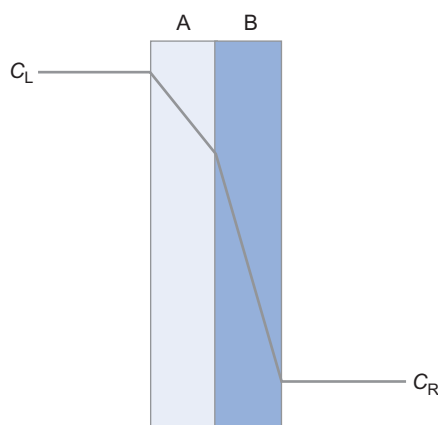


# Problem Set

## Membrane Transport 2.2

- The GLUT-1 transporter has a  $K_m$  for glucose of 1.5 mM. The normal, resting plasma glucose concentration is about 90 mg%. This is a clinical unit that is not part of the SI but you have to get familiar with it anyway. X mg% means that the solute has that many mg in a deciliter of plasma. One deciliter is 0.1 L = 100 mL. The molecular weight of glucose is  $180 \text{ g mol}^{-1}$ . From this information, calculate the rate of glucose transport by GLUT-1, as a percent of its maximum, when exposed to normal plasma. Assume saturation kinetics.
- When  $C_L$  was  $2.3 \times 10^{-6} \text{ M}$  and  $C_R$  was zero, the flux across a microporous membrane was found to be  $0.234 \text{ pmol cm}^{-2} \text{ s}^{-1}$ . The free diffusion coefficient of the material being measured was  $0.8 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ .
  - What is the permeability of the membrane to the material?
  - If the thickness of the membrane is  $10 \times 10^{-6} \text{ m}$ , what is the equivalent relative area available for diffusion of the material?
- Two membranes, A and B, have permeabilities  $P_A$  and  $P_B$  for a given solute. These two membranes are joined together to form a single composite, two-layered membrane, as shown in Figure 2.PS2.1. Examples are the successive filtration/diffusion barriers in the kidney glomerulus, successive permeability barriers in the lung, and successive permeability barriers of unstirred layers adjacent to intestinal epithelial membranes.



**FIGURE 2.PS2.1** Composite membrane formed from the sandwich of two membranes, A and B, with different characteristics.

- How would you define the overall permeability  $P$  of the two-layered membrane? *Hint:* Think about how you would define permeability for any membrane. Think about what you need to know to calculate  $P$ . Use  $C_L$  for the left concentration and  $C_R$  for the concentration on the right.
  - Why is the steady-state solute flux through the composite membrane the same through the membrane A layer and the membrane B layer? *Hint:* Think of the continuity equation and what it means.
  - What is the concentration profile through the composite membrane? *Hint:* Calculate the concentration at the interface of membranes A and B; call it  $C_m$ , in terms of  $C_L$  and  $C_R$ . *Hint:* Equate the flux through the two membranes.
  - Find an expression for  $P$  in terms of  $P_A$  and  $P_B$ .
  - Do the permeabilities act like inverse resistances in a series arrangement?
- When  $C_L$  was  $2.3 \times 10^{-6} \text{ M}$  and  $C_R$  was zero, the flux across a microporous membrane was found to be  $0.234 \text{ pmol cm}^{-2} \text{ s}^{-1}$ . This flux was determined with vigorous stirring, which virtually eliminated any unstirred layers adjacent to the membrane. When the stirrer was turned off, the flux decreased to  $0.157 \text{ pmol cm}^{-2} \text{ s}^{-1}$ . The free diffusion coefficient of the material being measured was  $0.8 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ .
    - What is the permeability of the membrane plus unstirred layer?
    - What is the permeability of the membrane alone?
    - Using the information in Problem #3, what is the thickness of the unstirred layer? (Assume that the diffusion coefficient in the unstirred layer is equal to the free diffusion coefficient.)
  - The surface area of the lungs is about  $75 \text{ m}^2$ , and the thickness of the alveolar diffusion layer is about  $0.5 \mu\text{m}$ . The  $P_{\text{O}_2}$  in alveolar air is 100 mmHg, while the  $P_{\text{O}_2}$  of venous blood is 40 mmHg. The diffusion coefficient of  $\text{O}_2$  in water is about  $1.5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ . The solubility of  $\text{O}_2$  is given by Henry's Law as  $[\text{O}_2] = 0.024 P_{\text{O}_2}$ ; here  $[\text{O}_2]$  is expressed in mL  $\text{O}_2$  at STPD (standard temperature and pressure, dry:  $0^\circ\text{C}$  and 1 atm pressure) per mL of water and  $P_{\text{O}_2}$  is in

