions γ_w, γ_s, γ_i which are different on the two sides of the membrane.

In correspondence with the previous notations

$$\Delta\mu_w = RT \ln \frac{\gamma_w^o}{\gamma_w^i} + \bar{v}_w \Delta p \tag{34}$$

so that, assuming the volume fractions of all the solutes to be small,

$$\Delta\mu_w = -\frac{RT \Delta c_s}{c_w} - \frac{RT \Sigma \Delta c_i}{c_w} + \bar{v}_w \Delta p \tag{35}$$

and

$$-RT \Sigma \Delta c_i = \Delta\pi_i$$

References p. 246.

where $\Delta\pi_i$ is the contribution of the non-permeable solutes to the difference in osmotic pressure.

As in the case of the two-component system

$$\Delta\mu_s = \frac{RT\,\Delta c_s}{c_s} + \bar{v}_s\Delta p$$

If the same flows are considered as before, the conjugated forces X_v and X_D may be derived from the dissipation function as follows:

$$\Phi = \dot{n}_s\Delta\mu_s + \dot{n}_w\Delta\mu_w = (\dot{n}_s\bar{v}_s + \dot{n}_w\bar{v}_w)\,X_v + \left(\frac{\dot{n}_s}{c_s} - \frac{\dot{n}_w}{c_w}\right)X_D \tag{36}$$

From the coefficients of $\dot{n}_s$ and $\dot{n}_w$ on both sides of equation (36) one obtains:

$$X_v = c_s\Delta\mu_s + c_w\Delta\mu_w$$
$$X_D = (1-\varphi)\,c_s\Delta\mu_s + \varphi c_w\Delta\mu_w$$

Introducing the values of $\Delta\mu_s$ and $\Delta\mu_w$ we get:

$$\begin{aligned} X_v &= \Delta p - \Delta\pi_i \\ X_D &= RT\,\Delta c_s + \varphi\Delta\pi_i \end{aligned} \tag{37}$$

We see that at equilibrium, both forces are zero, because the equilibrium pressure head is $\Delta\pi_i$ and the equilibrium distribution of the solute is such that

$$\Delta\mu_s = -\Delta\pi_i\bar{v}_s \quad i.e.\ RT\,\Delta c_s = -\varphi\Delta\pi_i \tag{38}$$

With the driving forces (37), the equations of flow corresponding to (31) and (32) in the presence of non-permeable solutes become

$$J_v = L_p\,(\Delta p - \Delta\pi_i) - \sigma L_p\,(RT\,\Delta c_s + \varphi\Delta\pi_i) \tag{39}$$

$$\dot{n}_s = L_p\,(1-\sigma)\,c_s\,(\Delta p - \Delta\pi_i) + [\omega - \sigma L_p\,(1-\sigma)\,c_s]\,(RT\,\Delta c_s + \varphi\Delta\pi_i) \tag{40}$$

and

$$\dot{n}_s = J_v\,(1-\sigma)\,c_s + \omega\,(RT\,\Delta c_s + \varphi\Delta\pi_i) \tag{41}$$

The term $\varphi\Delta\pi_i$ in (39), (40) and (41) in most cases represents only a small correction to $RT\Delta c_s$, but the contribution of $\Delta\pi_i$ to the first driving force in (39) and (40) is very important. Actually in many biological systems $\Delta p = 0$ whereas $\Delta\pi_i \neq 0$.

Comparing (39) with (6) it is seen that equation (6) describes the volume flow correctly only if none of the solutes present can pass the membrane (*i.e.* if $\sigma = 1$). The contribution of a solute characterized by reflection coefficient σ to the volume flow is σ times smaller than that of a non-penetrating solute at the same concentration.

We may now summarize the relation between the three coefficients L_p, σ, ω and the conventional constants k'_w and k_s.

(1) k'_w as defined by equation (6) can be identified with the filtration coefficient L_p if no permeable solute is present or if the latter is at equilibrium (equation (38)).

(2) The coefficient of solute flow k_s can be identified with ωRT if there is no volume flow.

