

FILTRATION, DIFFUSION, AND MOLECULAR SIEVING THROUGH POROUS CELLULOSE MEMBRANES*

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INTRODUCTION

In two recent publications (1, 2) Pappenheimer and his coworkers have developed a theory to describe restricted diffusion and molecular sieving through the walls of living capillaries. In view of the importance of this theory to the study of both biological and artificial membranes, it seems necessary to provide additional experimental evidence of its validity. Such is the aim of this paper. Measurements were made of ultrafiltration rates, molecular sieving during ultrafiltration, and diffusion rates of a variety of molecular species through inert porous membranes. Experimental results were compared with predictions based on the theory. Estimates derived thereby of membrane pore radii and membrane diffusion areas per unit path length were checked for internal consistency and compared with estimates obtained by the well known ultrafilter membrane calibration method of Elford and Ferry (3, 4) and the less widely known method of Manegold (5). Predictions based on the diffusion-filtration theory of Pappenheimer *et al.* were found to agree closely with experimental results, and to yield consistent values of pore radii and diffusion areas per unit path length. In contrast, estimates of pore radius based on the widely used calibration method of Elford and Ferry were found to be greatly in error.

Materials and Methods

General

1. *Diffusion.*—The diffusion rates of tritium-labelled water, urea, glucose, antipyrine, sucrose, raffinose, and hemoglobin through three types of cellulose membranes were measured. From these rates, the apparent diffusion area per unit path length ($A/\Delta x$) for each solute diffusing through each membrane was computed according to Fick's law:

$$\frac{dn}{dt} = D \frac{A}{\Delta x} \Delta c \quad (1)$$

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in which dn/dt is the diffusion rate, Δc is the concentration difference across the membrane, and D the free diffusion coefficient of the solute in the solvent which fills the pores of the membrane.

2. *Ultrafiltration*.—Water and aqueous solutions of urea, glucose, sucrose, maltose, raffinose, and hemoglobin were filtered under hydrostatic pressure through the same membranes. Filtration rates and ultrafiltrate compositions were measured. The sieving effect of ultrafiltration is described as the sieve coefficient (c_2/c_1), the ratio of the solute concentration of the filtrate to that of the filtrand.

3. *Other physical measurements* made on the membranes include thickness and water content. When combined with data on the filtration of water, these figures permit estimation of membrane diffusion areas and pore dimensions by the methods of Elford and Ferry and of Manegold.

Details

1. *Materials*.—(a) Visking "nojax" cellulose sausage casing (Visking Corporation, Chicago) obtained from the manufacturers as rolls of seamless tubing $2\frac{7}{8}$ inches in diameter. (b) Du Pont uncoated cellophane sheet, 450-PT-62 (kindly provided by Mr. W. G. Hunter, Film Department, E. I. du Pont de Nemours and Company, Wilmington). (c) Viscose wet gel, 300 weight (Sylvania Division, American Viscose Corporation, Fredericksburg). The first two materials were cut into discs of the appropriate size, and soaked in water before use. Viscose wet gel came immersed in water containing a preservative; discs were soaked in fresh water before use.

2. *Diffusion*.—Fig. 1 *a* is a diagram of the diffusion cells used. The membrane was clamped between the two chambers, which were kept well stirred by magnetically rotated steel rods. The stirrer in the upper chamber rested on the membrane but appeared to cause no damage. No thermostat was used; the temperature of the cells remained at $25 \pm 1^\circ\text{C}$. in an air-conditioned room. The lower chamber was filled with water, and at zero time, a dilute solution of the test solute was added to the upper chamber. After $\frac{1}{2}$ to 4 hours (17 hours for the single measurement on hemoglobin) the experiment was ended and samples of fluid from each chamber were taken for analysis. Values of $(A/\Delta x)$ were computed by means of the following equation:

$$\frac{A}{\Delta x} = \left(\frac{v_1 v_2}{v_1 + v_2} \right) \frac{2.3}{Dt} \log \frac{c_0}{c_0 - c_2(1 - v_2/v_1)} \quad (2),$$