atmospheres. Only the dissolved  $O_2$  diffuses. What is the initial rate of  $O_2$  diffusion from the aggregate alveoli to the blood? (Initial rate means to pretend that the venous  $P_{O_2}$  is clamped at 40 mmHg.) Give the answer in mL  $O_2$  per minute and in mol  $O_2$  per minute using the Ideal Gas Law. Assume  $37^\circ\text{C}$ ,  $R = 0.082 \text{ L atm mol}^{-1} \text{ K}^{-1}$ . Remember that  $1 \text{ atm} = 760 \text{ mmHg}$ .

6. Heart cells contain a Na–Ca exchanger with a stoichiometry of  $3\text{Na}:1\text{Ca}$ . The following questions pertain to this transporter.
- A. The free energy of transport of  $\text{Ca}^{2+}$  across the sarcolemma of the heart cell can be calculated from the following conditions during the rest phase of the heart beat:

$$[\text{Ca}^{2+}]_o = 1.2 \times 10^{-3} \text{ M}$$

$$[\text{Ca}^{2+}]_i = 0.1 \times 10^{-8} \text{ M}$$

$$E_m = -0.085 \text{ V}$$

Calculate the free energy for the reaction  $\text{Ca}_{\text{out}} \rightarrow \text{Ca}_{\text{in}}$ . Recall that  $R = 8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ,  $T = 310 \text{ K}$ ,  $F = 9.649 \times 10^4 \text{ C mol}^{-1}$ . Remember that  $\text{Ca}^{2+}$  has two electrical charges per atom.

- B. Calculate the free energy for the reaction  $\text{Na}_{\text{in}} \rightarrow \text{Na}_{\text{out}}$  for the following conditions during the rest phase of the heart:

$$[\text{Na}^+]_o = 145 \times 10^{-3} \text{ M}$$

$$[\text{Na}^+]_i = 14 \times 10^{-3} \text{ M}$$

$$E_m = -0.085 \text{ V}$$

- C. Which way does the Na–Ca exchange proceed at rest?

7. Ischemia refers to the condition of no blood flow. When the artery perfusing an area of tissue is blocked, oxygen can no longer be delivered to support energy metabolism. Under these conditions, the ATP concentration falls and ADP and  $\text{P}_i$  concentrations rise. What will this do to the free energy of ATP hydrolysis (also called the **affinity** of ATP hydrolysis)? What do you think will be the consequence of ischemia on the effectiveness of the ion pumps? (Kammermeir, Schmidt, and Jungling, Free energy change of ATP hydrolysis: a causal factor of early hypoxic failure of the myocardium? *J. Mol. Cell. Cardiol.* 14:267–277, 1982).
8. The membrane of the sarcoplasmic reticulum (an internal membrane in muscle cells) has multiple ion channels, so the membrane potential across this membrane is believed to be zero. This membrane has a Ca-ATPase pump that links two  $\text{Ca}^{2+}$  atoms to the hydrolysis of ATP. Using a free energy of ATP hydrolysis of  $-57.1 \text{ kJ mol}^{-1}$ , what is the thermodynamic limit of  $\text{Ca}^{2+}$  accumulation if the free  $[\text{Ca}^{2+}]$  on the cytosolic face is  $1 \times 10^{-7} \text{ M}$ ? (*Hint*: The thermodynamic limit is when the free energy change for the transport reaction is zero.)
9. Each of the reactions shown in Eqn [2.5.15] has a forward and reverse rate constant. Show that

for a passive transport process the product of all the forward rate constants is equal to the product of all the reverse rate constants. (*Hint*: Use a principle called **detailed balance**, which states that at equilibrium all steps in a reaction sequence must also be at equilibrium.)

