ERIC HEYER*
ALBERT CASS**
ALEXANDER MAURO†

The Rockefeller University
New York, New York 10021

A DEMONSTRATION OF THE EFFECT OF PERMEANT AND IMPERMEANT SOLUTES, AND UNSTIRRED BOUNDARY LAYERS ON OSMOTIC FLOW

Professor Donald Barron's scientific interests and achievements extend over a remarkably broad range of subjects. As a scholar and investigator he, however, did not relegate teaching to a secondary position. We hope that this modest communication will serve in a small way to remind us of the simple and lucid laboratory demonstrations that Professor Barron used so effectively and memorably in his teaching of physiology.

The behavior of a wide variety of artificial membranes has been invoked over the years to account for aspects of transport across naturally occurring membranes,[1] such as plasma membranes and those found in the systemic capillary and the nephron, as well as many others. Among the many transport processes to be considered, the investigation of osmosis, (osmotic equilibrium and osmotic flow) is central to an understanding of water permeability of biological membranes.

Generally, one may consider two kinds of artificial membranes, homogeneous or liquid membranes, and porous membranes, conveniently thought of as containing continuous aqueous channels. Cellular membranes exhibit to varying degrees the properties of both kinds of membranes,[2] and osmotic measurements provide useful operational distinctions between them. The flow osmometer to be described enables students to observe certain basic aspects of osmotic flow across a rigid porous membrane; these aspects are: 1) the equilibrium state of zero net flow, 2) the steady state flow established when the chemical potential of water is different in the two phases separated by the membrane, and 3) the dependence of flow on the permeability of the membrane to the solute.

The experiments described demonstrate the importance of the stable unstirred boundary layers adjoining the membrane in controlling the actual osmotic pressure difference across the membrane. In fact, the accumulation or depletion of impermeant solute in unstirred layers adjoin-

* Presently, Medical Scientist Trainee, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

** Presently, Postdoctoral Fellow, Department of Neurology, Albert Einstein College of Medicine.

† Associate Professor, Rockefeller University.

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ing the membrane is a problem so severe as to limit the validity of Starling's Law for such membranes.

Theory: Impermeant solute

Consider a membrane sufficiently rigid that it will sustain the application of pressure to the aqueous phases that it separates. Let one phase (I) be distilled water, and let a quantity of an impermeant solute be added to the other (II). The necessary and sufficient condition for equilibrium of water, the permeant species, is

$$\mu_w^{I} = \mu_w^{II}$$

That is, $\mu_w^{\circ} + RT \ln a_w^{I} + \overline{V}P^{I} = \mu_w^{\circ} + RT \ln a_w^{II} + \overline{V}P^{II}$ where μ_w is the chemical potential of water, μ_w° is the standard chemical potential of water, a_w the activity of water, $\overline{V}$ the partial molar volume, P the hydrostatic pressure, and R and T have their usual meanings. Thus, for $a_w^{I} = 1$,

$$P^{I} - P^{II} = \frac{RT}{\overline{V}} \ln a_w^{II} \qquad (1)$$

The osmotic pressure π is defined as the quantity on the right of expression (1) and is measured by the pressure that must be applied to phase II to establish equilibrium:

$$\pi = P^{II} - P^{I}$$

However, for an impermeant solute, if $\mu_w^{I} \neq \mu_w^{II}$, then net flow will ensue, and for the steady state:*

$$J_v = K' (\mu_w^{I} - \mu_w^{II})$$

$$J_v = K \left[(P^{I} - P^{II}) - \frac{RT}{\overline{V}} \ln a_w^{II} \right] \qquad (2)$$

where $K = K'\overline{V}$ or $J_v = K (\Delta P - \pi)$, which we shall refer to as Starling's Law. In these equations, J_v is the volume flow and K the hydraulic conductivity. Note that a_w^{II} refers to the activity of water at the membrane-solution interface. From (2), we note that the flow should be proportional to the difference $\Delta P - \pi$, provided that π refers to the values across the membrane. We shall see that owing to boundary effects, π may not always be equal to the difference between the values in the bulk

* It should be noted that this statement is unambiguous only for the case of impermeant solutes. If permeant solutes are present the sign and magnitude of the volume flow cannot be prescribed by the chemical potential difference of the solvent alone.

solutions. However, at equilibrium, $\Delta P = \pi$, and the values at the membrane must equal the bulk values. We can rewrite (1) such that

$$\pi = -\frac{RT}{\bar{V}} \ln a_w^{II}, \text{ and}$$

$$\frac{\ln a_w^{II}}{\bar{V}} \cong -\phi C_s$$

where C_s is the concentration of impermeant solute. The introduction of ϕ, the osmotic coefficient, explicitly acknowledges the possibility of nonideality of solutions. Of course, for dilute solutions,

$$\pi = RT C_s$$

the familiar van't Hoff relation. However, we shall be forced to explicitly acknowledge the nonideality of some of the solutions that we will encounter, though this will not be a central point that we wish to elucidate.

