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Osmotic Flow of Water across Permeable Cellulose Membranes

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ABSTRACT Direct measurements have been made of the net volume flow through cellulose membranes, due to a difference in concentration of solute across the membrane. The aqueous solutions used included solutes ranging in size from deuterated water to bovine serum albumin. For the semipermeable membrane (impermeable to the solute) the volume flow produced by the osmotic gradient is equal to the flow produced by the hydrostatic pressure RT ΔC, as given by the van't Hoff relationship. In the case in which the membrane is permeable to the solute, the net volume flow is reduced, as predicted by the theory of Staverman, based on the thermodynamics of the steady state. A means of establishing the amount of this reduction is given, depending on the size of the solute molecule and the effective pore radius of the membrane. With the help of these results, a hypothetical biological membrane moving water by osmotic and hydrostatic pressure gradients is discussed.

The processes of secretion and absorption in the body have often been assumed to require a movement of water in response to an osmotic gradient. By this hypothesis, a gradient in water activity is produced by the absorption or secretion of a solute. Usually this solute is a small ion or molecule, to which the membrane may be permeable. The influence of a given concentration difference of the solute on the osmotic movement of water depends on the extent of such permeability, or "leakiness." Staverman (1), in a theoretical treatment of this problem using the methods of irreversible thermodynamics, has introduced a correction factor σ into the van't Hoff expression for the osmotic pressure due to the solute. In the present work, the Staverman factor σ has been determined for a number of different solutes, in conjunction with three cellulose membranes which have been physically characterized. A systematic means of predicting σ from the properties of the membrane is given, and the use of σ in constructing models of the transporting membrane

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discussed. A preliminary report of part of this work was made to the First National Biophysics Conference in 1957 (2).

METHODS

In the experiments, the rate of increase in volume of the test solution was measured directly, using special lucite chambers which have been previously described (3). A cellulose membrane, chosen from dialysis tubing (Visking), wet gel (Sylvania, 300 weight), and uncoated cellophane (Dupont 450 PD 62), was mounted between two chambers. One contained 2 ml. of the test solution, and the other, 16 ml. of distilled water (or 50 mM NaCl if the test solute were albumin). Changes in volume of the smaller chamber could be measured with an accuracy of ± 1 microliter. Stirring was accomplished by means of a stream of gas in the large chamber, and by a glass-enclosed iron wire, driven by an external magnetic stirrer, in the small chamber.

The test solutions included deuterated water, urea, glucose, sucrose, raffinose, inulin (Pfanstiehl), and bovine serum albumin (Armour). Nearly pure D_2O (45 M) was used, and 1 M urea solution, since the net flow in these cases was small. Glucose, sucrose, and raffinose were used in 0.1 M solution. The solution of inulin, 5 per cent by weight, was dialyzed against distilled water; the resulting solute had a molecular weight of 3100, as determined from the freezing point of the solution. The bovine serum albumin was dissolved in 50 mM NaCl, and dialyzed against large volumes of 50 mM NaCl. The pH of the final solution was 5.3. The net volume flow was measured for two different concentrations of albumin (5 and 10 per cent by weight); the ratio of net flow to concentration was found to be constant.

For the characterization of the membranes used in these experiments, the hydraulic and diffusion flows of water were measured. In measuring the flow of water due to a pressure gradient across the membrane, both chambers were filled with distilled water. A perforated lucite disk was clamped in place to support the membrane, the disk having been covered with thick filter paper (Whatman No. 3) so that the whole area of the membrane was available for filtration. Pressure differences of 50 to 100 cm. of H_2O were used, stabilized with a large air bottle. Shortly before or after this measurement, the flux of tritiated water was measured across the same membrane. Measurements of net volume flow due to the presence of a solute were done on other membranes cut from the same batch of material. All experiments were performed at 25 ± 1°C.

