Donnan Equilibria in Biological Processes.

By K. R. Dixit,

Gujarat College, Ahmedabad.

(Received June 26, 1938.)

INTRODUCTION.

THE expression 'Donnan Equilibrium' or 'Membrane Equilibrium' is used to represent the state of thermodynamical or statistical equilibrium, between two homogeneous fluids separated by a membrane. The two fluids are at the same temperature and the membrane is permeable for some molecules or ions present in the two fluids but not for all. In other words, the Donnan Equilibrium deals with a system in which a mass of solvent is divided by a membrane into two phases, both of which contain *e.g.*, diffusible ions, and one of which contains a non-diffusible ion. The presence of the non-diffusible ions or molecules leads to an unequal distribution of the diffusible ions or molecules on the two sides of the membrane.

The study of the membrane equilibria is a well-known branch of physical chemistry. We get osmotic equilibria if the membrane is permeable only by the solvent. The conditions of this equilibria were studied by Willard Gibbs[1] in 1878, and according to him the chemical potential of the solvent must be the same on the two sides of the membrane. Gibbs' application of thermodynamics to the membrane equilibria, however, remained unnoticed for a very long time. In the year 1890, soon after the ionic theory of Arrhenius was known, William Ostwald[2] suggested the study of two solutions separated by a membrane,

which allows some ions, but not all ions to pass through. In 1910, Donnan and Harris[3] made a detailed examination of the molecular state of congo red in aqueous solution by means of measurements of osmotic pressure. They found unequal distribution of sodium and chlorine ions, and suggested that this behaviour may be explained with the help of thermodynamics. The thermodynamical theory of membrane equilibria was first developed by Donnan[4] in 1911. In this form the theory was applicable to dilute solutions in the ideal form. More recently Hückel[5] and Donnan and Guggenheim[6,7] have revised the theory to make it applicable to imperfect solutions of electrolytes and non-electrolytes. This equilibrium theory has been re-examined by Gatty[8] in terms of the number of degrees of freedom of systems containing any number of chemical components and containing any number of membranes. The statistical theory of such membrane equilibria has been worked out by Ganguli.[9]

The theory of Donnan has been applied to very many special problems, and we shall mention here only a few of such

[1] Gibbs, *Collected Works*, 1928, 1, 83.

[2] W. Ostwald, *Zeit. Physik. Chem.*, 1890, 6, 71.

[3] Donnan and Harris, *Journ. Chem. Soc.*, 1911, 99, 1554.

[4] Donnan, *Zeit. Elektrochem.*, 1911, 17, 572.

[5] Hückel, *Kolloid Zeit.*, 1925, 36, 204.

[6] Donnan and Guggenheim, *Zeit. Physik. Chem.*, (A), 1932, 162, 346.

[7] Donnan, *ibid.*, 1934, 168, 369.

[8] Gatty, *Phil. Mag.*, 1934, 18, 273.

[9] A. Ganguli, *Kolloid. Zeit.*, 1934, 67, 304.

applications. Thus Liu[10, 11] has discussed the theory in conjunction with the activity co-efficient of electrolytic solutions. The effect of adsorption of solute and solvent by the membrane on the direction of osmosis of systems not in equilibrium, and a classification of such systems has been given by Schreinemakers and Werre.[12] They have also discussed the properties of mosaic membranes whose permeability varies from point to point. The latter effect and its relationship to negative osmosis has been discovered by Söllner,[13] while the properties of membranes that are capable of doing work have been discussed by Straub.[14]

Now if we try to apply the theory of Donnan to biological processes we are confronted with a difficulty. All the biological processes are characterised by life and the living cell is really a physico-chemical transformer which assimilates various substances, and maintains itself in its dynamically stationary state. But any system at constant temperature obeying the laws of thermodynamics must tend towards that configuration in which its free energy is minimum. That is, a system cannot maintain its dynamically stationary state and at the same time obey the laws of thermodynamical equilibrium, or only non-living systems can be in a state of thermodynamical equilibrium. It would appear from this that it is not permissible to apply the theory of Donnan to the living cells. There is, however, a loop-hole in this argument and we can argue, that all living organisms are not living to the same extent, that is, do not possess the same amount of free chemical energy. Some parts of an organism may possess an amount of free chemical energy, which is equal to that which that part would be required to possess if the system obeyed the laws of thermodynamics. The theory of the membrane equilibria is strictly applicable only to those parts of a living organism where the free chemical energy is a minimum. The greater the deviation of the actual free chemical energy of the system from the

minimum chemical energy, the greater would be the inapplicability of the laws of Donnan to such a system. But even in such extreme cases we can say that the theory of Donnan equilibria if applied to biological processes is likely to give us some indication of the actual physico-chemical changes that are brought about in an organism.