which is an integrated solution of equation (1) for the geometry of the diffusion cell (6); v_1 is the volume of the upper chamber, v_2 that of the lower, t is the duration of the experiment in seconds, D is the free diffusion coefficient of the molecular species in water at the experimental temperature, c_0 the initial solute concentration in v_1 , c_2 the final solute concentration in v_2 . The final concentration of solute in v_1 was measured as a check. In a number of instances, measurements on a single substance were made at different c_0 's, and over widely different t 's; no significant variations in $(A/\Delta x)$ were observed.

The concentrations of test solutes, and the methods used for their analysis are

as follows: (a) tritiated water, sp. act. $0.1 \mu\text{c./ml.}$, analysis by internal G.M. counting of liberated tritiated hydrogen (the labelled water was supplied and analyses carried out through the courtesy of Dr. E. Stickley, of the Medical Department at Brookhaven); (b) urea, 20 mM/l. , analysis by the micro-method of Conway and O'Malley (7); (c) glucose, 0.2 and 20 mM/l. , method of Folin and Malmros (8); (d) antipyrine 5.3 mM/l. , analysis by direct spectrophotometry at $255 \text{ m}\mu$ (9); (e) sucrose, 0.2 and 2.0 mM/l. , method of Schreiner (10); (f) raffinose, 2.0 mM/l. , analysis as for sucrose; (g) hemoglobin, 0.5 per cent in 0.9 per cent saline, prepared from human red cells by the method of Hamilton *et al.* (11), and analyzed by direct colorimetry as HbO_2 (saline was used in the solvent chamber in this experiment).

3. *Filtration.*—Fig. 1 *b* is a diagram of the ultrafiltration apparatus. The chamber was of stainless steel, and had a capacity of 100 ml. The membrane was supported on a piece of filter paper on a perforated metal plate; this arrangement permits filtra-

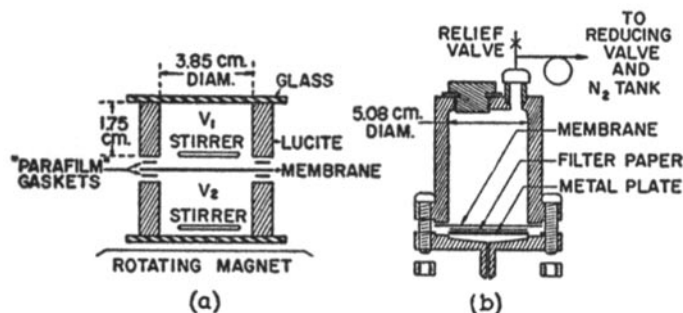


FIG. 1. (a) Diagram of diffusion cell, and (b) of ultrafiltration chamber.

tion through the entire area of the membrane. To wash out the dead space of the collecting funnel, which was 1.7 ml. , 5 ml. were allowed to flow from the filter in each experiment before samples were taken. Pressure was applied to the fluid in the chamber by means of a nitrogen tank and reducing valve, and was measured by a calibrated gauge. The fluid in the chamber was stirred by mechanical shaking. Ultrafiltrates were collected in graduated tubes, and rates of flow were measured with a stopwatch. The apparatus was kept at room temperature, $25 \pm 1^\circ\text{C.}$

Filtration rates of water over a range of pressures were measured for each membrane. At pressures below 3×10^6 and $6 \times 10^6 \text{ dynes/cm.}^2$, the relation between flow and pressure was linear, and the slope of the line is defined as the filtration coefficient of the membrane (K_f). 5 per cent aqueous solutions of the test materials were filtered at various rates, and the sieve coefficients measured. Concentrations were determined from densities measured by the falling-drop method of Barbour and Hamilton (12). In four experiments, 2.0 mM/l. solutions of sucrose were filtered and analyzed as described under Diffusion, above. Hemoglobin solutions ranging from 0.2 to 1.0 per cent in 0.9 per cent saline were also filtered, with analysis as described under Diffusion.