Calculations and Results

For convenience, the symbols used by Kedem and Katchalsky (4) are also used here. These authors consider a system of solvent, non-ionic solute, and membrane permeable to both solvent and solute. The net volume flow per unit area across the membrane is denoted by $J_v = \dot{n}_w\bar{v}_w + \dot{n}_s\bar{v}_s$, in which $\dot{n}_w$ and $\dot{n}_s$ are the solvent and solute flows respectively, in moles per second per unit area, and $\bar{v}_w$ and $\bar{v}_s$ are the corresponding partial molar volumes. The additional parameter necessary to describe solvent and solute flow is

the velocity of solute relative to solvent, J_D, defined as $(\dot{n}_s/c_s) - (\dot{n}_w/c_w)$, where c_s and c_w are the respective concentrations of solute and solvent in the region of interest. The flows, J_v and J_D, can be expressed in terms of the hydrostatic pressure difference Δp, and the concentration difference of solute, Δc_s, across the membrane:

$$J_v = L_p\Delta p + L_{pD}RT\Delta c_s \qquad (1\,a)$$

$$J_D = L_{Dp}\Delta p + L_DRT\Delta c_s \qquad (1\,b)$$

R is the gas constant, T the absolute temperature; L_p, L_{pD}, L_D, L_{Dp} are coefficients determined by experiment, as illustrated below.

(*a*) If no solute is present ($\Delta c_s = 0$), as in the measurement of hydraulic flow, Equation 1*a* becomes

$$J_v = L_p\Delta p \qquad (2)$$

and J_D is undefined.

(*b*) In the presence of a solute to which the membrane is impermeable, which is the case of van't Hoff, $J_v = \dot{n}_w\bar{v}_w$. Since the velocity of the solute is zero, $J_D = -\dot{n}_w/c_w$. For a dilute solution, $\bar{v}_w = 1/c_w$, so that $J_v = -J_D$. If $\Delta p = 0$,

$$\begin{aligned} J_v &= L_{pD}RT\Delta c_s \\ J_D &= L_DRT\Delta c_s \end{aligned} \qquad (3)$$

From Equation 3, $L_{pD} = -L_D$. Putting these values for J_D and L_D into the general equations, 1*a* and 1*b*, and adding,

$$(L_p + L_{Dp})\Delta p = 0 \qquad (4)$$

Since this must be true for any value of Δp, $L_p = -L_{Dp}$. By the general relationship of Onsager, $L_{Dp} = L_{pD}$. Equation 1*a* becomes, for the semipermeable membrane,

$$J_v = L_p(\Delta p - RT\Delta c_s) \qquad (5)$$

and hydrostatic or osmotic pressures are equally effective in producing net flow.

(*c*) In the general case, Staverman has introducee the coefficient $\sigma = -L_{pD}/L_p$. Depending on the system, σ takes on a value between 0 (nonselective membrane) and 1 (semipermeable membrane).[1] An idealized

[1] Sigma has been defined so as to include the conventional osmotic coefficient g, which is independent of the membrane and a function of the concentration and kind of solute. More exactly, σ varies between 0 and g. Since the major variations in σ are introduced by the membrane, g will be approximated by unity in this paper.

experiment may be used to evaluate σ. A pressure difference Δp is exerted across a permeable membrane, separating two very large and well stirred compartments containing the same solution ($\Delta c_s = 0$). In general, the solvent under pressure passes through faster than the solute (sieving). However, at zero time, equations (1) give

$$J_v = \dot{n}_s \bar{v}_s + \dot{n}_w \bar{v}_w = L_p \Delta p \tag{6a}$$

$$J_D = \frac{\dot{n}_s}{c_s} - \frac{\dot{n}_w}{c_w} = L_{Dp} \Delta p \tag{6b}$$

Using the simplification of dilute solutions ($\bar{v}_w \cong 1/c_w$), (6*a*) and (6*b*) may be added, and the result divided by (6*a*):

$$\frac{\dot{n}_s}{J_v}\left(\bar{v}_s + \frac{1}{c_s}\right) = 1 + \frac{L_{Dp}}{L_p} \tag{7}$$