Warburg[15] was the first to apply the theory of Donnan equilibria to red blood corpuscles. Considerable progress has been made in this direction by L. J. Henderson,[16] D. D. van Slyke[17] and their collaborators. Donnan's method has also been applied with success to various other biological processes. In this article we shall first consider the thermodynamics of the Donnan equilibria and then we shall turn our attention to some typical biological applications of the same. We shall also try to get a picture of the physico-chemical processes which must be accompanying the biological processes.

Osmotic Equilibrium.[18]

Membrane equilibria arise when two solutions are separated by a membrane which is permeable for some of the components but not for all. The most familiar case is that of an ordinary osmotic equilibrium where there are two components, the membrane being permeable for one (called the solvent) but not for the other (called the solute). In the simplest case of this type of equilibrium, the solution is present on one side of the membrane, the pure solvent on the other, i.e.,

Solution	Pure Solvent
Pressure $= P_1$	Pressure $= P_2$
(1)	(2)

If the hydrostatic pressures P_1 and P_2 (supposed uniform on both sides) are equal, the solvent diffuses from (2) to (1) and the solution phase (1) swells in volume.

[10] Liu, *Kolloid. Zeit.*, 1931, **57**, 139 and 285.

[11] Liu, *ibid.*, 1932, **58**, 144.

[12] Schreinemakers and Werre, *Proc. Aka. Amsterdam*, 1932, **35**, 42 and 162.

[13] Söllner, *Biochem. Zeit.*, 1932, **244**, 370.

[14] Straub, *Chem. Weekblad*, 1930, **27**, 672.

[15] E. Warburg, *Biochem. Journ.*, 1922, **16**, 153.

[16] L. J. Henderson, *Blood, a Study in General Physiology*, Dresden, 1932.

[17] D. D. van Slyke, *Factors affecting the Distribution of Electrolytes, Water and Gases in the Animal Body*, London, 1924.

[18] F. G. Donnan, *Journ. International Soc. Leather Trades' Chemists*, 1933, **17**, 136.

For any given temperature this osmotic movement of the solvent molecules can be prevented by sufficiently increasing P_1 or lowering P_2. For equilibrium if the phase (1) can be treated as an ideal solution,

$$P_1 - P_2 = \frac{RT}{V_0} \log \frac{1}{N_0} \qquad \qquad (1)$$

where $N_0 =$ mol. fraction of the solvent in the solution (1) and $V_0 =$ increase in volume of an infinite mass of solution on adding to it one mol. of the solvent. If the solution is an ideal one (as supposed) and if we neglect the compressibility of solution and solvent, then we can state that V_0 is simply the volume of one mol. of the pure liquid solvent at the temperature T. The pressure difference $\pi = P_1 - P_2$ is called the osmotic pressure of (1) against the pure solvent. We may write the foregoing equation in the form

$$\pi = -\frac{RT}{V_0} \log (1 - N_s) \qquad \qquad (2)$$

where $N_s =$ mol. fraction of the solute in the solution (1) at equilibrium, since $N_0 + N_s = 1$. This equation is valid for all concentrations of an ideal solution. If the solution is dilute enough to justify the neglect of higher powers of the fraction N_s in the series expansion of the logarithmic term $\log (1 - N_s)$, then we may write

$$\pi = \frac{RT}{V_0} N_s = \frac{RT}{V_0} \frac{n_s}{n_0 + n_s} \qquad \qquad (3)$$

where n_s and n_0 are the mol. numbers of solute and solvent respectively in solution (1) at equilibrium. If we may neglect n_s in comparison with n_0 then we obtain

$$\pi = RT \frac{n_s}{n_0 V_0} \qquad \qquad (4)$$

where $n_0 V_0 =$ volume of the solvent employed. We may employ this equation as it stands or introduce molar volume concentrations.