(3) σ is a third independent coefficient ignored by the conventional equations. It will be shown that it is closely related to Lepeschkin's constant.

References p. 246.

IV. APPLICATION TO SPECIAL SYSTEMS

Filtration and diffusion of water

The inconsistencies in the application of the conventional equations to the permeability data for heavy water are removed by the introduction of the reflection coefficient.

It is evident that membranes will have little ability to differentiate between ordinary and heavy water. σ for heavy water will therefore be either zero or have a very small positive value. If we introduce $\sigma = 0$ into equations (39) (41) we get

$$J_v = L_p (\Delta p - \Delta \pi_i) \tag{42}$$

$$\dot{n}_s = J_v c_s + \omega RT \Delta c_s \tag{43}^*$$

as the flow equations applying to the present case. We see therefore that for this case two coefficients, the filtration constant L_p and ω, corresponding to the diffusion constant at zero volume flow, suffice to describe the permeability behaviour.

Equation (43) demonstrates the pronounced dependence of solute on volume flow flow when $\sigma = 0$.

In the measurements with frog and fish eggs, for which, as we have seen, $\Delta p = 0$, J_v changes sign with the sign of $\Delta \pi_i$ (see (42)). The flow of heavy water $\dot{n}_s$ will thus be different depending on whether a simultaneous bulk flow of water enters or leaves the cell, an effect observed by ZEUTHEN AND PRESCOTT[10].

If σ for heavy water is to be determined, the experiments have to be made under special conditions. Thus if we arrange that $\Delta p - \Delta \pi_i = 0$ then $J_v = -\sigma L_p RT \Delta c_s$ and it becomes possible to evaluate σ from the magnitude of the small volume flow.

Permeability of plant cells

In part II, we pointed out that while the plasmolytic threshold concentration of the penetrating solute, c_s^{o*}, should equal, according to the conventional equations, the concentration c_{int}^i of the non-permeable cell constituents, c_s^{o*}/c_{int}^i differs enormously from unity. The following consideration will show that the coefficient actually measured by plasmolytic experiments is the reflection coefficient, σ, of the cell membrane.

At the plasmolytic point $\Delta p = 0$ and $J_v = 1/A \cdot dV^i/dt = 0$. Introducing into equation (37) we thus get:

$$J_v = 0 = L_p (\Delta \pi_i + \sigma RT \Delta c_s)$$

or

$$-\Delta \pi_i = \sigma RT \Delta c_s$$

As pointed out in II moreover the amount of solute penetrated at the plasmolytic point is negligible. This implies that $\Delta c_s = c_s^{o*}$ and hence

$$-\Delta \pi_i = \sigma RT c_o^{s*}$$

As

$$-\Delta \pi_i = RT c_{int}^i, \qquad \sigma RT c_s^{o*} = RT c_{int}^i$$

or

$$\frac{c_s^{o*}}{c_{int}^i} = \frac{1}{\sigma} \tag{44}$$

* We have neglected $\varphi \Delta \pi_i$ against $RT \Delta c_s$ despite the fact that φ is rather large in experiments with heavy water and may approach 0.1. However Δc_s is so large, throughout the greater part of the experiment, that the neglect is justified. For example in the case of the 10 % D_2O in water solutions Δc_s was 5.5 moles/liter at the beginning of the experiment. The initial value of $\Delta \pi_i$ for a cell immersed in frog Ringer × 0.5 expressed in moles/liter, is approximately 0.1, so that $\varphi \Delta \pi_i = 0.01$ as compared with 5.5.

References p. 246.

The plasmolytic threshold concentration therefore gives a direct measure of σ. Similarly the Lepeschkin constant $c_s^{o*}/c_{int}^{i} - 1 = \mu$ is another function of the reflection coefficient

$$\sigma = \frac{1}{1 + \mu}$$

From the measurements of RUHLAND AND HOFFMANN on the permeability of *Beggiatoa mirabilis*, quoted by HÖBER[11], it is seen that the threshold concentration of urea is $1.75 \cdot 10^3$ times larger than that of sucrose, *i.e.* $\sigma_{urea}/\sigma_{sucrose} = 5.7 \cdot 10^{-4}$.