10. Consider that a membrane separates two compartments, each containing a solution of 10 mL. The left compartment has an initial concentration  $C_L$ . The membrane has permeability  $p$  to the solute and area  $A$ .
- A. Derive an expression for  $C_L$  and  $C_R$  as a function of time.
- B. Suppose that you obtained experimental values for  $C_L(t)$  or  $C_R(t)$ . What plot of the data could you make to determine  $p$ ?
- C. What would happen if we doubled both the volume and the surface area to the time course of equilibration of  $C_L$  and  $C_R$ .
11. The surface area of the lungs is about  $75 \text{ m}^2$ , and the thickness of the alveolar diffusion layer is about  $0.5 \mu\text{m}$ . The  $P_{O_2}$  in alveolar air is 100 mmHg, while the  $P_{O_2}$  of venous blood is 40 mmHg. The diffusion coefficient of  $O_2$  in water is about  $1.5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ . The solubility of  $O_2$  is given by Henry's Law as  $[\text{O}_2] = 0.024 P_{O_2}$ ; here  $[\text{O}_2]$  is expressed in mL  $O_2$  per mL of water and  $P_{O_2}$  is in atmospheres. Only the dissolved  $O_2$  diffuses. The volume of blood in the lung is 70 mL, and the volume of air at the end of normal inspiration volume is about 2.8 L (at body temperature of  $37^\circ\text{C}$ ).
- A. Derive an equation for the time course of oxygen equilibration between blood and air in the lungs.
- B. Using the values given here, estimate the half-time of equilibration.
- C. Most of the oxygen in the blood is not free but is bound to hemoglobin within the red blood cells. Do you think this would accelerate or decelerate the rate of equilibration of blood and air oxygen pressures?
12. Vesicles of the sarcoplasmic reticulum have embedded in their membrane an active Ca-ATPase pump. Several different isoforms of this primary active pump are expressed in different tissues. When exposed to ATP, Mg, and Ca,  $\text{Ca}^{2+}$  ions are accumulated and eventually reach a steady-state uptake. If the pump is quickly quenched by adding extravesicular EGTA, which complexes activator  $\text{Ca}^{2+}$  and thereby stops the pump, the accumulated  $\text{Ca}^{2+}$  will leak back out. Monitoring the intravesicular  $\text{Ca}^{2+}$  with time allows one to estimate the permeability of the vesicles. Derive an expression for the amount of  $\text{Ca}^{2+}$  remaining in the vesicle as a function of time and suggest a plot to determine the permeability. What other information might you need to know to determine the permeability?
13. The osmotic coefficient for  $\text{CaCl}_2$  under physiological conditions is 0.85. Calculate the osmotic pressure of a solution of 10 mM  $\text{CaCl}_2$  at  $37^\circ\text{C}$ .

Recall here that  $R = 0.082 \text{ L atm mol}^{-1} \text{ K}^{-1}$ . Give the answer in both atm and mmHg (1 atm = 760 mmHg).

14. The kidney filters plasma to produce an **ultrafiltrate**, which is the first step in the formation of urine. This filtrate is called an ultrafiltrate because the kidney can retain even small particles like plasma proteins. The force behind ultrafiltration is the blood pressure. The kidney ultrafiltration occurs at a structure called the **glomerulus**, which is a group of small blood vessels (capillaries) that are closely joined to another structure, **Bowman's capsule**, that forms a double-walled cup for the collection of the ultrafiltrate. The filtration barrier is a combination of the capillary walls and structure in Bowman's capsule.
  - A. Calculate the filtration coefficient ( $L_p$ ) for the basement membrane of kidney glomeruli using the following approximations:
    1. Pore radius = 35 Å
    2. Pore length = 600 Å
    3. Fractional pore area = 5%
    4. Blood plasma viscosity = 0.02 poise ( $\text{dyne s cm}^{-2}$ )
 The "fractional pore" is the total area of the pores divided by the total area of the membrane. A dyne is a  $\text{g cm s}^{-2}$ .
  - B. Calculate the glomerular filtration rate (GFR) assuming a total area for both kidneys of  $1.5 \text{ m}^2$  and a driving force  $\Delta P = 20 \text{ mmHg}$ . The GFR is the total volume of ultrafiltrate produced per minute. Its units should be in  $\text{cm}^3 \text{ min}^{-1}$ . Make it so.
15. The observed osmotic pressure of solutions of plasma proteins increases more rapidly than concentration. Empirical fits to the concentration dependence of osmotic pressure are given by Landis and Pappenheimer (*Handbook of Physiology*, vol 2, section 2, pp. 961–1034, 1963):

$$\pi_{\text{albumin}} = 2.8C + 0.18C^2 + 0.012C^3$$

where  $\pi$  is in units of mmHg and  $C$  is in units of g% (i.e., g of protein per deciliter of plasma).