If the solutions are not ideal, we must write the original equation in the form

$$\pi = \frac{RT}{V_0} \log \frac{1}{N_0 f_0} \qquad \qquad (5)$$

where $f_0 =$ the activity coefficient of the solvent. We may also write this equation in the form

$$\pi = \frac{gRT}{V_0} \log \frac{1}{-} \qquad \qquad (6)$$

where g is called the osmotic coefficient of the solution.

In general when a membrane equilibrium occurs, some of the solute components, i.e., the ones for which the membrane is permeable will be present in the solutions on both sides of the membrane.

THERMODYNAMICAL EQUILIBRIUM IN SIMPLE SYSTEMS.[19-22]

The theory of Donnan in its original form[4] is applicable only to dilute ideal solutions of dissociated electrolytes. (The solutions are said to be ideal when the interaction between the molecules of the solute can be neglected, further the interaction between the molecules of the solute and the solvent is independent of the concentration.) In this simple form the theory has been applied to a large number of biological processes. Let us first consider the two types of biological processes, those that are likely to show thermodynamical equilibria, and those which can show chemical equilibria.

According to the laws of diffusion, such substances as salts and sugars, which are soluble in water and are absorbed by the cell, must continue to enter the cell until the concentration of each substance becomes equal, both outside and inside the cell. As the plant usually obtains rather dilute solutions of nutritive substances, it is evident that their entrance into and accumulation within the cell require special conditions. A most important pre-requisite is the chemical change of the absorbed substances. For instance, when carbohydrates are stored in the tubers of the potato, the sugar obtained by the cells from the leaves is transferred directly into starch, which is insoluble in water. The concentration of sugar in the cells of the growing tuber is therefore extremely low and does not impede the diffusion of new amounts of sugar. The same is observed in ripening oil-bearing seeds. The only difference in this case is

[19] F. G. Donnan, *Kolloid. Zeit.*, 1932, **61**, 160.

[20] T. R. Bolam, *Kolloid. Beihefte*, 1934, **39**, 139.

[21] Padoa and Tedeshi, *Biochem. Zeit.*, 1933, **266**, 452.
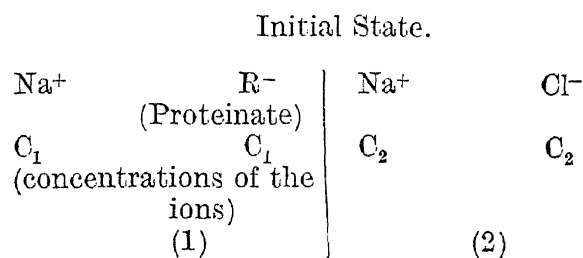
[22] Maximow, *Text-book of Plant Physiology*, McGraw Hill, 1930, 119.

that the fats are now accumulated at the expense of the soluble carbo-hydrates. Protein compounds are formed from amino acids and so on. As a general rule, the substances entering the cell are subject to chemical transformation which assures their uninterrupted absorption.
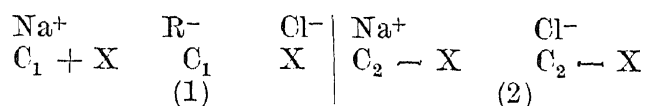
This general mechanism of the absorption and accumulation of substances in the cell, however, does not always hold true. There are cases when solute substances accumulate in great quantity, and remain in the same state in which they are when entering the cell, for example, in the bulb of the common onion, are stored considerable amounts of glucose. In pigweed and other plants much potassium nitrate accumulates. At the present time, the explanation of these phenomena is sought in the so-called membrane equilibria of Donnan.

If a membraneous sac impermeable to colloids containing a readily ionisable salt, one of whose ions is of a colloidal character, such as sodium proteinate—a sodium salt in which the rôle of an acid is played by some protein—is immersed in water, the sodium ions though able to pass through the membrane, will not diffuse out of the sac, being retained by the electrostatic attraction of anions and cations. If some easily penetrating salt is added to water, for instance sodium chloride, then in the process of osmosis the Na^+ and Cl^- ions will diffuse through the septum and finally there will be established an equilibrium.

To apply thermodynamics to these processess, let us consider the solution of a salt NaR, separated from the solution of $NaCl$ by a membrane, and let us suppose that the membrane is permeable for all particles except the anion R^- and the undissociated salt NaR. The initial and the final states may be represented diagrammatically as follows. The vertical line represents the membrane.