There is no way of obtaining the solute mobility ω from the plasmolytic concentration. It should be stressed however that the classical measurements of OVERTON, and of COLLANDER AND BÄRLUND[14] give this constant in a clear and definite manner. Permeability measurements on plant cells under non-plasmolytic conditions are here carried out at constant cell volume so that $J_v = 0$. Hence from equation (41), the solute flow is determined by the single coefficient ω:

$$\dot{n}_s = -\frac{V^i}{A}\frac{\mathrm{d}\Delta c_s}{\mathrm{d}t} = \omega RT \Delta c_s \tag{45}$$

Stationary state measurements

The evaluation of the coefficients becomes relatively simple if one of the flows vanishes. This condition is known in the thermodynamics of irreversible processes as a "stationary" state and is characterized by several important features. In the case of permeability studies, the flow which can be made to vanish by experimental conditions is generally the volume flow *i.e.* $J_v = 0$. In the case of this stationary state we get from equation (39) and (38)

$$(\Delta p - \Delta \pi_i) = \sigma RT \Delta c_s \text{ (for negligible } \varphi \Delta \pi_i) \tag{46}$$

and

$$\dot{n}_s = \omega RT \Delta c_s \tag{47}$$

respectively, so that σ and ω can be obtained directly from pressure measurements and from solute flow.

The first to carry out systematic stationary state studies on biological material were PAPPENHEIMER, RENKIN AND BORRERO[15]. These workers circulated plasma through a dog's hind leg maintaining a hydrostatic pressure in the blood vessels which kept the weight of the limb constant. It can be shown that the condition of constant weight is practically equivalent to zero volume flow.

With plasma fluid alone, the "isogravimetric" hydrostatic pressure was found to be nearly equal to the osmotic pressure of the plasma proteins (actually 97% of the osmotic pressure as measured by artificial membranes). On addition of permeable solute to the plasma fluid, the pressure had to be raised to a value Δp_i in order to avoid filtration. The increase was sudden and large and declined only slowly with time.

In these experiments it was impossible to determine the volume of the tissue fluid into which diffusion takes place during the experiment. It was also difficult to determine the effective membrane surface. Therefore an exact relation between $\dot{n}_s$ and Δc_s could not be obtained. PAPPENHEIMER *et al.* gave the value of $\dot{N}_s$, *i.e.* the number of moles of solute penetrating per unit time through the membrane area A^g per 100 g tissue

$$\frac{\dot{N}_s}{A^g} = \dot{n}_s$$

References p. 246.

From equation (47) therefore

$$\dot{N}_s = A^g \omega RT \Delta c_s \tag{48}$$

It was observed that $\dot{N}_s/(\Delta p_i - \Delta \pi_i)$ is constant during a given experiment. From (48) and (46) we see that

$$\frac{\dot{N}_s}{\Delta p_i - \Delta \pi_i} = \frac{A^g \omega}{\sigma} \tag{49}$$

which is indeed a constant. PAPPENHEIMER *et al.* assumed however that $\Delta p - \Delta \pi_i = RT\Delta c_s$ instead of equation (46) and deduced that

$$\frac{\dot{N}_s}{\Delta p_i - \Delta \pi_i} = A^g \frac{k_s}{RT}$$

$$(\text{At } J_v = 0, \quad k_s = \omega RT)$$

The value of the permeability constant obtained in this way may be many times larger than the true value as already demonstrated by GRIM[16]. Nevertheless, the data of PAPPENHEIMER *et al.* permit the evaluation of σ and $A^g\omega$, as σ can be determined from equation (46) at zero time, when $\Delta c_{s,o}$ is known, (provided the time required for the distribution in the plasma fluid is short compared to the time for penetration). Δp_i cannot be measured exactly at $t = 0$; however, it can be deduced by extrapolating the plot of $\ln \Delta p_i$ against t (which is a straight line) to $t = 0$.