- A. According to this equation, as  $C$  becomes more dilute the relation approaches van't Hoff's Equation. Keeping in mind the units of the variables, what is the molecular weight of albumin? Assume that the temperature is  $37^\circ\text{C}$ . (By the way, osmotic measurements were the first measurements of protein molecular weights.)
- B. What is the contribution of albumin to the osmotic pressure of plasma when it contains 4.0 g% of albumin?
- C. The osmotic pressure of plasma proteins and associated ions is called the **oncotic pressure**. If the plasma oncotic pressure is 25 mmHg, how much of the oncotic pressure is contributed by globulins, fibrinogen, and other components?

16. Assume that serum albumin is a sphere of diameter 31 Å. Assume that the glomerular membrane is pierced by pores of equivalent diameter of 35 Å.
  - A. Give an estimate of  $\sigma$  for albumin for the glomerular membrane.
  - B. Calculate the concentration of albumin in the ultrafiltrate.
  - C. If the GFR is  $120 \text{ mL min}^{-1}$ , calculate the daily filtered load of albumin (how much is filtered every day). How does this compare with the recommended dietary intake of 0.8 g protein per kg body weight per day?
17. The value of  $L_p$  for the red blood cell is about  $1.8 \times 10^{-11} \text{ cm}^3 \text{ dyne}^{-1} \text{ s}^{-1}$ . Its surface area is about  $1.35 \times 10^{-6} \text{ cm}^2$  (Solomon, *Methods in Enzymology*, pp. 192–222, 1989).
  - A. What is the initial osmotic flow if the osmolarity inside is initially 300 mOs M and the osmolarity outside is 275 mOs M? (Assume  $\sigma = 1.0$  for all solutes.)
  - B. If the volume of the cell is  $100 \times 10^{-12} \text{ cm}^3$ , how long would it take to double its volume provided that the osmotic pressure and area of the membrane and  $L_p$  did not change?
  - C. In the case described, how much water would be required to enter the cell to equilibrate the osmotic pressure between inside and outside? Assume that the outside bath is essentially infinite so that its osmotic pressure is kept constant.
18. Osmotic pressure is one of a class of properties of solutions that are called **colligative properties**. The others in this class include **vapor pressure depression**, **boiling point elevation**, and **freezing point depression**. These properties are different expressions of the same phenomenon: the lowering of the activity of water by dissolution of solutes. Various osmometers have been made using one or another of these properties. Table 2.PS2.1 shows several solutions of sucrose and glucose, their water concentrations,

**TABLE 2.PS2.1** Solute Concentration, Water Concentration, and Freezing Point Depression in Sucrose and Glucose Solutions

Sucrose Solutions			Glucose Solutions		
[Sucrose] (M)	[Water] (M)	$\Delta$ ( $^\circ\text{C}$ )	[Glucose] (M)	[Water] (M)	$\Delta$ ( $^\circ\text{C}$ )
0	55.45	0	0	55.46	0
0.029	55.12	0.06	0.028	55.28	0.05
0.059	54.77	0.11	0.056	55.11	0.10
0.081	54.42	0.16	0.084	54.94	0.16
0.118	54.07	0.23	0.112	54.76	0.21
0.179	53.72	0.35	0.140	54.59	0.27

Source: Data from Handbook of Chemistry and Physics, CRC Co, Cleveland, OH, 1965.

and their freezing point depression. Because dissolving solutes invariably dilutes the solvent, water, you can see from the table that the water concentration also decreases with increasing solute concentration. Sucrose, however, is twice as large as glucose, so we might expect that dissolution of sucrose would dilute the water further. Therefore, the colligative properties of solutions cannot be proportional to both solute and solvent concentration. Plot separately the freezing point depression against the water concentration and against the solute concentration. Which relationship shows the same dependence? From this result, do you expect osmosis to be dependent on solvent water concentration or on solute concentration?