As before, $L_{Dp} = L_{pD}$. The ratio L_{Dp}/L_p is therefore $-\sigma$. Furthermore, for dilute solutions, $\bar{v}_s$ is negligible in comparison to $1/c_s$. Rearranging Equation 7,

$$\sigma = 1 - \frac{\dot{n}_s}{J_v c_s} \tag{8}$$

The quotient $\dot{n}_s/J_v c_s$ is equal to the moles of solute passing through the membrane per unit time, divided by the moles of solute arriving at the membrane during that time. This ratio may be equated (5, 6) to the ratio A_{sf}/A_{wf}, where A_{sf} is the effective pore area available to solute molecules, and A_{wf} to solvent molecules. Using this substitution, Equation 8 takes the form (3):

$$\sigma = 1 - A_{sf}/A_{wf} \tag{9}$$

An equation has been given for the effective filtration area A_{Xf} for the molecule X passing through a membrane with the geometrical pore area A_0, under a pressure gradient (6):

$$A_{Xf} = A_0\left[2\left(1 - \frac{a}{r}\right)^2 - \left(1 - \frac{a}{r}\right)^4\right] \cdot \left[1 - 2.104\frac{a}{r} + 2.09\left(\frac{a}{r}\right)^3 - 0.95\left(\frac{a}{r}\right)^5\right] \tag{10}$$

in which a is the effective radius of the molecule X and r the effective pore radius.

It is impossible, at the present time, to give more than an approximate estimate of the effective radius a for the smaller molecules used in these experiments. The interpretation of the experiments does not, however, depend

TABLE I
DIFFUSION COEFFICIENTS AND RADII OF TEST SOLUTES

Solute	$D_{25°}$ cm.²/sec. × 10^5	Reference	Radius used
			Å
D_2O	2.3	(10)	1.9
Urea	1.4	(11)	2.7
Glucose	0.67	(12)	4.4
Sucrose	0.52	(12)	5.3
Raffinose	0.43	(12)	6.1
Inulin	—	—	12
Bovine serum albumin	0.066	(11)	37

critically on the exact values of these radii. Consequently, an arbitrary procedure has been adopted for the estimates. In the case of heavy water, the radius has been taken to be the radius of a sphere of equal weight and density, $= (3\ M/4\pi\rho N)^{1/3}$, in which M is the molecular weight, N Avogadro's number, and ρ the density. This expression yields a value of 1.9 Å for the water radius, as listed in Table I. A value of 1 Å would have been obtained from the (incorrect) use of the Stokes-Einstein relationship, $a_{SE} = RT/6\pi\eta DN$, in which η is the viscosity of the medium and D the diffusion coefficient of the solute in that medium. In effect, the factor 6π has been replaced by a factor slightly greater than 3π, as suggested by Longsworth (7).

The value of $a_w = 1.9$ Å for the radius of the water molecule has next been used to evaluate the constant K in the quasiempirical relationship, modified from an expression derived by Gierer and Wirtz (8):

$$a = a_{SE}[(Ka_w/a) + (1 + 2a_w/a)^{-1}] \qquad (11)$$

TABLE II
PHYSICAL CHARACTERISTICS OF EXPERIMENTAL CELLULOSE MEMBRANES

	L_p cm.⁵ × 10^{11} / dyne·sec.	L_p μl. / 24.4 atmospheres·min.	$A_w/\Delta x$	r
			cm.$^{-1}$	Å
Dialysis tubing	1.7	25	15	23
Cellophane	6.4	95	17	41
Wet gel	25	370	16	82

The unit of L_p in the second column contains as the unit of pressure RT, = 24.4 atmospheres. In the third column, the area/thickness ratio for water is given per unit geometrical area of membrane. The pore radii obtained may be compared with the values obtained by Renkin (6) for other batches of similar material: dialysis tubing, 19 Å; cellophane, 31 Å; wet gel, 77 Å.