Permeant solutes

In general, we will find that

$$J_V = K(\Delta P - \sigma\pi)$$

where the quantity σ, known as the reflection coefficient,[8] may vary from zero, for solutes that are not distinguished from water, to one, for impermeant solutes. The dependence of this quantity on the size of small hydrophilic solutes is an important experimental feature of the behavior of the porous membrane, as the ability of such molecules to penetrate a pore determines the nature of the flow process they are able to induce.

METHODS

We hope that these flow cells will prove useful for students, and accordingly we shall describe the apparatus and procedure in some detail. In general, cellulose acetate membranes of the type used for desalination[4] were used to separate either an aqueous solution or distilled water (phase II) from a chamber containing distilled water only (phase I). A capillary connected to phase I was open to the atmosphere, and enabled measurement of the rate of flow across the membrane by viewing the rate of movement of the meniscus in the capillary. One of the two types of cells used in this study enabled the application of pressure to phase II. Thus, with distilled water in phase II, the hydraulic conductivity of the membrane was determined from the rate of movement of the meniscus as a function of the applied pressure.

With a solution in phase II, pressure could be applied to obtain an equilibrium state of zero flow of solvent. Alternatively, with phase II

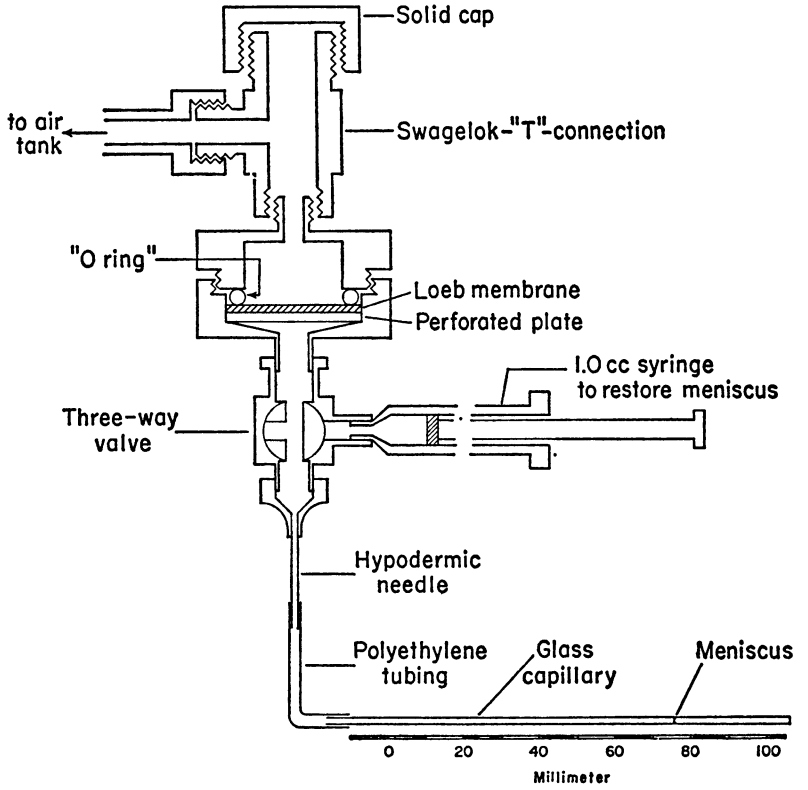

FIG. 1. Diagram of the large membrane flow cell. The membrane area was 3.8 cm^2 whereas the membrane area in the small membrane flow cell was 0.8 cm^2. The pressure gauge and reduction valve was a Swagelok unit (All Tube Tee, Part No. 500-3). 5/16" copper tubing was used to connect the gauge to the cell. (For pressure less than five pounds per square inch, a Cenco mercury manometer was used to measure the pressure applied.) The capillary diameter was about 0.6 millimeter.

at atmospheric pressure, the steady state of osmotic flow could be observed. The cell used for these purposes, the "large membrane cell," was constructed from a Micro-Syringe Filter Holder (Millipore Filter Co., Cat. No. XX30 025 00) as detailed in Figure 1.

