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Relation Between Pressure and Concentration Difference Across Membranes Permeable to Solute and Solvent.* (20306)

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Most of the osmotic systems encountered in biology involve membranes that are permeable to solute as well as to solvent molecules, hereinafter called leaky membranes. In the study of material transport across such membranes, the relation between the solute concentration difference and the hydrostatic pressure difference across the membrane at any time is of fundamental importance. The purpose of this note is to demonstrate that this relation is not given by the classical van't Hoff osmotic pressure equation. In 1887, van't Hoff(1) pointed out that the perfect gas law could be applied directly to the observations of Pfeffer on the equilibrium hydrostatic pressure difference developed across the nearly semipermeable copper-ferrocyanide membrane when that membrane separated a solution of a non-electrolyte from water. In any system with a strictly semipermeable membrane (a membrane permeable to solvent only) separating solutions of non-electrolytes, the hydrostatic pressure difference, Δp, existing across the membrane *at equilibrium* (by definition, the osmotic pressure difference, $\Delta\pi$) is directly proportional to the difference in activity, or neglecting activity coefficients, the difference in concentration, Δc, of the solute in the two solutions separated by the membrane. The proportionality factor in this relation is RT, where R is the gas constant and T is the absolute temperature. The "van't Hoff relation" describing the equilibrium state is thus given by the equation:

$$\Delta p_{eq} = \Delta\pi = \Delta c_{eq} RT \qquad (1)$$

In systems in which the membrane is permeable, with various degrees of restriction,

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to molecules of the solute as well as to solvent molecules, it is the changes in Δp and Δc occurring during the approach to equilibrium rather than the equilibrium state itself which is of major interest; for, at equilibrium the composition of the two solutions separated by the membrane have become identical through interdiffusion; *viz.*, Δp and Δc equal zero. Even in the simplest case involving aqueous solutions of non-electrolytes (the one to be considered here) the relation between Δp and Δc at any time preceding equilibrium is more complex than that expressed by equation (1).

Two cases will be considered here. The first applies to a system in which the volumes of the two solutions separated by a leaky membrane are maintained constant by changes in the pressure difference across the membrane to compensate for the changing solute concentration difference. The second case is concerned with systems in which the pressure difference is maintained constant and equal to zero and the volumes of the solutions change with time. Recently, the mathematical relation between concentration and pressure difference across a leaky membrane separating a non-electrolyte solution from water has been developed by Laidler and Schuler (2). Their general equation (II-5), with appropriate changes in symbols to agree with those used here, is:

$$\frac{dV_i}{dt} = K\left[\frac{Q_1V_1^2}{RT}(\Delta c_2RT - \Delta p) - \frac{Q_2V_1V_2}{RT}(\Delta c_2RT)\right] \quad (2)$$

where the subscript i refers to the solution inside a test-tube shaped membrane and the subscripts 1 and 2 refer to water and non-electrolyte, respectively. The new symbols have the following meaning: K is essentially a constant for any given membrane, being dependent upon the total area, A^m, and the thickness, Δx, of the membrane. Q is the permeation coefficient, has the same units as the diffusion coefficient, D, and may be considered equal to the product of D and the distribution ratio for the particular molecular species in question at the interface between membrane and solution. As pointed out by Laidler and Schuler, this ratio may be considered to be qualitatively equal to the ratio of pore area available for diffusion of the particular molecular species to the total membrane area; *i.e.*, A^p/A^m. V is the partial molar volume.

For case I (*i.e.*, $dV_i/dt = 0$), this equation reduces to:

$$Q_1V_1(\Delta c_2RT - \Delta p) - Q_2V_2(\Delta c_2RT) = 0 \quad (3)$$

Rearranging and collecting terms:

$$\Delta p = \left(1 - \frac{Q_2V_2}{Q_1V_1}\right)\Delta c_2RT = k\Delta c_2RT = k\Delta\pi \quad (4)$$

Thus a new proportionality factor which will be called k is added to van't Hoff's relation when applied to leaky membranes, the factor being dependent on the diffusion coefficients, partial molar volumes, and the pore areas available for diffusion of the solute and solvent molecules. Qualitatively, equations (2), (3), and (4) are merely statements of the fact that the volume and/or pressure changes on both sides of the membrane are dependent upon solute as well as solvent transport across the membrane. In the special case in which the membrane is semipermeable (Q_2 equals zero), equation (4) reduces to the classical van't Hoff equation; *e.g.*, in the case of plasma proteins and the capillary membrane. For the usual case with non-electrolytes and biological membranes, Q_2V_2 is smaller than Q_1V_1; hence $k < 1$ and $\Delta p < \Delta\pi$. Theoretically, of course, k may equal zero or be greater than one. The error introduced in calculating Δp from a measured Δc or vice versa by means of the van't Hoff equation will depend on the value of k and as will be shown later can easily be as great as 2 or 3 orders of magnitude.