The value of K is found to be 1.57. Equation 11 may then be solved for the radius a of larger molecules, by successive approximations. The values for urea, glucose, sucrose, and raffinose in Table I have been obtained in this manner. For large molecules, Equation 11 reduces to the Stokes-Einstein equation, which has been used for bovine serum albumin. For inulin, the radius has been interpolated from the radii of raffinose and albumin, using the experimentally determined molecular weight of 3100 and the assumption that a varies as the cube root of the molecular weight.

The effective pore radius, r, has been determined for the cellulose membranes in conventional manner, by combining measurements of the rate of flow due to a hydrostatic pressure, assumed to be given by Poiseuille's law,

TABLE III
RATES OF NET VOLUME FLOW

Solute	Net volume flow (μl./ molar·min.)		
	Dialysis tubing	Cellophane	Wet gel
D_2O	0.06		0.084
Urea	0.6	0.6	1.5
Glucose	5.1	4.2	5.8
Sucrose	9.2	7.0	10.4
Raffinose	11	8.5	13
Inulin	19	41	84
Bovine serum albumin	25.5	98	270

with the rate of diffusion of labeled water through the membrane. The pore radius is given by

$$r = \sqrt{\frac{8L_p\eta}{A_w/\Delta x}} \tag{12}$$

in which

$$A_w/\Delta x = \dot{n}_{wu}\bar{v}_w/D_w \tag{13}$$

Here A_w is the pore area available to water per unit geometrical area of membrane, assumed the same for diffusion or filtration; Δx is the effective thickness of the membrane; $\dot{n}_{wu}$ is the unidirectional flow of water, obtained from the diffusion of labeled water; η and D_w are the viscosity and diffusion coefficients of water, respectively. The contribution of diffusion flow under a hydrostatic pressure has been assumed negligible in comparison to Poiseuille flow. Table II gives the results obtained for L_p, $A_w/\Delta x$, and r for the three membranes. The pore radii listed are in substantial agreement with those obtained previously by Renkin (6) for similar membranes (r_p in his notation).

It should be also noted that Renkin found good agreement between the value of r obtained from Equations (12) and (13), and the value estimated from the measurement of the rate of diffusion of a series of solute molecules, graded in size, across the membrane.

Net volume flow was measured for the solutes listed in Table I, using the

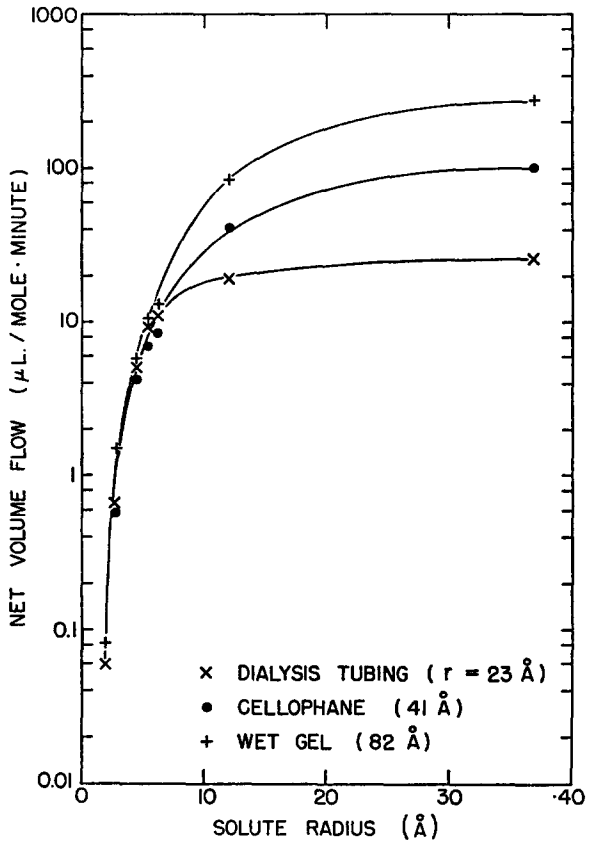

FIGURE 1. Experimentally determined rates of net volume flow, expressed in microliters per unit time and unit concentration difference of solute. The actual concentrations used are given in the text. The measured pore radius r is indicated for each membrane.