In order to correct for the change in filtrand concentration during molecular sieving, the following experimental procedure was used: (1) About 50 ml. 5 per cent

solution was placed in the chamber. (2) Pressure was applied, shaking begun, and 5 ml. collected and discarded. (3) The chamber was opened and sample 1 removed (1 or 2 ml.). (4) Pressure was applied again, the shaker turned on, and four samples of ultrafiltrate collected (1 to 2 ml. each). (5) The chamber was opened and sample 6 was taken. From samples 1 and 6, the concentrations of the filtrand at the mid-points of each collection period were computed, and used with the concentrations of the samples to obtain four values for the sieve coefficient. The average was then taken as a single experimental point. Variations of c_2/c_1 during such an experiment were small and irregular.

4. *Other Physical Measurements.*—(a) The thickness (d) of wet membranes was measured with a vernier micrometer equipped with a ratchet to insure uniform pressure. (b) Water content (S) was taken as the difference between the weight of the wet, blotted membrane and the same dried 2 to 4 hours at 105°C. It is expressed as the fractional volume of the wet membrane made up of water.

Geometrical Approximations

For mathematical simplicity, the pores in the membranes are assumed to be uniform cylinders, and the diffusible molecules spherical in shape (2, 4). The actual structure of cellulose membranes is presumably a thick fibrous meshwork, the thickness of which is over one thousand times the width of the interstices between the fibers (see values in Table II). These interstices are filled with solvent, and form irregular anastomosing channels from one surface of the membrane to the other, the pathways by which diffusion and ultrafiltration take place. The obvious oversimplification of the assumed uniform geometry must be kept in mind in comparing experimental results with theory. Geometrical idealizations other than cylindrical pores are possible (5) but appear to have no advantage over those used here. Electron micrographs published by Bugher (13) show general agreement between the size of the real channels and the calculated radii of their cylindrical equivalents.

The simplest estimate for the radius of a molecule is the radius of a sphere of equal weight and density (a_0):

$$a_0 = \sqrt[3]{\frac{3M}{4\pi\rho N}} \quad (3)$$

in which M is the gram molecular weight, N is Avogadro's number, and ρ is the density of the substance. Another estimate is the radius of a sphere which would have the same free diffusion coefficient as the given molecule (a_e), as calculated by the Stokes-Einstein equation (6):

$$a_e = \frac{RT}{6\pi\eta DN} \quad (4),$$

in which R is the gas constant, T the absolute temperature, η the viscosity of the solvent, and D the free diffusion coefficient of the molecule in the solvent.

This equation is valid only for solute molecules much larger than the solvent molecules. For solute and solvent molecules of comparable size, a correction of equation (4) has been derived by Gierer and Wirtz (14):

$$a'_s = \left(1.5 \frac{a}{b} + \frac{1}{1 + a/b} \right) a_s \quad (5),$$

b is the radius of the solvent molecules and a that of the solute. This equation may be solved graphically, or by successive approximations.

The three estimates of molecular radius, and the data from which they were calculated are listed in Table I. For the smallest molecules, uncorrected Stokes-

TABLE I
Estimation of Molecular Dimensions
See text for explanation.

Substance	M	ρ (15)	D_{25° (16)	Calculated molecular radius			Molecular radius used
				a_0	a_s	a'_s	
	gm./mol.	gm./cm. ³	cm. ² /sec. $\times 10^5$	A	A	A	A
H ₂ O	18	1.000		1.92			1.97
H ³ HO	20		2.36 (17)		1.01	2.02	
Urea	60	1.335	1.45	2.61	1.68	2.79	2.70
Glucose	180	1.544	0.68	3.59	3.55	4.75	3.57
Antipyrine	188	1.19	0.65*	3.96			3.96
Sucrose	342	1.588	0.55	4.40	4.40	5.55	4.40
Maltose	342	1.540		4.44			4.44
Raffinose	594	1.465	0.42	5.43	5.85	6.95	5.64
Hemoglobin (18)	67,000	1.34	0.078	27.2	30.8	30.8	30

* Estimated from molecular weight.