19. From the data in Table 2.PS2.1, calculate the coefficient relating freezing point depression to molarity in the equation:

$$[2.PS2.1] \quad T_f - T = \Delta = k_f C$$

where  $T_f$  is the freezing point of the pure liquid,  $T$  is the freezing point of the solution,  $k_f$  is the coefficient, and  $C$  is the concentration.

20. Chapter 2.7 gives us several expressions for the osmotic pressure. The most complete is derived from Eqn [2.7.6] and is

$$[2.PS2.2] \quad P_L - P_R = \pi = \frac{RT}{\bar{V}_w} \ln \frac{a_{\text{pure water}}}{a_{\text{solution}}}$$

Here the subscripts L and R refer to the left and right sides of the semipermeable membrane, respectively, where pure water is on the right side. If the solution was ideal, we can use the mole fraction for the activity and this equation becomes

$$[2.PS2.3] \quad \pi = -\frac{RT}{\bar{V}_w} \ln X_w$$

In the further approximation of a dilute solution, this equation was transformed to

$$[2.PS2.4] \quad \pi = RT C_s$$

The activity of water in Eqn [2.PS2.2] is measured by the vapor pressure. Table 2.PS2.2 tabulates the calculations of the osmotic pressure from the vapor pressure measurements, the mole fraction of water and from the concentration of solute. Plot the ratio of each of the observed osmotic pressures to the calculated osmotic pressure, in columns 3, 4, and 5, against the sucrose concentration. The values you calculate for the ratio of the observed  $\pi$  to the calculated  $\pi$  in column 3 is  $\varphi (= \pi/RT \ln C_s)$ , the osmotic coefficient. The ratio of the observed  $\pi$  to column 4 (i.e.,  $(\pi/[-RT/\bar{V}_w \ln x_w])$ ) defines the **rational osmotic coefficient**,  $g$ .

- A. Why is the equation using the mole fraction of water a better predictor of the osmotic pressure than the van't Hoff Equation?

**TABLE 2.PS2.2** Values for the Observed Osmotic Pressure and the Osmotic Pressure Calculated from the van't Hoff Equation, from the Mole Fraction of Water, and from the Measured Vapor Pressure of Solutions

[Sucrose] (M)	Observed $\pi$ (atm)	$RT C_s$ (atm)	$-RT/\bar{V}_w \ln x_w$ (atm)	$RT/\bar{V}_w \ln (P_o/P_s)$ (atm)
0.098	2.47	2.40	2.48	2.50
0.824	27.2	20.4	24.6	26.6
1.399	58.4	35.1	48.8	57.3
1.823	95.2	45.5	72.6	93.2
2.146	139.0	55.7	96.0	135.6
2.55	187.3	64.5	118.9	186.5

Source: Data from Glasstone, Textbook of Physical Chemistry, van Nostrand, 1946.