Introducing $\Delta p_{i,o}$ and $\Delta c_{s,o}$ into equation (46) we derive the σ values given in the following Table. With the aid of equation (49) the corresponding values of $A^g\omega$ are obtained.

TABLE I

REFLECTION COEFFICIENTS AND SOLUTE MOBILITIES
(calculated from PAPPENHEIMER, RENKIN AND BORRERO[15])

	σ	$A^g \omega \cdot 10^{12}$ $cm^2 \cdot \frac{1}{sec} / \frac{dyn}{mol}$
Glucose	0.04	1.05
Sucrose	0.058	0.99
Inulin	0.375	0.55

As expected, the selectivity increases with molecular weight while ω decreases.

In experiments with artificial membranes in which $\Delta \pi_i$ was equal to zero, GRIM was able to demonstrate that there exists a very large difference between stationary state pressure Δp_i and $RT\Delta c_s$. He interpreted the ratio between Δp_i and $RT\Delta c_s$ on the basis of the kinetic theory of LAIDLER AND SHULER[2], which will be discussed in the next paragraph in the light of irreversible thermodynamics.

Relaxation measurements and the kinetic theory of LAIDLER AND SHULER

In relaxation experiments, one starts with a system in a non-equilibrium state and subsequently allows it to go to equilibrium. The observer does not interfere with the run of the process and only records the mode of approach to the final state. Normally, the evaluation of coefficients from the results of relaxation experiments is not as simple as that from stationary state studies, however the data give more information and all the coefficients can be obtained from a single curve.

References p. 246.

A typical permeability relaxation experiment was carried out by SHULER, DAMES AND LAIDLER[17] on the permeation of water and non-electrolyte through collodion membranes. SHULER *et al.* immersed a collodion bag filled with the solution under consideration into a large thermostatted water bath and followed the changes in the volume and pressure by means of an attached narrow capillary. Immediately after the immersion, the solution rose rapidly in the capillary, passed a maximum and descended slowly to its equilibrium level. The pressure difference between the bag and surroundings is proportional to the height h of the column in the capillary, while the volume flow is proportional to the change of this height with time. Calculations of flows as function of time are simplified by the fact that the bag volume remains practically constant during the experiment. Furthermore the external bath is so much larger than the collodion bag that

$$-\frac{\mathrm{d}\Delta c_s}{\mathrm{d}t} = \dot{n}_s \frac{A}{V^i} \tag{50}$$

where A is the membrane area and V^i the volume of the bag.

The pressure difference $\Delta p = -hg$, if the density of the solution is assumed to be close to unity, and

$$\frac{\mathrm{d}\Delta p}{\mathrm{d}t} = -\frac{\mathrm{d}h}{\mathrm{d}t} g \tag{51}$$

where g is the gravitational acceleration.

If the cross section of the capillary is a, the volume flow per unit membrane area is

$$J_v = \frac{a}{A} \cdot \frac{\mathrm{d}h}{\mathrm{d}t} \tag{52}$$

or introducing (51)

$$J_v = -\frac{a}{Ag} \frac{\mathrm{d}\Delta p}{\mathrm{d}t} \tag{53}$$

LAIDLER AND SHULER recognized the difference between volume flow and water flow and used the correct expression

$$J_v = \dot{n}_w \bar{v}_w + \dot{n}_s \bar{v}_s \tag{17}$$

However, they calculated the flows on a two-coefficient system according to the equations:

$$\dot{n}_w = \frac{Q_1 c_t \bar{v}_w}{d' RT} (\Delta p - RT \Delta c_s) \tag{54}$$

$$\dot{n}_s = \frac{Q_2 c_t \bar{v}_w}{d'} \Delta c_s \tag{55}$$

where Q_1 and Q_2 are permeability coefficients for solvent and solute respectively, d' is the thickness of the membrane, c_t is "the total concentration of all species present" assumed to be constant, $c_t \bar{v}_w$ being close to unity.