A cellulose acetate membrane, backed up by a one millimeter thick stainless steel plate uniformly perforated with holes (approximately 1 mm in diameter) was sealed by means of an "O-ring" between the upper half of the cell and the membrane. Although not shown in Figure 1 a thin stainless steel screen (supplied with the filter holder) was placed between the membrane and the thick stainless steel plate. The cell was assembled under water to eliminate air bubbles, by placing membrane, screen, plate, and

"O-ring" into the bottom half of the cell, and screwing in the upper half of the cell. The lower half of the cell always contained only glass distilled water, and the capillary for measurement of flow was joined to this half by a three-way valve (Luer B-D, MS 10), a hypodermic needle (Yale 20), and polyethylene tubing. One position of the valve enabled adjustment of the meniscus by injection or withdrawal of distilled water, whereas another position connected the flow cell directly to the capillary (movement of the meniscus by one millimeter corresponded to a volume flow of about 0.3 microliter). The upper half of the cell screwed into a Swagelok-"T"-connection; one limb of the connector was fitted with a removable cap, and the other limb was connected with copper tubing to a pressure tank through a pressure regulator graduated in pounds per square inch (psi).*

Solutions in the upper half (phase II) were changed by removing the solid cap and sucking up the solution through a polyethylene tube connected to a vacuum bottle. The chamber was then rinsed with at least 40 ml. of distilled water, and about 20 ml. of the new solution were then used to rinse the 2 ml. volume of the upper half of the cell.

The second flow cell, also fashioned from a Swinny Filter Holder,† differed from the first in its smaller membrane area and in its arrangement for changing solutions. Both sides of this cell were open to the atmosphere. Thus pressure could not be applied to the solution phase. A stainless steel capillary inserted through the wall of the upper half of the cell introduced solutions just above the surface of the membrane, and a second capillary, near the very top of the cell, was connected to a vacuum bottle and removed fluid from the top of the cell. As before, the flow cell was rinsed with about 40 ml. of distilled water between runs, and 2 ml. of solution then irrigated a chamber whose volume was about 1/3 of a milliliter. This cell is referred to as the "small membrane flow cell."

Membranes

The unique feature of the present flow cell is the use of cellulose acetate membranes of the type used for desalination;[4] the high permeability of such membranes to water combined with their relative impermeability to small solutes such as glucose and sucrose enable a very convincing demonstration of osmotic flow with relatively modest differences in osmotic pressure. These membranes, developed by Loeb, *et al.*[5] for desalination on a commercial scale, are made by casting solutions of cellulose acetate containing inorganic ions on glass plates, and allowing the upper, or active

* The regulator was a National No. 540 with ¼" Swagelok fittings, and an oxygen-nitrogen gas mixture was used.

† Millipore Filter Co., Cat. No. XX30 012 00.

surface to dry in air at elevated temperatures.[4] The underside of the membrane thus formed is a region of spongy, relatively large pores that does not contribute significantly to the osmotic properties of the membrane; thus, the upper or rough surface must be placed in contact with the solution phase. The membranes used in the present experiments were supplied by S. Loeb in the form of sheets from which membranes of the appropriate size were cut with a cork borer. Care must be taken to keep the membranes moist at all times.*

Solutions

The solutes used were urea, glycerol, erythritol, glucose, and polymers of polyethylene glycol (PEG) of several different number average molecular weights:

	Number average molecular weight
PEG "600"	——
PEG "1540"	2050
PEG "4000"	4700
PEG "6000"	6900

Measurements

The rate of flow across the membrane into the various solutions was determined by recording the position of the meniscus in the capillary at various times following the introduction of a given solution. All experiments were performed at room temperature.

The osmotic pressure of the impermeant solutes (PEG "1540," "4000," and "6000") was determined from the equilibrium pressure ($\Delta P = \pi$) required to maintain the flow at zero. In addition, the flow resulting from pressure less than, or greater than the osmotic pressure of the solution was noted. The osmotic pressure of the permeant solutes was measured from the freezing point depression employing the Fiske Osmometer Model G.