Initial State.

Na^+	R^- (Proteinate)	Na^+	Cl^-
C_1	C_1 (concentrations of the ions)	C_2	C_2
(1)		(2)	

Final State.

Na^+	R^-	Cl^-	Na^+	Cl^-
$C_1 + X$	C_1	X	$C_2 - X$	$C_2 - X$
	(1)			(2)

The resultant electrical charge on each side of the membrane must be zero and hence

$$(Na^+)_1 = (Cl^-)_1 + (R^-)_1$$

$$\text{and } (Na^+)_2 = (Cl^-)_2$$

Further, as the system is in equilibrium, the work done in taking equal quantities of Na^+ and Cl^- ions in the same direction must be equal, hence

$$dn \text{ RT } \log \frac{(Na^+)_2}{(Na^+)_1} + dn \text{ RT} \log \frac{(Cl^-)_2}{(Cl^-)_1} = 0 \quad (7)$$

Therefore in the state of equilibrium

$$(Na^+)_1 \times (Cl^-)_1 = (Na^+)_2 \times (Cl^-)_2$$

This unequal distribution of the charged particles gives rise to a difference of potential E on the two sides of the membrane,

$$E = \frac{RT}{F} \log \frac{(Na^+)_2}{(Na^+)_1} \qquad \qquad (8)$$

This difference of potential which may be supposed to be localised on the membrane is called the membrane potential. F = Faraday's equivalent in volt-coulombs. This difference of potential can also be expressed in terms of Cl^- ions, and we get

$$E = \frac{RT}{F} \log \frac{(Cl^-)_1}{(Cl^-)_2} \qquad \qquad (8\,a)$$

The expression for E in terms of any positive ions will be similar to 8, while any negative ions will be similar to $8a$. Hence we get

$$\frac{(A^+)_1}{(A^+)_2} = \frac{(B^+)_1}{(B^+)_2} = \frac{(C^-)_2}{(C^-)_1} = \frac{(D^-)_2}{(D^-)_1} \qquad (9)$$

Similarly, by applying the equation 7 we get for doubly charged positive ions, if simultaneously present with singly charged sodium ions, the relation

$$\frac{(Ca^{++})_1}{(Ca^{++})_2} = \frac{(Na^+)_1^2}{(Na^+)_2^2} \qquad \qquad (9\,a)$$

We can also express the membrane potential E in terms of the corresponding pH values

$$E = \frac{RT}{F} \log \frac{(Na^+)_2}{(Na^+)_1} = \frac{RT}{F} (pH_1 - pH_2) \qquad (10)$$

Let us apply these considerations to the case of NaR and NaCl which has been already represented by a diagram. According to Donnan, at equilibrium the products of the concentrations of the diffusible ions must be equal on both sides of the septum. Then we get

$$(C_1 + X) X = (C_2 - X)^2$$

From this equation may be calculated the relation between the ions of sodium and of chlorine on both sides of the septum. This calculation shows that higher the concentrations of sodium proteinate in comparison to the concentration of sodium chloride, the less complete will be the equalisation of the concentrations of NaCl in the surrounding solution and the osmometer, and the more it is retained in the surrounding liquid. Let us give some figures illustrating the membrane equilibria of Donnan.

TABLE I.

| Initial concentrations		Final concentrations NaCl	
NaR	NaCl	Inner solution	Outer solution
0·01	1	0·497	0·503
0·1	1	0·476	0·524
1·0	1	0·33	0·66
10	1	0·083	0·917
100	1	0·01	0·99

These figures show that the large amount of sodium proteinate makes the membrane appear as if impermeable to NaCl and this salt ceases to penetrate into the sac of the osmometer.

If a salt with another cation than the one connected with the proteinate within the osmometer is taken, for instance KCl instead of NaCl, another correlation will be obtained. As before this can be represented diagrammatically.

Initial State.

Na+	R−	K+	Cl−
C₁	C₁	C₂	C₂

Final State.