Introducing (54) and (55) into (17) and (50) and making use of (53) one obtains

$$-\frac{\mathrm{d}\Delta p}{\mathrm{d}t} = \lambda_1 (\Delta p - RT \Delta c_s) + \lambda_2 RT \Delta c_s \tag{56}$$

$$= \lambda_1 \Delta p - (\lambda_1 - \lambda_2) RT \Delta c_s$$

References p. 246.

and

$$\frac{d\Delta c_s}{dt} = \lambda_3 \Delta c_s \tag{57}$$

respectively, where

$$\lambda_1 = \frac{A}{a}\frac{g}{d'}\frac{Q_1 c_t \bar{v}_w^2}{RT} \tag{58}$$

$$\lambda_2 = \frac{Ag}{a}\frac{Q_2 c_t \bar{v}_w \bar{v}_s}{d'RT}$$

and

$$\lambda_3 = \frac{A}{Vi}\frac{Q_2 c_t \bar{v}_w}{d'}$$

It will be observed that despite the use of three parameters λ_1, λ_2 and λ_3, there are in LAIDLER AND SHULER's system only two independent coefficients. The coefficient λ_2 is derived from λ_3 by multiplication with a constant independent of the membrane.

Integration of (56) and (57) carried out by LAIDLER AND SHULER leads to the expressions

$$\Delta p = RT\Delta c_{s,o}\frac{\lambda_1 - \lambda_2}{\lambda_1 - \lambda_3}(e^{-\lambda_3 t} - e^{-\lambda_1 t}) \tag{59}$$

$$\Delta c_s = \Delta c_{s,o} \cdot e^{-\lambda_3 t} \tag{60}$$

in which the initial condition $\Delta p = 0$ and $\Delta c_s = \Delta c_{s,o}$ at time $t = 0$ was taken into account. The authors found that equation (59) describes the experimental results satisfactorily. It was found that a short while after maximal pressure was attained, $\ln\Delta p$ *versus* t gives a straight line. This fact shows that equation (59) can apply if λ_3 differs widely from λ_1 and after a certain time one of the terms $e^{-\lambda_3 t}$ or $e^{-\lambda_1 t}$ will vanish in comparison with the other.

SHULER, DAMES AND LAIDLER assumed $\lambda_3 > \lambda_1$. It is seen immediately that in the limiting case of an ideal semipermeable membrane, λ_1 must be larger than λ_3, as in this case $Q_2 = 0$ and λ_3 is thus zero. That the assumption $\lambda_1 > \lambda_3$ is reasonable in the case of a penetrating solute, can be shown as follows. From (59) and (60)

$$\frac{\Delta p}{RT\Delta c_s} = \frac{\lambda_1 - \lambda_2}{\lambda_1 - \lambda_3}(1 - e^{-(\lambda_1 - \lambda_3)t})$$

If $(\lambda_1 - \lambda_3)$ is a sufficiently large positive number, $e^{-(\lambda_1 - \lambda_3)t}$ becomes negligible as compared to unity after a short time, and thus the ratio of Δp and Δc becomes constant. If, on the other hand, $\lambda_1 - \lambda_3$ is an equally large negative number, $e^{-(\lambda_1 - \lambda_3)t}$ becomes much larger than 1 and the ratio of Δp to Δc would increase logarithmically.

The values given for λ_1 and λ_3 have therefore to be interchanged. In Table II the experimental parameters from the measurements of SHULER *et al.* for the penetration of some sugars through the same membrane are given, assuming $\lambda_1 > \lambda_3$.

The values of λ_2/λ_3 calc. given in the last column of Table II were calculated from equation (58) introducing for V^i and a the values given by SHULER, DAMES AND LAIDLER. The partial molar volumes were derived from the densities of the solutions, cited in Landoldt-Börnstein's Tables.

The large difference between the calculated and experimental ratio λ_2/λ_3 indicates that the permeability of the synthetic membrane cannot be expressed by two straight coefficients alone and that the neglect of the cross coefficient leads to intrinsic contradictions.

References p. 246.