three membranes of Table II. Volume changes were recorded over several consecutive intervals, beginning just after the instillation of the test solution in the small chamber ($t = 0$). If necessary, as was the case for urea and deuterated water, the rates of flow were plotted against time, and the rate at zero time used. All rates of flow have been calculated in units of microliters per minute, per one molar concentration difference of solute, as listed in Table III. The flow rate depends on the radius of the solute molecule,

TABLE IV

STAVERMAN COEFFICIENTS AS A FUNCTION OF THE RATIO OF SOLUTE RADIUS TO PORE RADIUS

Solute	Dialysis tubing		Cellophane		Wet gel	
	a/r	σ	a/r	σ	a/r	σ
D_2O	0.083	0.002			0.023	0.001
Urea	0.117	0.024	0.066	0.006	0.033	0.004
Glucose	0.19	0.20	0.107	0.044	0.054	0.016
Sucrose	0.23	0.37	0.13	0.074	0.065	0.028
Raffinose	0.26	0.44	0.15	0.089	0.074	0.035
Inulin	0.52	0.76	0.29	0.43	0.146	0.23
Bovine serum albumin	1.6	1.02	0.90	1.03	0.45	0.73

as shown by the plot of these data in Fig. 1. For each membrane, the rate of flow tends towards a plateau at large solute radii. Since the effective area/thickness ratios are comparable for the three membranes, the increasing height of this plateau indicates the greater Poiseuille flow with larger pore radius.

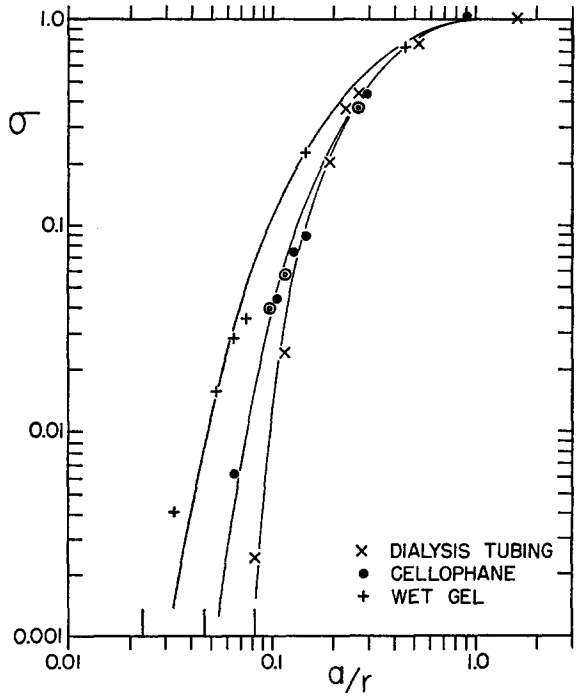

FIGURE 2. The results plotted without dimension. The abscissa is the ratio of solute radius to pore radius; the ordinate, the ratio of the measured net flow in μl./molar·minute to the hydraulic flow, calculated in μl. per minute and pressure difference of RT. The asymptotes in the lower left are the appropriate ratios of water radius to pore radius.