RESULTS

1. *Hydraulic conductivity*

With distilled water on both sides of the membrane, the flow ensuing upon application of pressure to phase II was directly proportional to the pressure, over the range zero to sixty pounds per square inch. Thus, the hydraulic conductivity, $K = J/\Delta P$, was apparently unchanged by the application of pressure under the present experimental conditions.

* Cellulose acetate membranes of this type may be obtained commercially from Eastman Chemical Products Incorporated, Kingsport, Tennessee.

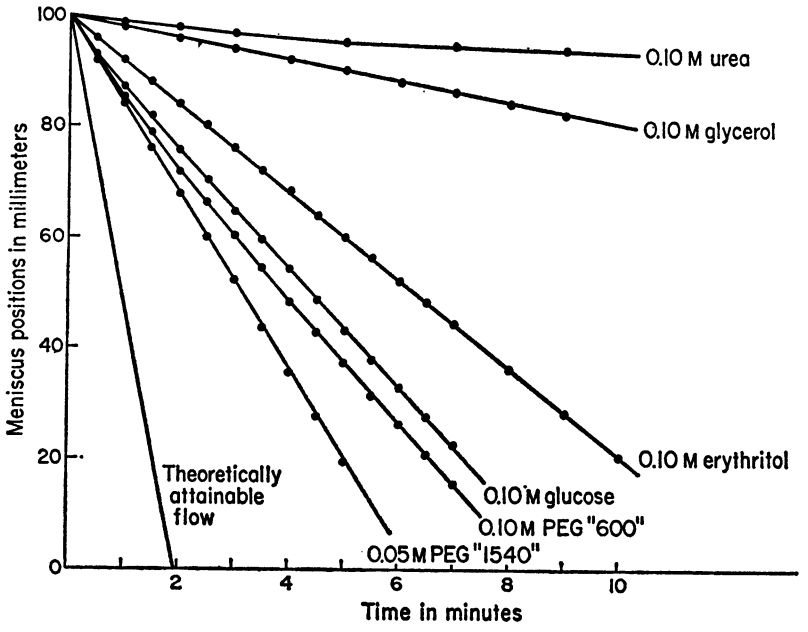

FIG. 2. Position of the meniscus in millimeters as a function of time in minutes for the various solutes indicated at the same value ϕ RT C_s. The osmotic coefficient of PEG "1540" is two at this concentration, whereas the other solutes have an osmotic coefficient of one.

2. *Osmotic flow with permeant and impermeant solutes*

Figure 2 is a plot of the position of the meniscus as a function of time for solutions having the same osmotic pressure. These data were obtained with the small membrane flow cell without a pressure difference across the membrane, i.e. both phases were at atmospheric pressure. An important feature of the experimental results is that the rate of movement of the meniscus, which is proportional to the volume flow, is constant over a long period of time for a given solution at a specified osmolarity (Figure 2). The slope of each line is proportional to the steady state volume flow across the membrane. The line to the left of the family of curves indicates the theoretically predicted volume flow given by the product of the hydraulic conductivity and the osmotic pressure of the test solutions.

A striking feature of osmotic flow is seen in the increase in slope with increase in size of the permeant species, as can be seen in the data obtained for urea, glycerol, erythritol, glucose, and PEG "600." A reflection coefficient (σ) can be calculated for a given solute by taking the ratio of

TABLE 1.

Solution	*Reflection coefficient (σ)*
0.01 M Urea	0.014
0.01 M Glycerol	0.038
0.01 M Erythritol	0.154
0.01 M Glucose	0.216
0.01 M PEG "600"	0.249

the slope obtained for that species to the theoretically predicted slope, and these values are given in Table 1.* It should be noted that, unlike the other solutes, the species PEG "1540" is impermeant, in which case the deficiency in the volume flow relative to the theoretically attainable value, i.e. $\sigma < 1$, must be related not to the leakiness of the particle but rather to another mechanism which will be treated in the discussion.