Einstein radii (a_0) are too low, while the corrected radii (a_s) for the larger molecules are too high. For each molecule, however, two of the three estimates are nearly alike, and the average of these has been selected for use in the present work. Comparison of the selected radii with estimates based on viscosity measurements and crystallographic data (1) generally shows good agreement.

RESULTS

1. *Estimation of Pore Sizes.*—On the assumption that the pores in a membrane are all perpendicular to the surface, and that flow through the pores follows Poiseuille's law, Guérout (19) proposed the following equation to determine pore radius:

$$r_p = 2 \sqrt{\frac{2K_F \eta d}{S}} \quad (6).$$

This equation has been used extensively to estimate effective pore radius in membranes used for particle size determinations (3, 4, 13, 20). S is the water content of the membrane, K_F the filtration coefficient, η the viscosity of water, and d the membrane thickness. The subscript e is used to identify this particular estimate. Bjerrum and Manegold assumed that the pores were oriented randomly with respect to the plane of the membrane (5). The mean pore length (Δx) is then equal to $3d$, and the equation becomes:

$$r_m = 2 \sqrt{\frac{6K_F \eta d}{S}} = r_e \sqrt{3} \quad (7),$$

in which m is used as a distinguishing subscript.

Part of the water in a membrane may be adsorbed to the cellulose fibers, or trapped in blind pores; and because of anastomoses between the pores, the mean Δx may lie somewhere between d and $3d$. These difficulties may be avoided by substituting for S/d in the above equations $(A/\Delta x)_w$ which can be measured by the diffusion of isotope-labelled water. Pappenheimer *et al.* (1) give the following equation:

$$r_p = 2 \sqrt{\frac{2K_F \eta}{(A/\Delta x)_w}} \quad (8).$$

This estimate of pore radius is essentially independent of the preceding two. Values of r_e , r_m , and r_p for each membrane are listed in Table II, which also gives the data from which they were calculated.

Discussion.—It is to be noted that r_e is considerably smaller than r_p , r_m , and the other values of r in Table II. (These were determined in diffusion and ultrafiltration experiments described below.) Since all these estimates are in fairly close agreement, it appears that r_e is in error. The average pore radius obtained in calibration of ultrafilter membranes by the widely used method of Elford and Ferry (3, 4) is identical with r_e , and consequently particle sizes estimated on the basis of this calibration are seriously in error. The source of error must lie in the assumptions (1) that the pores are perpendicular to the membrane surface and (2) that all water in the membrane is free. The estimate r_p , due to Pappenheimer *et al.* (1), is based on the direct measurement of $(A/\Delta x)_w$ in the membrane with isotope-labelled water, and is independent of both assumptions. It is therefore recommended as a standard method for membrane calibration.

2. Restriction to Diffusion.—Table II shows that $(A/\Delta x)$ for diffusion of various solutes decreases with increasing molecular weight in all three membranes, and that the decrease is greater in the membranes with smaller pores. The relations observed between $(A/\Delta x)$ and molecular weight are similar to those reported by Pappenheimer *et al.* for living capillary membranes (1).

Discussion.—The pore diffusion theory of Pappenheimer *et al.* proposes two

TABLE II
Experimental Results

All figures are for 1.0 cm.² membrane at 25°C.