Na+	K+	Cl−	R−	K+	Na+	Cl−
C₁−Z	X	Y	C₁	C₂−X	Z	C₂−Y
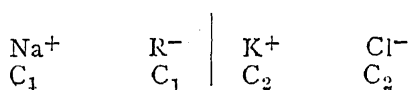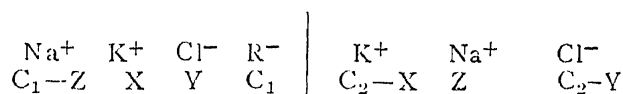
The concentration X, Y and Z are connected by the equation $Z = X - Y$. Further, the conditions of equilibrium give us the following equations :—

$$\frac{C_1 - (x - y)}{x - y} = \frac{x}{C_2 - x} \qquad \qquad (a)$$

$$\frac{C_1 - (x - y)}{x - y} = \frac{C_2 - y}{y} \qquad \qquad (b)$$

hence $\qquad \dfrac{x}{C_2 - x} = \dfrac{C_2 - y}{y}$

or $\qquad x + y = C_2$

substituting in a and b we get

$$x = \frac{(C_1 + C_2) C_2}{C_1 + 2C_2}$$

$$y = \frac{C_2^2}{C_1 + 2C_2}$$

or $\quad \dfrac{(Na^+)_1}{(Na^+)_2} = \dfrac{(K^+)_1}{(K^+)_2} = \dfrac{(Cl^-)_2}{(Cl^-)_1} = \dfrac{C_1 + C_2}{C_2}$

The cations of this electrolyte KCl introduced into the surrounding solution, will be attracted towards the interior of the membrane by the anion of the protein. The anion on the contrary will be forced outwards. Donnan illustrates this relation by the following table :—

Table II.

Initial concentration		Final concentration					
		K		Na		Cl	
NaR	KCl	Inside	Outsibe	Inside	Outside	Inside	Outside
0·1	1	0·5	0·5	0·05	0·05	0·5	0·5
1·0	1	0·66	0·33	0·66	0·33	0·33	0·66
10	1	0·90	0·10	9·2	0·8	0·10	0·90
100	1	0·99	0·01	99·0	1·0	0·01	0·99

With the great abundance of the colloidal ions within the osmometer, if compared to

the concentration of salts in the surrounding liquid, for instance 100 : 1 in the last line of our table, the almost complete disappearance of K (99 per cent. of the original amount) from the surrounding medium and its accumulation within the osmometer may be observed. At the same time the almost complete expulsion of the Cl ion from the osmometer is observed, in spite of the fact that both ions pass readily through the membrane and enter into no chemical reactions within the osmometer.

In an analogous way may be explained the accumulation of different kinds of ions in the plant cells containing a considerable amount of protein substances which show the properties of ampholytes, that is, substances which act as weak acids and weak bases and therefore are able to induce the accumulation of cations as well as of anions. As the composition of the colloidal substances in the cells changes continually, quantitatively as well as qualitatively, the conditions of Donnan equilibria prove very complicated in them. The protoplasma being impermeable not only to the colloids but also to many electrolytes, for instance to organic acids, these combinations too may determine the conditions for the establishment of Donnan's equilibrium between the cell and the surrounding medium, and may promote the accumulation in the plant of many anions and cations in much higher concentrations than are found in the surrounding medium, for instance in the soil solution. At the present time, attempts are being made to reconstruct the whole theory as to the entrance of substances from the soil into the roots on the basis of the conditions discovered by Donnan. This necessitates, however, an extension of Donnan's simple theory, to non-ideal solutions.

The Exact Theory of Membrane Equilibrium.[6, 7, 23]

The theory of membrane equilibrium in its most general form was established by Gibbs. He showed that if a fluid mass be divided into two parts by a rigid diaphragm, permeable by some of the components but impermeable by others, the conditions that are necessary for equilibrium can be stated as follows. In the first place, the absolute temperature T' in the fluid phase designated L' must be the same as the temperature T'' in the phase L'' on the opposite side of the membrane. In the second place, the partial free energy or chemical potential of each one of the substances which are free to diffuse accross the membrane must be the same in both phases. The potentials and other properties of the substances designated S_1, S_2, $S_3 \cdots \cdot S_r$ are distinguished by subscripts as in formula 11.

$$\mu_1' = \mu_1'', \qquad \mu_2' = \mu_2'' \qquad \cdot \cdot (11)$$

where $\mu_1' =$ the chemical potential of the substance S_1 in the phase L'. Such equations, applicable to all substances that can diffuse across the membrane, give the criteria for an exact thermodynamical equilibrium.

In the case of membrane equilibrium the hydrostatic pressures P_1 and P_2 on the two sides of the membrane are not necessarily equal, the same is the case with the potentials of the substances which cannot pass through the membrane.