For case II (*i.e.*, $\Delta p = 0$), equation (2) reduces to:

$$\frac{dV_i}{dt} = K\Delta c_2(Q_1V_1^2 - Q_2V_1V_2) \quad (5)$$

The volume change rate or "osmotic transport rate" is a function of the nature of the non-electrolyte as well as the solute concentration difference; and depending on the non-electrolyte, it may be positive, negative, or zero. With a semipermeable membrane in such a

system ($Q_2 = 0$), the magnitude of the osmotic transport rate becomes for a given membrane a function of the concentration difference only and is independent of the nature of the non-electrolyte (as might be predicted from an extrapolation of the van't Hoff relation to such systems). This has been shown experimentally by Berkeley and Hartley(3). If, in case II, the outside compartment is filled with solution of a non-electrolyte different from but at the same concentration as the inside non-electrolyte, then, with a semipermeable membrane, it would be found that the net volume change of the inside solution would be zero, since the osmotic transport rate is a function of the concentration difference only. With a leaky membrane, however, it would be expected that a net volume change would occur, since the QV values of the two different non-electrolytes would most likely be unequal. Any net osmotic transport at zero time in such a system with two iso-osmotic solutions obviously could not be explained by an extrapolation of the van't Hoff law.

Experimental. The first experiments are concerned with a system treated as case I in the preceding theoretical discussion. It provides a direct comparison between the observed hydrostatic pressure difference across the membrane required to maintain the isovolumetric state and the osmotic pressure calculated by the van't Hoff equation from the measured concentration difference existing across the membrane at the same time. For such an experiment, the procedure was as follows: Test-tube shaped unoxidized collodion membranes of 35 mm diameter and 95 mm length were prepared by the method of Sollner and collaborators(4). A typical specimen was tied to a Lucite stopper which was attached to a capillary manometer of approximately 0.5 mm diameter. The membrane was filled with a measured volume of glucose solution of known concentration through a small hole in the Lucite stopper which was subsequently sealed. The membrane was then immersed in a measured volume of water. Stirring of the solutions on either side of the membrane was initiated immediately, the outside solution by two streams of water-saturated oxygen and inside by means of two small Alnico magnets, magnetically rotated on axes attached to a rod suspended from the Lucite stopper into the center of the inside solution. By means of a syringe in communication with the manometer system through a side arm just above the junction of the manometer and Lucite stopper, the pressure inside the membrane was increased as rapidly as possible until the liquid level in the manometer remained at a constant height, showing that the hydrostatic pressure difference across the membrane was just sufficient to prevent any net movement of liquid across the membrane. The height and time (usually one to two minutes after the initial immersion) was noted. At frequent intervals during a subsequent period of 2 to 3 hours the height and time were again noted. Simultaneous with each of several of these readings, exactly one milliliter was pipetted from the outside solution and subjected to analysis for its glucose content. From the concentration of glucose in the outside solution, it is possible to obtain the concentration inside by difference; hence the concentration difference, $\triangle c$, can be determined. From the values of $\triangle c$, $\triangle \pi$ was calculated by means of the van't Hoff equation.

In Fig. 1, the results of such an experiment

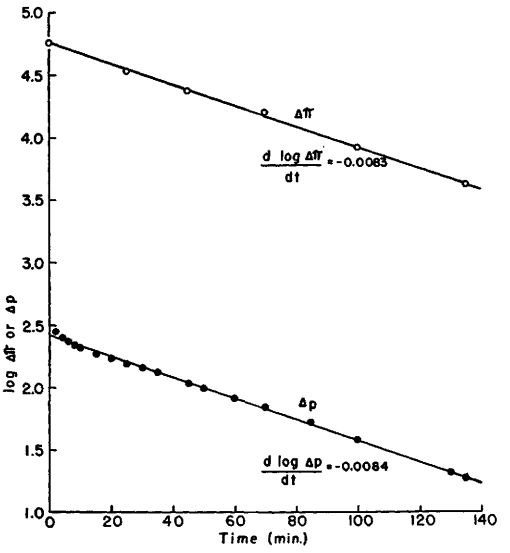

FIG. 1. Relation between observed and "van't Hoff" hydrostatic pressure differences across a leaky membrane.