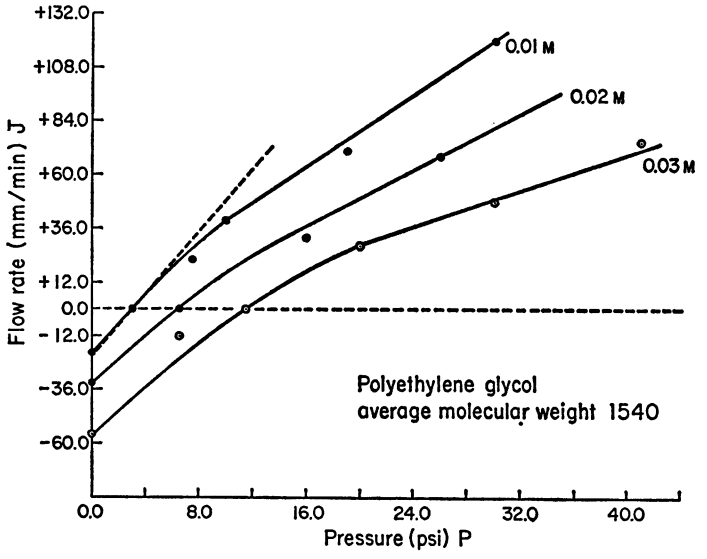

FIG. 3. Osmotic flow as a function of the pressure applied to the solution phase for three different concentrations of PEG "1540." The dotted line is the theoretically expected flow from $K(\Delta P - \pi)$ for the concentration 0.01 M. A dotted line may be drawn through $\Delta P = \pi$ for each concentration. Experiment at room temperature (27°C.) with large membrane.

* Inasmuch as these values of σ are calculated assuming that the bulk concentration difference obtains across the membrane, they must be regarded as lower limits for the true value of σ.

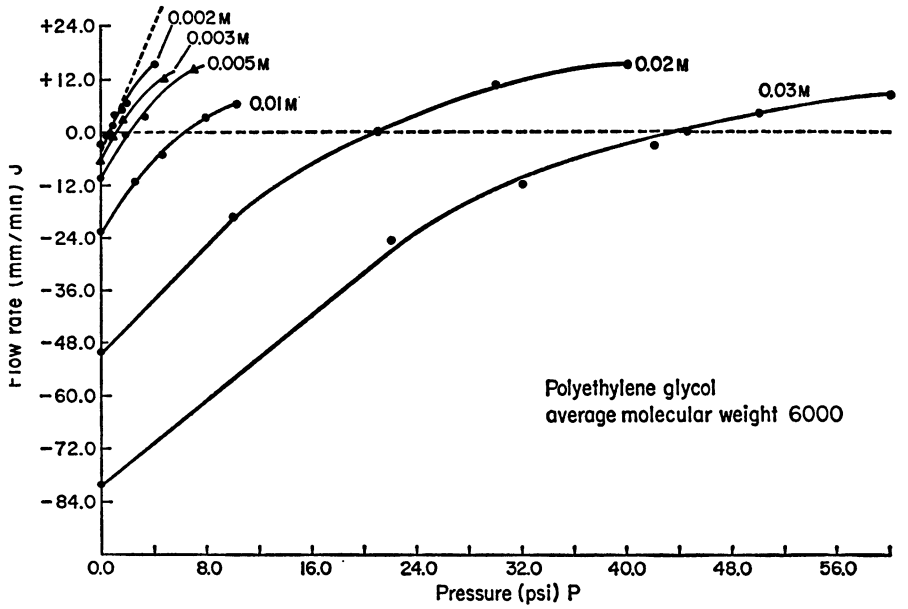

FIG. 4. Osmotic flow as a function of the pressure applied to the solution phase for six different concentrations of polyethylene glycol "6000." The dotted line at the left is the theoretically expected flow from $K(\Delta P - \pi)$ for the concentration 0.002 M. A dotted line may be drawn through $\Delta P = \pi$ for each concentration. Experiment at room temperature (27°C.) with large membrane.

3. *The equilibrium state:* $\Delta P = \pi$

All of the following results were obtained using the larger membrane cell. Figures 3 and 4 are plots of the rate of flow observed with different concentrations of PEG "1540" and PEG "6000," respectively, as a function of the pressure applied to the solution phase (II). For these impermeant solutes, the value of ΔP at which the flow is zero is equal to π, the osmotic pressure of the solution, which is given by ϕ RT C_s. For polyethylene glycol solutions, the osmotic coefficient ϕ, a quantity which is equal to one for an ideal solution, is in fact much greater than one, and is dependent on the concentration of the polymers (Figure 5). This nonideality is, in general, to be expected for long chain polymers[8] and, as a thermodynamic property of their solutions, is reflected in the colligative properties of such solutions.