TABLE II

VALUES OF λ, AS DEFINED BY EQUATION (58), FOR A NUMBER OF SUGARS[17]

From SHULER, DAMES AND LAIDLER's paper[17] (their Table II), λ_1 and λ_3 interchanged.

	$\lambda_1 \cdot 10^2$ min^{-1}	$(\lambda_1 - \lambda_2) \cdot 10^4$ min^{-1}	$\lambda_3 \cdot 10^2$ min^{-1}	$\lambda_2 \cdot 10^2$ min^{-1}	λ_2/λ_3 *exp.*	λ_2/λ_3 *calc.*
Sucrose	7.8	1.4	1.00	7.49	7.79	0.0936
Lactose	9.1	2.9	1.00	9.07	9.07	0.094
Raffinose	8.0	1.9	0.89	7.98	8.95	0.19

The following values of partial molar volume were used in the derivation of $\lambda_2/\lambda_{3\,calc}$. Sucrose: 202 c.c./mol. Lactose: 203 c.c./mol. Raffinose 405 c.c./mol.

If we introduce (50) and (53) into the thermodynamically derived equations (31) and (33), we find

$$-\frac{d\Delta p}{dt} = \frac{Ag}{a} L_p \, (\Delta p - \sigma RT \Delta c_s) \tag{61}$$

$$-\frac{d\Delta c_s}{dt} = \frac{A}{V^i} [\omega RT \Delta c_s - (1 - \sigma) J_v c_s] \tag{62}$$

As is easily verified $(1 - \sigma) J_v c_s$ can be neglected as compared with $\omega RT \Delta c_s$ in these experiments. Consequently comparison of (61) and (62) with (56) and (57) leads to the following identifications:

$$\lambda_1 = \frac{Ag}{a} L_p$$

$$\lambda_3 = \frac{Ag}{V^i} \omega RT \tag{63}$$

$$\lambda_1 - \lambda_2 = \frac{Ag}{a} \sigma L_p$$

It is clear from this that λ_1, λ_2 and λ_3 should be independent. In particular $\lambda_2/\lambda_3 = gV^i/aRT \cdot (1 - \sigma) L_p/\omega$ is seen to depend on the membrane system. From (63) and Table II, the following values are obtained for σ and $A\omega$:

TABLE III

REFLECTIVITY CONSTANTS AND SOLUTE MOBILITIES FOR SOME SUGARS IN COLLODION MEMBRANES

From SHULER, DAMES AND LAIDLER's measurements.

	σ	$A\omega \cdot 10^{13}$ $cm^2 \cdot \frac{1}{sec} / \frac{dyn}{mol}$
Sucrose	0.0018	3.96
Lactose	0.0029	3.96
Raffinose	0.0024	3.53

The values show that the collodion membranes are less selective than the natural membranes investigated by PAPPENHEIMER *et al.* The reflection coefficient of collodion membranes can also be determined from the measurements of GRIM[16]. GRIM measured the ratio of Δp and $RT\Delta c_s$ at zero volume flow, which gives directly the reflection coefficient (equation (31)). From these experiments the reflection coefficient of sucrose penetrating through a collodion membrane was 0.004, which is of the same order of magnitude as the corresponding value in Table III.

References p. 246.

The permeability of collodion membranes differs of course according to the mode of preparation.

V. DISCUSSION OF THE REFLECTION COEFFICIENT

As pointed out previously, the values of the coefficients σ, ω and L_p are independent of each other, subject to a restriction corresponding to the condition $L_pL_D - L_{pD}^2 > 0$ Any explicit correlations between them have to be derived kinetically on the basis of models for the transport mechanism. However, without considering a detailed model, it is possible to delimit the range of σ, for a given pair of ω and L_p, more closely on the basis of the following general assumption.

We assume that solvent and solute interact with each other and this interaction endows each of them with a velocity component *in the direction of the force acting on the other*. The extent to which this interaction takes place in the passage through the membrane depends on the nature of the system. Cases of lowest interaction are systems where solute and solvent follow different paths through the membrane, as encountered in aqueous solutions of liquid-soluble substances passing through a mosaic membrane. Highest interaction of solute and solvent occurs in free diffusion and is approached in coarse capillary membranes.