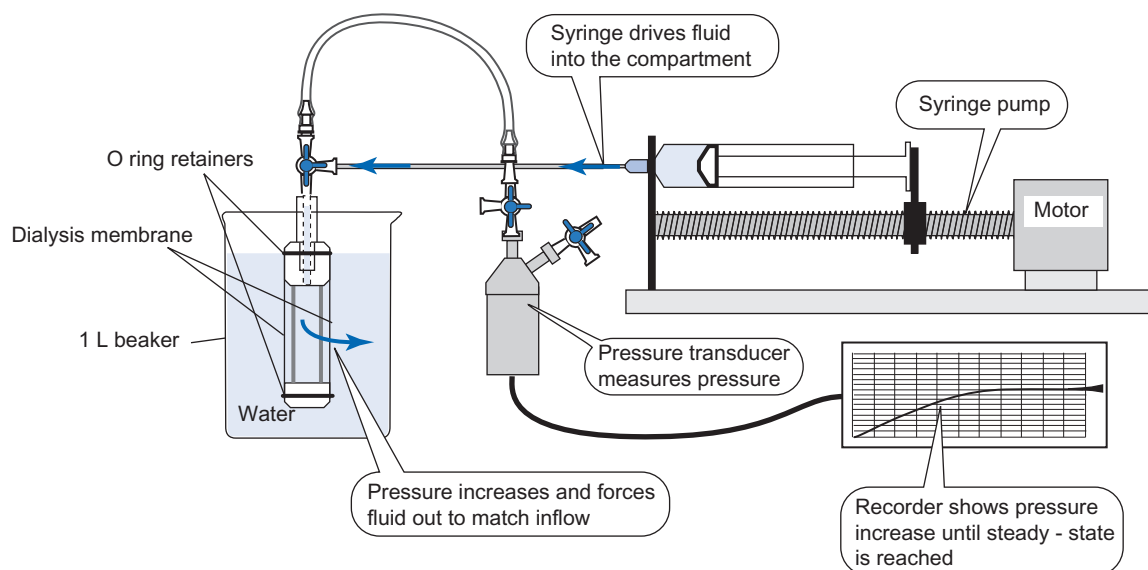
**TABLE 2.PS2.3** Molecular Weight and Concentration of Plasma Proteins.

Protein	Average Molecular Weight	Concentration in Plasma (g dL <sup>-1</sup> )
Albumin	69,000	4.2
Fibrinogen	330,000	0.3
Immunoglobulin G	150,000	0.8
Immunoglobulin M	750,000	0.3
$\alpha_2$ Globulins	100,000	0.7
$\alpha_1$ Globulins	50,000	0.5
$\beta$ Globulins	100,000	0.8

- B. Why is the equation using the vapor pressure a better predictor of the osmotic pressure than the equation using the mole fraction of water?

- C. Are these methods good predictors of the osmotic pressure in dilute solutions?

21. Krebs–Henseleit buffer has the following composition: 119 mM NaCl, 25 mM NaHCO<sub>3</sub>, 3.2 mM KCl, 1.2 mM MgSO<sub>4</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 11 mM glucose, and 1.4 mM CaCl<sub>2</sub>. Calculate its osmolarity and osmotic pressure, at 37°C, assuming  $\varphi = 1$  for all solutes. Would you expect this solution to be hypertonic, hypotonic, or isotonic to mammalian cells?
22. Plasma contains a variety of proteins that exert osmotic pressure. A list of these, with their molecular weights and approximate protein concentrations, is given in Table 2.PS2.3. From these data, calculate the approximate osmotic pressure in plasma that is due just to plasma proteins.
23. A. The device shown in Figure 2.PS2.2 was used to determine the steady-state flow and pressure across a dialysis membrane. The data



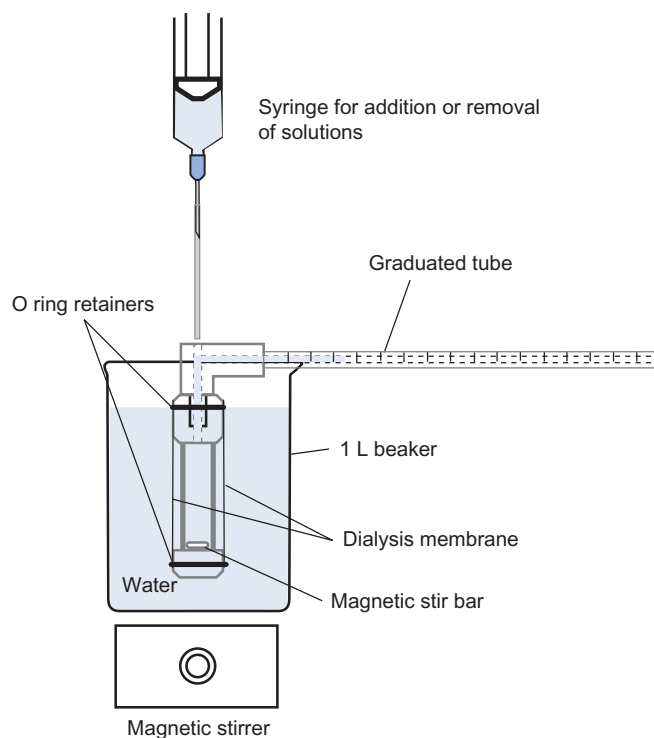
**FIGURE 2.PS2.2** Device used to determine  $L_p$ . Water was injected into the inner chamber at a known rate using a syringe pump. This increases the pressure within the chamber and forces fluid out through the membrane. The pressure increases as more water is injected and eventually a steady state is reached in which the rate of injection matches the rate of filtration through the membrane. The steady-state pressure is measured continuously by a pressure transducer and recorded.