Gibbs' conditions for equilibrium have been expressed by Donnan and Guggenheim[6,7] in a form which is easily applicable to experimental investigation.

$$\mu_1 = \mu_1{}^*(T) + P v_1{}^*(1 - \tfrac{1}{2} K_1 P) + RT \log N_1 f_1 \quad (12)$$

where $\mu_1{}^*$ $(T) =$ a constant at a constant temperature, which need not be determined. $P =$ the pressure in the solution. $v_1{}^* =$ the volume occupied by 1 mol. of S_1 in a very dilute solution at zero pressure. $K_1 =$ the compressibility of S_1 in very dilute solutions. (The equation 12 is subject to a small correction for the effects of changes in compressibility at very high pressures.) $R =$ the gas constant. $T =$ the absolute temperature. $N_1 =$ molar fraction $=$ mols. of S_1 divided by the total number of mols. in the solution. $f_1 =$ the activity coefficient which may be a measure of the deviations from the ideal solution laws. The symbol f_1 denotes the activity coefficient at pressure P and f'_1 at pressure P'.

Donnan and Guggenheim obtained the equation 13 from 11 and 12, eliminating the constant term $\mu_1{}^*$ (T) which must be the same in both phases.

$$P' v_1{}^* (1 - \tfrac{1}{2} K_1 P') + RT \log N_1' f_1' =$$
$$RT \log N_1'' f_1'' + P'' v_1{}^* (1 - \tfrac{1}{2} K_1 P''). \quad (13)$$

23 G. S. Adair, *Trans. Farad. Soc.*, 1937, 33, 1106.

They suggested an abbreviated notation in which $[v_1]$ represents

$$v_1^* [1 - \tfrac{1}{4}K_1 (P' + P'')]$$

the volume at the mean pressure $\dfrac{P' + P''}{2}$.

In order to allow for the changes in compressibility with increasing pressure, the symbol $[v_1]$ is here defined by the formula

$$[v_1] = \left(\int v_1 \, dP\right) / (P' - P'') \quad \ldots \quad (14)$$

where v_1 denotes the volume occupied by 1 mol. of S_1 in a very dilute solution. With this definition the equations 15 to 21 are exact.

$$(P' - P'') [v_1] + RT \log N'_1 f'_1 = RT \log N_1'' f_1'' \quad \ldots \quad (15)$$

If the substance S_1 be solvent water, it may be more convenient to use osmotic coefficients g_1' and g_1'' as a measure of the deviations from the ideal solution laws.

$$(P' - P'') [v_1] + g_1' RT \log N_1' = g_1'' RT \log N''_1 \quad \ldots \quad (16)$$

Equations like 13 and 15 can be applied to any non-electrolyte that can diffuse across the membrane.

$$(P' - P'') [v_2] + RT \log N_2' f_2' = RT \log N_2'' f_2'' \quad \ldots \quad (17)$$

If we eliminate the pressures, using equations 15 and 17 we obtain the equation 18 given by Donnan and Guggenheim.

$$\frac{(N_2' f_2')}{(N_1' f_1')^r} = \frac{(N_2'' f_2'')}{(N_1'' f_1'')^r} \quad \ldots \quad \ldots \quad (18)$$

where $r = [v_2] / [v_1]$.

A slight modification of their treatment with special reference to systems in which the pressures can be determined with greater accuracy than the molar fractions is given by Adair.[23] With the abbreviation

$$h_1 = [v_1] (P' - P'') / RT \quad \ldots \quad (19)$$

the equation 15 may be restated in the form

$$N_1' f_1' e^{h_1} = N_1'' f_1''$$

where $e^{h_1} = 1 + h_1 + \tfrac{1}{2}h_1^2 + \cdots \cdot \quad (20)$

A similar formula can be applied to the substance S_2 and in a system where S_1 and S_2 can diffuse across the membrane we get

$$\frac{N_2' f_2' e^{h_2}}{N_1' f_1' e^{h_1}} = \frac{N_2'' f_2''}{N_1'' f_1''} \quad \ldots \quad \ldots \quad (21)$$

In practical applications of the theory of membrane equilibrium it is customary to use a simpler, but approximate formula

$$m_2' f_2' = m_2'' f_2'' \quad \ldots \quad (22)$$

where m_2 = the molality in aqueous solutions expressed in gram-mols of S_2 per 1000 grams water. The estimation of the errors due to the use of the approximate formula has been made by Adair, and his calculations show that although the error in investigations of the membrane equilibria, of small molecules, in systems where the pressure difference $P' - P''$ is small, can be disregarded, the exact formula will have to be used in the interpretation of experiments with membranes permeable by large molecules as, for example, in the ultrafiltration of proteins described by Elford,[24] and the work of Moran[25] on equilibria with gelatine gels and sodium chloride at pressures exceeding 2000 atmospheres.