Measurement	Visking cellulose	Du Pont cellophane	Sylvania viscose wet gel
Thickness (d) cm. $\times 10^3$	5.47	7.86	8.43
Water content (S).....	0.664	0.763	0.838
Filtration coefficient (K_F), $\frac{\text{cm.}^3}{\text{dyne-sec.}} \times 10^{12}$	9.5	22.4	194.
($A/\Delta x$), cm.			
HPHO.....	19.0 \pm 1.6 (7)*	16.6 \pm 2.5 (2)	23.6 \pm 0.7 (2)
Urea.....	17.2 \pm 1.5 (5)	18.3 \pm 1.4 (2)	22.6 \pm 1.2 (2)
Glucose.....	9.6 \pm 1.6 (4)	14.7 \pm 1.4 (2)	23.7 \pm 0.5 (4)
Antipyrine.....	11.9 \pm 0.4 (2)		24.6 \pm 1.7 (2)
Sucrose.....	6.60 \pm 0.19 (6)	11.4 \pm 1.1 (3)	21.1 \pm 1.6 (3)
Raffinose.....	5.14 \pm 0.03 (2)	9.9 \pm 0.7 (2)	20.1 \pm 1.3 (2)
Hemoglobin.....	0†	0 (1)	2.4 (1)
Calculated pore radius, A			
Previous methods:			
1. Guérout (19), Elford and Ferry (3).	7.5	12.6	37.3
2. Manegold (5).	13.0	21.9	64.6
New methods: Pappenheimer <i>et al.</i> (1, 2)			
3. Diffusion of water.	18.9	31.0	76.7
4. Restricted diffusion and molecular size.	15.	30.	80 - 100
5. Molecular sieving in ultrafiltration.	15.	35 - 40	~200
5a. Distribution of pore radii.....	14 \pm 7	30 \pm 20	

* Mean \pm standard deviation (number of measurements). When only two measurements were made, the range is given.

† Impermeability to hemoglobin was established by ultrafiltration.

factors to account for the fall in apparent diffusion area with increasing molecular weight. The first, originally described by Ferry (21) establishes the condition that for entrance into a pore, a molecule must pass through the opening without striking the edge. The center of the molecule must, therefore, pass through a circle of radius $(r - a)$ within the mouth of the pore, in which r is the pore radius and a is that of the molecule. The effective area of the opening (A) is:

$$A = A_0 \left(1 - \frac{a}{r}\right)^2 \quad (9)$$

in which A_0 is the total cross-sectional area of the pore. The second factor corrects for the friction between a molecule moving within a pore and its walls. For this factor, Pappenheimer used an empirical equation obtained by Ladenburg (22) for the motion of a sphere in a narrow column of liquid. In the present paper, the following equation, derived on theoretical grounds by Faxén (23), and applied to membrane diffusion by Lane (24), is used:

$$\frac{A}{A_0} = 1 - 2.104 \left(\frac{a}{r}\right) + 2.09 \left(\frac{a}{r}\right)^3 - 0.95 \left(\frac{a}{r}\right)^5 \quad (10).$$

This equation gives nearly the same values of (A/A_0) as does Ladenburg's at values of (a/r) below 0.08. It has been shown experimentally to hold without significant deviation to (a/r) 's at least as high as 0.32, where Ladenburg's equation is inaccurate (25).

The total restriction to diffusion, due to the combined effects of steric hindrance at the entrance to the pores (Equation 9) and frictional resistance within the pores (Equation 10), is given by:

$$\frac{A}{A_0} = \left(1 - \frac{a}{r}\right)^2 \left[1 - 2.104 \left(\frac{a}{r}\right) + 2.09 \left(\frac{a}{r}\right)^3 - 0.95 \left(\frac{a}{r}\right)^5\right] \quad (11).$$

A graph of this function is found in Fig. 2.

In Fig. 3 *a*, *b*, *c*, curves drawn according to equation (11) for various values of r are compared with the experimental data. These are in general agreement with the shape of the curves, and the curves of best fit give values for pore radius which are in agreement with r_m and r_p . (See Table II; this new value of pore radius is designated r_d .) Deviations from theoretical curves show no consistency, and are attributed to the geometrical oversimplifications.