A comparison of the results may be conveniently made by expressing the data of Table III without dimension, dividing the solute radius by the appropriate pore radius (a/r), and the measured rate of flow by the observed hydraulic flow under the hydrostatic pressure, RT. This is the pressure equal to the van't Hoff osmotic pressure for $\Delta c_s = 1$ molar. By definition, the ratio of rates of flow so obtained is equal in absolute value to L_{pD}/L_p, or the Staverman factor σ. The results, in these units, are listed in Table IV. The dependence of σ on the ratio of solute radius to pore radius is demonstrated by

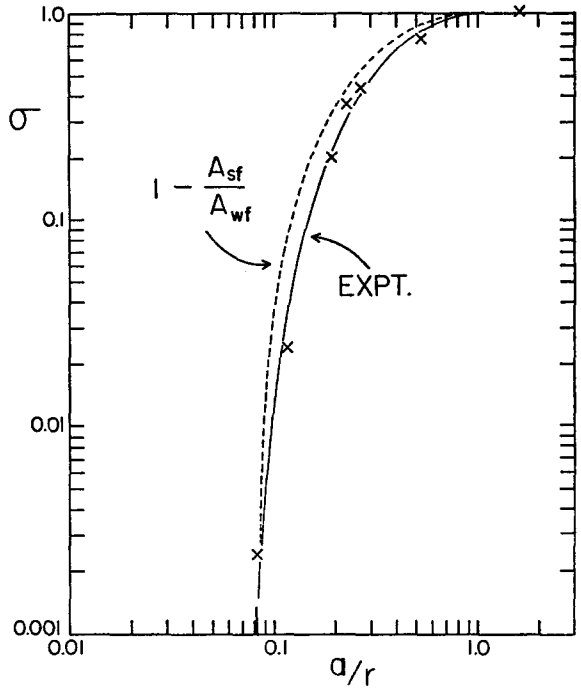

FIGURE 3. The data (crosses) and solid line correspond to the experimentally determined values of σ for dialysis tubing. The dotted line has been calculated from the expression for $\sigma = 1 - A_{sf}/A_{wf}$.

the plot of the results in Fig. 2. The passage of the three curves through the point (1, 1) is in agreement with the preceding theoretical prediction that osmotic and hydraulic pressures should produce equal volume flows across semipermeable membranes [*cf.* (13)]. As the solute radius approaches the radius of the water molecule ($a/r \rightarrow 1.9/r$), the net volume flow disappears, and log σ approaches minus infinity. The asymptotes, $1.9/r$, for the three values of pore radius are shown as short vertical lines in the lower left of Fig. 2.

Another means of obtaining an estimate of the Staverman coefficient σ is by the use of Equations 9 and 10. Having determined the effective pore

radius, one may calculate the effective pore area for water or solute from Equation 10. This has been done for a range of solutes, in the case of Visking dialysis tubing; the corresponding values of σ have been plotted in Fig. 3 as the dotted line. The experimental points and solid line refer to the observed values of σ, from Table IV. The agreement between solid and dotted lines is surprisingly good, considering the number and variety of assumptions necessary to make the comparison. Perhaps the major uncertainty lies in the application of Equation 10, derived for macroscopic tubes or pipes, to filtration on the molecular scale.

DISCUSSION

The Staverman factor σ has been determined directly for a collodion membrane (9), for the red blood cell (14), and by calculation, for the isolated cat hind limb (4, 5). Meschia and Setnikar (9) found for a collodion membrane these values of σ: glucose, 0.01; sucrose, 0.013; raffinose, 0.019; dextran (mean molecular weight 52,000), 1.0. The first three values, compared with the data of Table IV, indicate for the collodion membrane a pore radius somewhat greater than that of wet gel, perhaps 100 to 150 Å. This being so, the membrane would be permeable to the dextran used, and the corresponding value of σ, less than unity. The source of this disagreement is not known; perhaps it is due, at least in part, to inhomogeneity of the dextran used.