THE MEMBRANE-EQUILIBRIUM OF IONISED ELECTROLYTES.

In a system where an acid, a base for a salt can diffuse across the membrane, the chemical potential of the electrolyte must be the same in both phases as stated in 11. If the electrolyte dissociates, yielding r_i ions of the species S_i and r_j ions of the species S_j, the diffusion of an ion is subject to the condition that an equivalent quantity of ions of opposite sign pass across the membrane in the same direction. The condition for equilibrium is

$$r_i \mu_i' + r_j \mu_j' = r_i \mu_i'' + r_j \mu_j'' \quad \ldots \quad (23)$$

we can compare this with $(Na^+)_1 \times (Cl^-)_1 = (Na^+)_2 \times (Cl^-)_2$ given by Donnan.

In a system where two ions of the same sign, S_i and S_k can diffuse across the membrane, we get the equation 24, since the possible variations dm_i' and dm_k' in the composition of the phase L' are subject to the condition that

$$dm_i' / n_i = - dm_k' / n_k$$

$$\frac{\mu_i'}{n_i} - \frac{\mu_k'}{n_k} = \frac{\mu_i''}{n_i} - \frac{\mu_k''}{n_k} \quad \ldots \quad \ldots \quad (24)$$

or $(\mu_i' - \mu_i'')n_k = (\mu_k' - \mu_k'')n_i \quad \ldots \quad (25)$

In a system where two salts containing the ions S_i, S_j and S_k can diffuse across the membrane, equations 23 and 24 are both applicable, it is possible to restate these formulæ in terms of molar fractions, as in formula 19

$$(N_i' f_i' e^{h_i})^{r_i} (N_j' f_j' e^{h_j})^{r_j}$$
$$= (N_i'' f_i'')^{r_i} (N_j'' f_j'')^{r_j} \quad \ldots \quad (26)$$

24 Elford, *ibid.*, 1937, **33**, 1100.

25 Moran, *Report of Food Investigation Board for 1935*, 20.

The activity coefficients of the individual ions can be replaced by $f\pm$, the mean activity coefficient. If the pressure difference $P' - P''$ be small, and f the activity coefficient of water be the same in both phases, we get the equation 27, which is applicable to a salt with two univalent ions

$$\frac{(f_\pm')^2}{(f_\pm'')^2} = \frac{m_{Na}'' \times m_{Cl}''}{m_{Na}' \times m_{Cl}'} \qquad \qquad (27)$$

It appears that equation 27 is useful in considerations of the state of equilibrium between the blood and the aqueous humour discussed by Ridley[26] and by Davson, Duke-Elder and Benham.[27]

If the osmotic pressure be low and both of the ions be univalent, equation 27 is replaced by the simple but approximate equation 28.

$$\frac{m_i' f_i'}{m_k' f_k'} = \frac{m_i'' f_i''}{m_k'' f_k''} \qquad \qquad (28)$$

MEMBRANE POTENTIALS AND THE POTENTIALS OF INDIVIDUAL IONS.

From the thermodynamical point of view, no criterion for the equilibrium of an individual ion across the membrane is necessary because the possible variations in the state of the system are comprehended by equations 23 and 24 applicable to pairs of ions. Under certain conditions, it is, however, convenient to supplement these equations by another equation involving the potentials of the ion S_i in both phases L' and L'' and the electrical potential difference $(E' - E'')$ between the phases.

$$\mu_i' = \mu_i'' - n_i F (E' - E'') = \mu_i'' - n_i' FE \quad (29)$$

F = Faraday's equivalent in volt-coulombs. $E = E' - E''$, n_i the valence of the ion of the species S_i is negative for anions. This formula is comparable with the approximate formula

$$pH' = pH'' - \frac{E}{0\cdot05416} \times \frac{273}{273 + t} \quad (30)$$

used by Loeb[28] and by Adair and Adair.[29] The last mentioned authors correlate the membrane potentials and pH values, they

also give a precise method for the measurement of membrane potentials with certain types of solutions containing proteins.