Let us now derive σ for given L_p and ω in a system where the solute passes the membrane by dissolution and the solvent goes separately through the membrane capillaries. The driving force on the solute is

$$\Delta\mu_s = \bar{v}_s\Delta p + \frac{RT\Delta c_s}{c_s}$$

and the velocity of solute penetration in this type of system will evidently be determined only by $\Delta\mu_s$ and will be independent of $\Delta\mu_w$. Let us now consider the solute flow under two different experimental conditions. One: $\Delta p = 0$, $RT\Delta c_s/c_s = a$ and another: $\bar{v}_s\Delta p = a$, $\Delta c_s = 0$. As $\Delta\mu_s$ is the same in both cases, $\dot{n}_s^I$ for the first case and $\dot{n}_s^{II}$ for the second will be equal. Introducing the values for cases I and II into equation (40) we get

$$\dot{n}_s^{\mathrm{I}} = ac_s\,[\omega - \sigma L_p\,(1-\sigma)\,c_s] = \dot{n}_s^{\mathrm{II}} = \frac{L_p\,(1-\sigma)}{\bar{v}_s}\,a\,c_s$$

which, rearranging terms and neglecting $\sigma\varphi$ as compared to 1, becomes

$$\sigma = 1 - \frac{\omega\,\bar{v}_s}{L_p} \tag{64}$$

Passing to systems where hydrodynamic interaction occurs in the membrane, we can easily show that $\dot{n}_s^{\mathrm{I}}$ will no longer equal $\dot{n}_s^{\mathrm{II}}$. In case I, the driving force on the solute is opposite in direction to that on the solvent and the latter will thus tend to diminish solute flow. In case II, on the other hand, the pressure difference Δp operates on both solute and solvent in the same direction. Hence

$$\dot{n}_s^{\mathrm{I}} < \dot{n}_s^{\mathrm{II}}$$

or, translated in terms of σ

$$\sigma < 1 - \frac{\omega\bar{v}_s}{L_p} \tag{65}$$

The inequality (65) together with equation (64) thus delimits the range of values of

References p. 246.

the reflection coefficient in dependance on the extent of hydrodynamic interaction between solute and solvent in the membrane.

The condition $\sigma = 1$ may thus be regarded as sufficient experimental evidence for semi-permeability, as in view of (65) σ can approach unity only when $\omega \to 0$.

Finally it is clear that for very coarse membranes, σ goes to zero. For given ω and L_p, we have in general

$$0 \leq \sigma \leq 1 - \frac{\omega \bar{v}_s}{L_p} \tag{66}$$

The above consideration may thus help to decide from measurements of σ, ω and L_p what mechanism of solute transfer is involved. In fairly permeable membranes values of σ close to $(1 - \omega\, \bar{v}_s/L_p)$ indicate independent passage of solute and solvent, while $\sigma \ll 1 - \omega\, \bar{v}_s/L_p$ indicates capillary mechanism.

ACKNOWLEDGEMENT

The authors are indebted to Prof. A. J. STAVERMAN for helpful discussions.

SUMMARY

The application of the conventional permeability equations to the study of biological membranes leads often to contradictions. It is shown that the equations generally used, based on *two* permeability coefficients—the solute permeability coefficient and the water permeability coefficient—are incompatible with the requirements of thermodynamics of irreversible processes.

The inconsistencies are removed by a thermodynamic treatment, following the approach of STAVERMAN, which leads to a *three* coefficient system taking into account the interactions: solute–solvent, solute–membrane and solvent–membrane.

The equations derived here have been applied to various permeability measurements found in the literature, such as: the penetration of heavy water into animal cells, permeability of blood vessels, threshold concentration of plasmolysis and relaxation experiments with artificial membranes.

It is shown how the pertinent coefficients may be derived from the experimental data and how to choose suitable conditions in order to obtain all the required information on the permeability of the membranes.

The significance of these coefficients for the elucidation of membrane structure is pointed out.

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Received June 14th, 1957