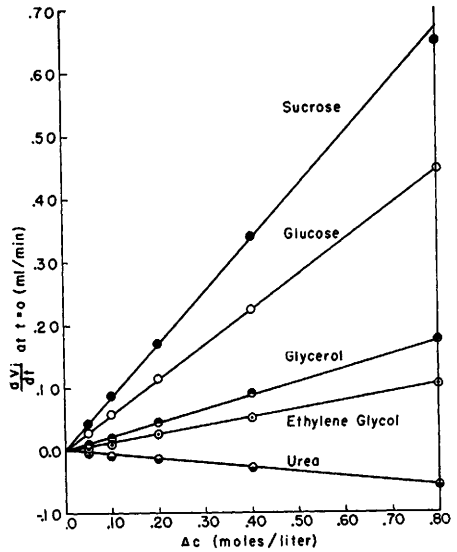

FIG. 2. Influence of concentration difference and nature of the solute on osmotic transport rates across leaky membranes.

are shown. The upper curve is a plot of log ($\triangle\pi$), the pressure difference calculated from the van't Hoff equation, versus time; and the lower curve is a similar plot of log ($\triangle$p), the observed pressure difference. Each set of values falls on a straight line as is to be expected for a diffusion process. Of primary importance is that at all times, $\triangle$p is much smaller than $\triangle\pi$. Furthermore, as expected from equation (4), the $\triangle$p values are directly proportional to the $\triangle\pi$ values as indicated by the nearly equal slopes of the 2 curves; *i.e.*, $\triangle p = k\triangle\pi$ with k in this particular case approximately equal to 0.0046.

The following experiments are concerned with case II and were made in some earlier work of the author(5) performed for a different purpose. In the first group of experiments, the hydrostatic pressure difference was maintained essentially constant and approximately equal to zero, the volume of the solution inside the membrane varying with time. By observing the initial rate of volume change of the inside solution associated with the initial known concentration difference, the relation between the magnitude of the osmotic transport rate across the membrane and the concentration difference, as well as the nature of the non-electrolyte is obtained. In Fig. 2, the results of such experiments are shown, the initial rate of volume change of the inside solution being plotted against the initial concentration difference. For a given non-electrolyte the rate of volume change is directly proportional to the concentration difference as expected from equation (5). Further, the volume change rates are different for the various non-electrolytes at the same concentration difference, an occurrence expected from the inequality of the values for Q_2V_2. Of particular interest is the case of urea in which the sign of the volume change rate is opposite to that of the concentration difference; *i.e.*, negative osmosis occurs, the net movement of liquid being from the solution to the solvent side of the membrane. The explanation of the latter case, on the basis of the theoretical discussion, is that Q_2V_2 is greater than Q_1V_1; *i.e.*, a greater volume of urea than water is transported per unit time across the membrane.

In the second group of experiments associated with case II, the outside compartment was filled with an iso-osmotic solution of a different non-electrolyte instead of distilled water. The results are shown in Table I and demonstrate that, contrary to what would be expected on the basis of an extrapolation of the van't Hoff relation; there is a net movement of solution across the membrane despite the zero concentration difference. Of especial interest again is the observation that the osmotic transport rate is greater in the system in which the outside compartment contains urea solution instead of distilled water.

Discussion. The preceding observations have important implications in relation to the conclusions of a recent paper by Pappenheimer, Renkin, and Borrero(6) on material transport across the leaky capillary membrane. These workers have introduced a

TABLE I. Osmosis in Iso-Osmotic Systems.

Solute in the inside solution	Solute in the outside solution	$\frac{dV_1}{dt}$ (ml/min.)
Sucrose	Glucose	.11
"	Glycerol	.23
"	Ethylene glycol	.26
"	Distilled water	.31
"	Urea	.35

method for determining the area available for diffusion for various molecules per unit path length through the capillary wall. The method involves the measurement, for a test molecule, of the variables in the Fick equation:

$$\frac{dm}{dt} = -DA^p \cdot \frac{\Delta c}{\Delta x} \qquad (6)$$

where m is the net amount of test molecule crossing the membrane and the other symbols denote the same quantities as earlier in this paper. Substituting these values into the equation, a value for $A^p/\Delta x$, the diffusion area per unit path length is obtained.

In their experiments, the test molecule is introduced into the perfusing fluid of an isolated cat limb, and after a short time interval, the perfusing fluid flow rate and the arteriovenous difference of concentration are measured. From these values the quantity, dm/dt is readily obtained. Unfortunately, the quantity, Δc, is not obtained by direct measurement. Their procedure is to measure the increase in the mean capillary hydrostatic pressure required to prevent movement of fluid from the interstitial spaces into the capillaries as a result of the "partial osmotic pressure" exerted by the added test molecules; *i.e.*, the pressure increase necessary to maintain the isovolumetric state (see case I above).† The value for this pressure increment is then erroneously substituted into the van't Hoff equation as being equal to the "increase in osmotic pressure due to the added test molecules", and the equation is solved for Δc. By this means, they arrive at the following equation for the diffusion area per unit path length:

$$\frac{A^p}{\Delta x} = \frac{Q_b (c_a - c_v) RT}{D \cdot \Delta p} \qquad (7)$$