MEMBRANE EQUILIBRIUM IN SYSTEMS WHERE THE COMPOSITION OF ONE PHASE IS CONSTANT.

In studies on proteins, it is often desirable to investigate solutions containing a mixture of inorganic electrolytes. If the protein solution L' be enclosed in a membrane permeable by all the components except the protein, in equilibrium with the dialysate L'' of constant temperature, pressure and composition, the properties of the system are determined by one independent variable, the concentration of the protein in L', even if the number of diffusible substances be unlimited. Adair[30, 31] has shown that the potential of the protein, or the protein salt μ_{ps} is correlated with the observed osmotic pressure π and the volume V of solution per mol. of protein by the formula

$$d\mu_{ps} = V d\pi \qquad \qquad (31)$$

Some special cases of this type of equilibria have been considered by Adair. For example, he gives the relationship between osmotic pressures, protein concentrations, molecular weights of hæmoglobin[32, 33] and serum proteins in systems where L' is constant and also the effects of proteins[34] on the activity coefficients of diffusible ions.[29, 32, 34, 35] Adair[32, 34] also suggested a method for extending the theory to ideal systems with low osmotic pressures, including colloidal solutions in mixed solvents, in which the composition of the solution L' is expressed in 'corrected concentrations' in gram mols. per litre of the mixed solvent.

$$[S]_c' = [S]_0' \times 100/(100 - V_1 C) \quad (32)$$

$[S]_c'$ = corrected concentration of substance S in L'.

$[S]_0'$ = observed concentration in mols. per litre of solution.

C = grm. colloid per 100 c.c. solution.

V_1 = volume occupied by 1 grm. colloid, including water of hydration.

[26] Ridley, *Brit. Journ. Exp. Path.*, 1930, 11, 217.

[27] Davson, Duke-Elder and Benham, *Biochem. Journ.*, 1936, 30, 773.

[28] Loeb, *Proteins and the Theory of Colloidal Behaviour*, New York, 1922.

[29] Adair and Adair, *Biochem. Journ.*, 1934, 28, 199.

[30] Adair, *Journ. Amer. Chem. Soc.*, 1929, 51, 696.

[31] Adair, *Trans. Farad. Soc.*, 1935, 31, 98.

[32] Adair, *Proc. Roy. Soc.*, 1928, A, 120, 573.

[33] Adair, *Proc. Camb. Phil. Soc. (Biol.)*, 1924, 1, 75.

[34] Adair and Robinson, *Biochem. Journ.*, 1930, 24, 1864.

[35] Adair and Adair, *ibid.*, 1934, 28, 1230.

The theory of membrane equilibrium may be applied to the process of dialysis, because the rate of removal of a diffusible impurity S_n from a colloidal solution L', enclosed in a membrane surrounded by pure water is partly determined by the distribution ratio $r_n = [S_n]_c' / [S_n]_c''$

$$- \frac{d}{dt} [S_n]_c' = [S_n]_c' \left(\frac{1}{r_n} \right) k_n \qquad .. \quad (33)$$

where t = time, k_n is a coefficient, directly proportional to the area of the membrane, and inversely proportional to the volume of the solution L' and the thickness of the membrane. k_n is an undetermined function of the shape of the membrane, the rate of stirring and other factors. On the assumption that k_n is a constant, the reciprocal of the distribution ratio, is a measure of the rate of dialysis. Adair's[23] work shows that the effect of salts on the rate of removal of impurities is of importance in studies on systems where the composition of the dialysate is *constant*.

Before we proceed to describe the applications of this theory of membrane equilibrium, it may be pointed out that the presence of a membrane is not absolutely necessary for the existence of the Donnan equilibrium. The theory is applicable to any system which contains some kind of ions, which are not allowed to diffuse to some part of the system. The diffusion of the ions may be hindered either by the formation of aggregates round the ion, or by the absorption of the ion on the surface of the membrane, or some other peculiar property of the ion. For all practical purposes we can assume that the theory of Donnan is applicable even when the ion is able to diffuse, but the rate of diffusion is very small.