**TABLE 2.PS2.4** Pressure at Steady-State Flow Across a Dialysis Membrane

Flow Rate ( $\text{cm}^3 \text{min}^{-1}$ )	Pressure (mmHg)
0	0
0.0097	180
0.0194	360
0.0388	680

that were obtained are given in Table 2.PS2.4. The area of the membrane that was exposed to flow was  $90.5 \text{ cm}^2$ . Determine  $L_p$  for the membrane.

- B. The membrane in part A was used to separate pure water on the outside from a  $0.75 \text{ M}$  sucrose solution on the inside. The flow across the membrane was measured using the device shown in Figure 2.PS2.3.
24. When vesicles of the cardiac sarcoplasmic reticulum (CSR) are incubated with ATP,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ , they take up  $\text{Ca}^{2+}$  and reach a pseudo steady state. This is a steady state that changes, but slowly. The uptake of  $\text{Ca}^{2+}$  is mediated by the SERCA2a Ca-ATPase. The uptake reaction can be quenched by adding EGTA to the external solution, which binds the  $\text{Ca}^{2+}$  outside of the vesicles, or by adding glucose plus hexokinase, that converts the ATP to ADP and glucose-6 phosphate. When the uptake reaction is stopped,  $\text{Ca}^{2+}$  that was already taken up by the vesicles leaks out passively.
- A. The amount of  $\text{Ca}^{2+}$  taken up by the vesicles is generally normalized to the amount



**FIGURE 2.PS2.3** Device for measuring osmotic flow at constant  $\Delta P = 0$ . The inner compartment could be drained and then filled with various experimental solutions. The outer compartment contained pure water. As fluid enters the compartment across the dialysis membrane as a result of the osmotic pressure difference, it forces fluid down the horizontal tube without any increase in the hydrostatic pressure. The rate of fluid flow can be estimated from the rate of fluid movement down the tube.

of CSR protein in  $\text{mg}$  rather than being expressed as a concentration. A typical steady-state  $\text{Ca}^{2+}$  uptake is  $40 \text{ nmol mg}^{-1}$ . In separate experiments, the enclosed



- volume of the CSR vesicles was determined to be  $5 \mu\text{L mg}^{-1}$ . What is the approximate concentration of  $\text{Ca}^{2+}$  inside the vesicles at steady state?
- B. The average vesicle size determined by electron microscopy is about 150 nm. What is the volume and surface area of a vesicle this size, assuming it is a sphere?
- C. Given that the enclosed volume of the aggregate vesicles is  $5 \mu\text{L mg}^{-1}$ , how many vesicles are there per mg of CSR protein? How much surface area is there per mg of CSR protein?
- D. The initial passive efflux at a load of  $40 \text{ nmol mg}^{-1}$  when the pump is stopped is  $16 \text{ nmol min}^{-1} \text{ mg}^{-1}$ . Convert this to a flux in units of  $\text{nmol cm}^{-2} \text{ s}^{-1}$  by dividing by the surface area per mg of CSR protein and converting min to s.

E. From the information in part D, What is the passive permeability to  $\text{Ca}^{2+}$  in units of  $\text{cm s}^{-1}$ ?

25. Oral Rehydration Solution used for Oral Rehydration Therapy, as recommended by WHO/UNICEF, has the following composition:
- 2.6 g NaCl
  - 2.9 g  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2 \text{ H}_2\text{O}$  (trisodium citrate dihydrate)
  - 1.5 g KCl
  - 13.5 g glucose
- per L of solution.

Calculate the osmolarity of this solution. Trisodium citrate will dissociate fully at neutral pH to 3  $\text{Na}^+$  ions and one citrate $^{-3}$  ion. Assume complete dissociation of NaCl and KCl.