4. *Osmotic flow:* $\Delta P < \pi$

Upon moving from the equilibrium state by decreasing the pressure exerted on the solution, a steady state of flow is established. However, it is apparent that the flow does not equal the expected value as given

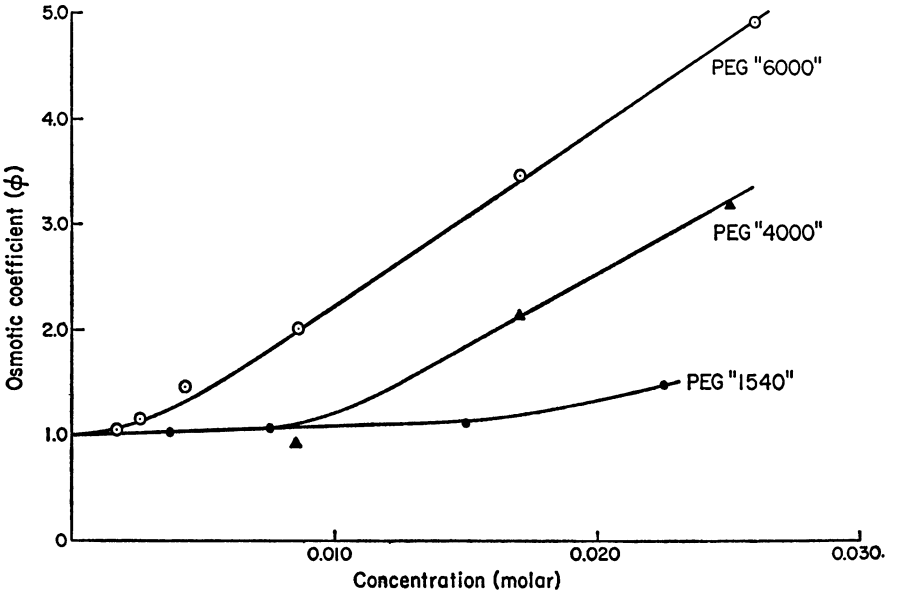

FIG. 5. The osmotic coefficients (ϕ) of PEG "1540," "4000," and "6000" as a function of their molar concentration and as determined from the equilibrium osmotic pressures in these experiments. For a comparison of the data for PEG "6000" with data obtained by Alexandrowicz[13] by light scattering, see Table 2. As can be seen, the solutions are highly nonideal. Room temperature was 27°C.

by the product of the conductivity, K, and $\Delta P - \pi$, the magnitudes observed being considerably less than predicted (Table 2). In the limit as π, and therefore J_V goes to zero, we see from Figure 4 that the flow approaches $K(\Delta P - \pi)$ as a limit, and the system therefore tends toward Starling's Law for very dilute solutions.

5. *Osmotic flow: $\Delta P = 0$*

For the case of pure osmotic flow, i.e. $\Delta P = 0$, we see most clearly this altogether new aspect of osmotic flow (Fig. 6). This is seen in the fact that even for dilute solutions, e.g. with π values of 3 - 4 psi, the expected osmotic flow ($-K\ \pi$) is not observed experimentally. Moreover, the disparity between the expected osmotic flow and that observed becomes more marked with increasing molecular weight of the polyethylene glycol molecule, i.e. increasing chain length. We have not attempted to analyze the convective-diffusive regime near the membrane in detail, but it is evident that the basis for the effect encountered here is a marked depletion of the solute species that arises as a consequence of the induced osmotic flow.

TABLE 2.

Solution	*Concentration** *(molar)*	π *(psi)*	*Osmolarity*	ϕ†	ϕ††	*Flow rate (mm/min.)*	*Ideal flow rate (mm/min.)*
PEG "1540"	0.0037	1.4	0.0039	1.0		−8.0	−9.7
	0.0075	3.0	0.0084	1.1		−19.8	−20.8
	0.0150	6.5	0.0181	1.2		−35.1	−45.2
	0.0225	11.5	0.0320	1.4		−55.5	−80.0
	0.0450	17.0	0.0472	1.0		−65.7	−118
	0.0600	30.0	0.0834	1.4		−83.5	−208
	0.0680	39.0	0.1082	1.6		−98.0	−271
PEG "4000"	0.0085	3.0	0.0084	1.0		−15.0	−20.8
	0.0170	14.0	0.0389	2.3		−43.3	−97.2
	0.0255	27.0	0.0750	3.3		−68.0	−188
	0.0340	48.0	0.1331	3.9		−97.5	−334
PEG "6000"	0.0017	0.7	0.0019	1.1	1.1	−2.9	−4.8
	0.0026	1.3	0.0035	1.3	1.2	−6.8	−8.5
	0.0043	2.1	0.0058	1.4	1.5	−10.2	−14.6
	0.0086	6.1	0.0169	2.0	2.2	−22.6	−42.2
	0.0170	21.0	0.0582	3.4	3.8	−50.0	−310
	0.0260	45.0	0.1250	4.8	5.5	−77.0	−715

* The concentrations are corrected for the experimental number average molecular weight.