Kedem and Katchalsky (4) have recalculated some of the results of Pappenheimer, Renkin, and Borrero (5) to obtain σ for several solutes across the capillary membranes of the cat hind limb: glucose, 0.04; sucrose, 0.058; inulin, 0.375. A comparison of these values of σ with the results listed in Table IV suggests an effective pore radius for the capillary membrane slightly greater than that (41 Å) of uncoated cellophane. Assuming a value of 45 Å for r, the respective values of a/r may be calculated, and σ plotted for these solutes. The resulting points are shown by double circles in Fig. 2; the agreement of these points with the curve for cellophane is good. The value of 45 Å is consistent with the range of values, 30 to 45 Å, suggested by Pappenheimer for the effective pore radius (15). However, he gives a different correction factor for the van't Hoff law, $(1 - D'/D'_w)$, in which D' is the restricted diffusion coefficient of the test solute and D'_w the corresponding coefficient of water. This factor predicts a considerably smaller deviation of σ from unity than was actually observed in the present work.

The Staverman coefficient σ is useful in analyzing models of membranes which perform active transport, a point which has also been discussed by Curran (16). If secretion or absorption is nearly isoosmolar, the membrane must be readily permeable to water, yet tight enough to maintain an osmotic

gradient of transported solute. In addition, the irreversible energy loss due to back-diffusion of the transported substance can thereby be minimized. Such a hypothetical structure is illustrated in Fig. 4. It consists of two membranes, a thin membrane, M_1, supplying the solute S by means of an internal transport mechanism driven by metabolism, and a relatively thick support, M_2, which serves as a pathway for nutrients, etc. The purpose of the transport is assumed to be the production and transfer of S to solution 2; an example might be the secretion of HCl into the gastric lumen. Accordingly M_1 should

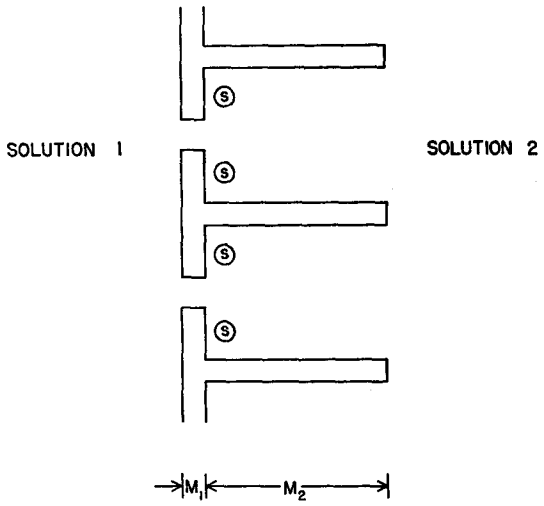

FIGURE 4. An idealized, compound membrane, secreting the solute S into solution 2.

have fine pores, with σ for S nearly unity, and M_2 coarse pores, with σ for S near zero. Under these circumstances, any osmotic gradient due to S will essentially be found across M_1. Even a small difference in concentration of S across M_1, if the thickness of the membrane were of the order of a few Ångstroms, could produce an enormous osmotic gradient and, conceivably, the required water flow.

In the steady state, the influx of water from solution 1 due to the secretion of S will result in a gradient of hydrostatic pressure across M_2, just large enough to deliver solute plus water to solution 2. If the pores of M_2 are large, no separation of solute from water occurs; the amount of pressure difference can therefore be minimal, justifying its neglect in comparison with the osmotic gradient across M_1 in the determination of the primary water flow.

Leaf (17) has pointed out that the behavior of such a duplex membrane is analogous to that of conductances in series. Thus, if the area/thickness ratio of membrane M_2 is much less than that of M_1 for water, the over-all membrane will behave as M_2 for the diffusion of labeled water. If, because

of the fine pores of M_1, M_1 has an area/thickness ratio considerably less than that of M_2 for other solute molecules, the membrane behavior is dominated by M_1 for these solutes. Additional submembranes may be introduced, either in series or in parallel, and the area to thickness ratio for a particular solute obtained for the whole membrane by a similar, simple procedure.

In summary, the model illustrates the usefulness of the Staverman coefficient σ in establishing the direction of net volume flow, and potentially, its magnitude. Moreover, like biological membranes, it has the property of readily permitting the diffusion of water while severely limiting the passage of small solutes.

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