The correct equation, taking cognizance of the permeability of the capillary membrane to the test molecules involved should include the factor, k, in the numerator of the right-hand side of equation (7). All of the values given in the Pappenheimer paper for $A^p/\Delta x$ are, thus, too large by a factor which must be determined experimentally for each test molecule with the capillary membrane. The magnitude of the error might be estimated from the data obtained with the collodion membranes of this study. These artificial membranes are similar in many properties to the capillary wall. They are impermeable to serum albumin and slowly permeable to oxyhemoglobin. They have a filtration coefficient of approximately 2×10^{-5} ml/min/mm Hg/cm^2 as compared to filtration coefficients calculated from Pappenheimer's data for the capillary wall in the range of 2.5×10^{-4} to 2.3×10^{-6}. An important difference, of course, is that the collodion membranes used here are approximately 100 times as thick as the capillary wall (100 μ as compared to 1 μ); however, this difference is partially counterbalanced by the fact that A^p/A^m for the collodion membranes used here is of the order of 0.2 which is much larger than similar values for the capillary wall. On the basis of approximate comparability of the two kinds of membranes, Pappenheimer's values are too large by approximately two orders of magnitude. Such errors could conceivably be relevant to Pappenheimer's criticism of the work of Flexner, Cowie, and Vosburgh(7) made on the basis that his (Pappenheimer's) $A^p/\Delta x$ values were appoximately 200 times larger than similar values calculated from the data of Flexner *et al.*

A note of caution in the study of transcapillary diffusion of electrolytes may be appropriate here. In the case of electrolyte solutions and membranes bearing fixed dissociable groups, the factor, k, becomes an unknown function of the epsilon-potential across the membrane and the zeta-potential on the membrane, as well as a function of Q and *V*. It has been shown experimentally by the author that with certain electrolyte solutions k may even exceed one; *i.e.*, $\Delta p > \Delta \pi$.

Summary. 1. Experiments are presented which demonstrate, as expected from theoretical considerations, that the classical van't Hoff equation cannot be employed to ascertain the relation between pressure difference and concentration difference across a membrane in the dynamic situation existing in osmotic

† In Pappenheimer's work it was actually the nearly equal isogravimetric state.

systems involving membranes permeable to solute as well as to solvent. It is shown that the pressure difference necessary to prevent a net osmotic movement of fluid across a leaky membrane may be smaller by several orders of magnitude than the pressure difference necessary to accomplish the same effect with a semipermeable membrane; *i.e.*, a membrane to which the van't Hoff equation applies. It is also shown that, contrary to what would be expected on the basis of reasoning from the van't Hoff equation, the osmotic transport rate across a leaky membrane is a function not only of the solute concentration difference but also of the nature of the solute molecules. Further, it is demonstrated that net transport of liquid may occur across leaky membranes separating iso-osmotic solutions of different non-electrolytes. 2. In all these cases, the use of equations derived for the equilibrium state across a semipermeable membrane (*e.g.* the van't Hoff equation) would lead to decidedly erroneous results. 3. These observations demonstrate the use of van't Hoff's equation in a recent analysis of capillary permeability to be incorrect.

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Mack Virus—Serum and Gamma Globulin Neutralization of Unidentified Agent Isolated From Suspected Nonparalytic Poliomyelitis.* (20307)

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We wish to describe certain observations on an unclassified virus isolated from tissue-cultures of the feces of a child (Mack) who developed thermostable specific antibodies in convalescence. We believe that the child's illness was caused by the agent which is tentatively referred to as *Mack* virus. The 5-year-old girl's illness occurred during a poliomyelitis outbreak in Cincinnati during August, 1947, with symptoms of headache, pain and "stiffness" of the neck, temperature of 103°F and pleocytosis (40 leucocytes per cu mm, 65% lymphocytes.) Recovery was complete in a few days. Although objective nuchal-spinal rigidity was not detected the clinical findings were compatible with a short-lived aseptic meningitis syndrome. For additional clinical and epidemiological details the reader is referred to patient No. 7 (K.M.) included in a study reported by Sabin and Steigman(1).

Characteristics of Mack virus in tissue-culture—isolation and titration. The roller tube tissue-culture system described by Robbins, Weller, and Enders(2) for poliomyelitis virus was used with some modification. Normal rhesus and cynomolgus monkey testicles were used as the source of fibroblastic proliferation. Natural and synthetic(3) nutrient media which are used in cultivating poliomyelitis virus were used equally well in these studies. The presence of the *Mack* virus in the culture system is indicated by cytopathogenic effects upon the fibroblastic outgrowths which are specifically inhibited by appropriate

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