† Osmotic coefficient determined from the present equilibrium measurements; $\phi = \frac{\pi}{RT\ C_s}$.

†† These osmotic coefficients were obtained from Alexandrowicz' data.[18]

6. *Osmotic flow:* $\Delta P > \pi$

We may now profitably examine the flow for values of $\Delta P > \pi$; from Figures 3 and 4, it is apparent that the flow deviates from the expected values of $K(\Delta P - \pi)$ even more than the flow deviates for values of $\Delta P < \pi$. Here, the effect of flow is to *pile up* solute at the membrane-solution interface rather than to deplete the interface of solute. This tendency of the flow to choke itself off is particularly conspicuous in the plot for 0.03 molar PEG "6000" (Figure 4).

Though it is not plotted in this paper, this piling up is illustrated most strikingly by the instantaneous removal of the pressure; there is initially a very high rate of flow that subsequently declines to the values given, for example, in Figure 6. One can also see the attainment of the steady state upon applying excess pressure starting from $\Delta P = \pi$; the flow initially is higher than the value to which it ultimately relaxes. Thus, the transient

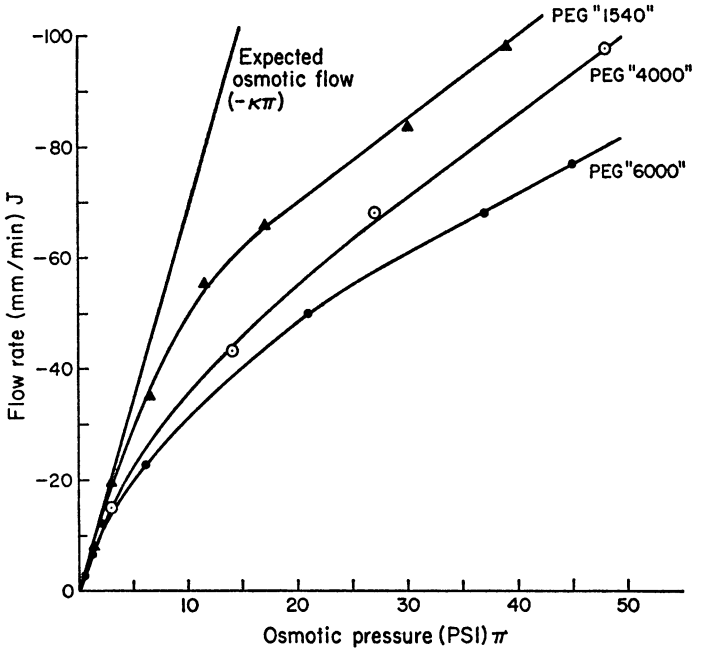

FIG. 6. Osmotic flow ($\Delta P = 0$), as rate of movement of the meniscus in millimeter / minute, as a function of osmotic pressure in psi ($\pi = \phi$ RT C_s) for PEG "1540," "4000," and "6000." Note that the flow approaches the theoretically expected value $-K\pi$ as π approaches zero. Data obtained at room temperature (27°C.) with large membrane flow cell.

response upon increasing or decreasing the pressure from the equilibrium value confirms the existence and behavior of the convective-diffusive regime established during osmotic flow.

DISCUSSION

Using a rigid porous membrane of the type developed by Loeb for desalination, we have observed with the apparatus described several important and basic features that occur in "pure" osmotic flow ($\Delta P = 0$) and in the more general case when both hydrostatic pressure (ΔP) and osmotic pressure (π) act to produce flow across the membrane.

The flow with permeant solutes is less than the flow with impermeant solutes

In recent years, the marked diminution in osmotic flow with decreasing size of the solute particle in the solution phase has received attention in the introduction of the term "reflection coefficient."[8] It is fitting, however,

to note that this important feature of osmotic flow was well known to physiologists in the time of Starling,[7] as seen, for example, in the work of his contemporary Lazarus-Barlow,[8] who worked with solutions of urea, sodium chloride and glucose on "peritoneal membrane of calf." With remarkable clarity, Lazarus-Barlow emphasized that one cannot predict even the direction of flow let alone the magnitude of that flow solely from the tonicities of the solutions bathing the membrane. He found that the flow for solutions of a given tonicity with the smaller solutes was less than that observed with the larger solutes, and he demonstrated that flow can occur from a solution of higher tonicity into a solution of lower tonicity. This effect has been dramatically demonstrated in recent years by Meschia and Setnikar,[9] and the implications of such observations for the mechanisms of osmosis across rigid porous membranes are explored by Mauro.[10,11] The results presented in this paper (Fig. 2) also demonstrate this aspect of osmotic flow.

In this context, it is useful to state the criterion that we used to determine whether a solute penetrated the membrane: after a period of osmotic flow, the solution phase was exchanged for distilled water; if the meniscus remained stationary after this procedure, we inferred that within the limits of our observation, solute had not penetrated. With the solutes that we have termed permeant, however, we observed using this procedure movement of the meniscus in the direction opposite to that usually encountered; in other words, enough solute had penetrated into phase I during the period of osmotic flow to establish a significant osmotic pressure difference. The solutes urea, glycerol, erythritol, glucose and PEG "600" were permeant by this criterion.

The equilibrium state accurately establishes the osmotic pressure of the solutions

We have determined the osmotic pressure at equilibrium for the impermeant polyethylene glycols "1540" and "6000" (Figs. 3 and 4). Although these are nonideal solutions, the thermodynamic activity of water is established in agreement with independent measurements carried out by Alexandrowicz[12] on similar polymers using light scattering (Table 2, Fig. 5). Thus, students may confidently expect to measure the osmotic pressure of solutions of impermeant solutes using this apparatus.

Depletion or accumulation of solute in the unstirred layer: the relation $J_V = K(\Delta P - \pi)$

So far as may be judged from Figures 3 and 4, certain features of the stationary states that result for values far removed from $\Delta P = \pi$ are in apparent contradiction to Starling's Law, but in general are anticipated

properties of these flow regimes. The flow rates for the impermeant solutes, PEG "1540" and PEG "6000," are substantially less than the values predicted from the product of the hydraulic conductivity K and the driving force ($\Delta P - \pi$) *not* because the solute molecules penetrate, but rather because the concentrations at the membrane-solution interface are either greater than or less than the bulk values for the cases of ($\Delta P - \pi$) > 0 and ($\Delta P - \pi$) < 0, respectively. A number of investigators[13-15] have shown that when these membranes are used to filter brackish water under very high pressure (about 1000-1500 psi) salts pile up at the membrane-solution interface; we infer this "piling up" from our results as well. First of all, the fact that a steady state rate of flow ensues at all indicates that mixing and convection at the interface serve to maintain the concentration of solutes at some constant value. Since distilled water is pouring out into a concentrated solution at the boundary, the combined effects of convection arising from density gradients and diffusion of solute back to the membrane maintain a concentration of solute approaching the bulk value in the limit of zero flow. Although we have not been able to obtain quantitative data, it is clear that a finite amount of time is necessary after the exchange of solutions for the steady state to be established.* The attainment of the steady state occurs more rapidly with dilute than with concentrated solutions presumably because the thickness of the boundary region, which is a function of the osmotically induced flow, is less with the more dilute solution. A quantitative treatment of flow processes within this region is not to our knowledge available, but it would almost certainly be highly nonlinear with respect to both ΔP and π, a feature which is clearly seen in the data presented.

SUMMARY

Both the large and small cellulose acetate membrane osmometers are useful in demonstrating osmotic equilibrium and osmotic flow for solutions of permeant and impermeant solutes. For permeant solutes the particle size determines the osmotic flow. However, for impermeant solutes osmotic flow depends on the accumulation or depletion of solute in unstirred layers adjoining the membrane; in addition, by applying pressure on these solutions which are separated from water by a membrane, the flow as a function of pressure may be explored. From this function the osmotic pressure of a solution can be determined.

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* The reader should note that the "dynamic" method of measuring osmotic pressure first used by Berkeley and Hartley, depends upon observing the *initial* rates of flow. Their classical paper[16] deals with the problem of determining osmotic pressure by observing volume flow in a manner similar to that employed in the present work.

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