A striking difference between the permeability of the cellulose membranes used in this study and the living capillary endothelium is illustrated by their respective $(A/\Delta x)$'s for antipyrine. In the cellulose membranes, $(A/\Delta x)$ for this substance is in close agreement with predictions based on molecular size. In capillary walls, this quantity is disproportionately great. In addition to

their system of water-filled pores, the capillaries provide an additional diffusion pathway for lipid-soluble substances, of which antipyrine is an example (9). The cellulose membranes have only a system of water-filled pores.

3. *Molecular Sieving in Ultrafiltration.*—Fig. 4 shows that retention of solute molecules by Visking cellulose is dependent on both molecular size and filtration rate, and independent of solute concentration. Similar results were obtained on the other membranes, but were less marked, since pore size was

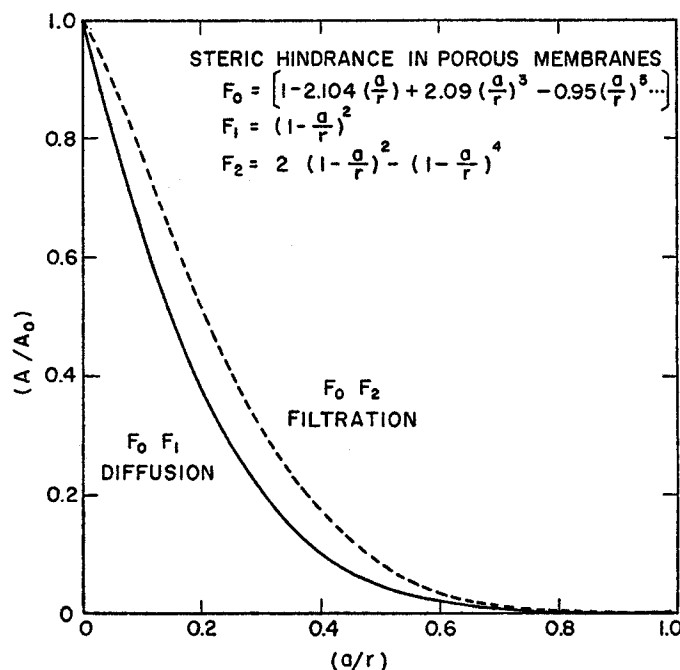


FIG. 2. Theoretical equations for steric hindrance in porous membranes.

greater. These experimental values are found in Fig. 5 *b, c*. The sieving of hemoglobin by membranes of viscose wet gel was very erratic, presumably due to plugging of the pores, and will not be reported here; the other membranes let none of this substance through.

Discussion.—The dependence of molecular sieving on filtration rate or pressure has been observed by several investigators (26–29). Ferry's theory (21) relates sieving to the ratio of particle radius to pore radius, but does not account for the effect of diffusion taking place simultaneously with filtration. The initial sieving sets up a concentration gradient:

$$c_2 = \left(\frac{A_x}{A_w} \right) c_1 \quad (12)$$

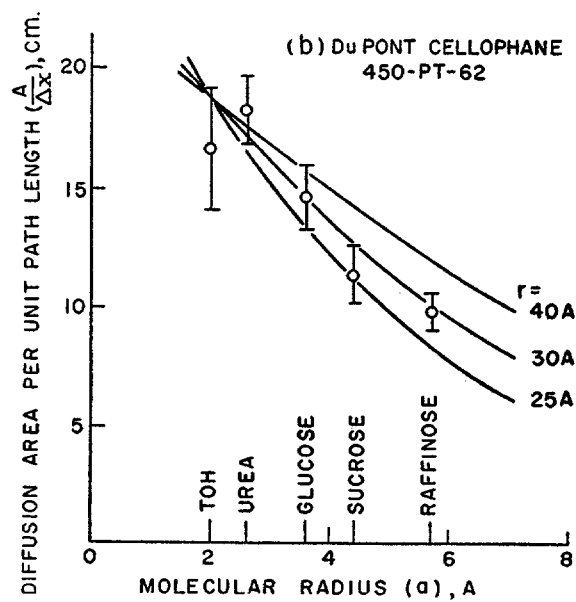
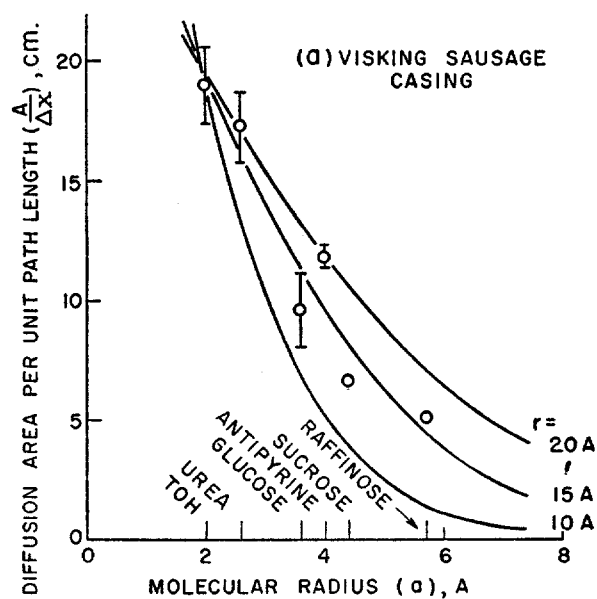


FIG. 3 *a*, *b*, and *c*. Restricted diffusion in porous cellulose membranes.

in which c_1 is the solute concentration of the filtrand, c_2 , of the filtrate, A_s is the effective pore area for solute molecules, and A_w for solvent molecules. The

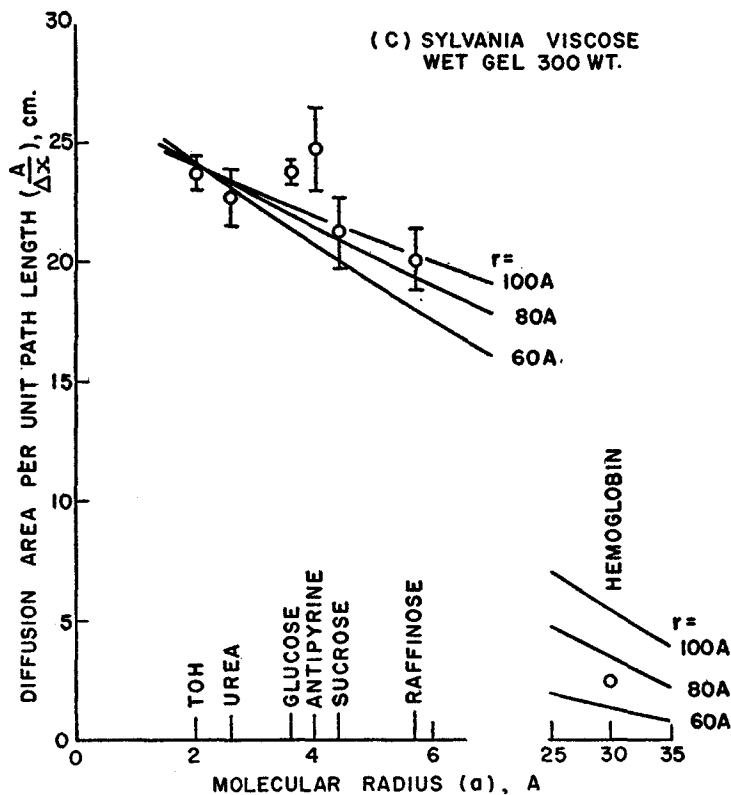


FIG. 3 c

concentration difference across the membrane ($c_1 - c_2$) leads to the diffusion of solute in the same direction as the filtration:

$$\frac{dn}{dt} = D_s \left(\frac{A}{\Delta x} \right)_s (c_1 - c_2) \quad (13),$$

in which dn/dt is the diffusion rate, D_s the free diffusion coefficient of the solute in the solvent, and $(A/\Delta x)_s$ is the effective diffusion area per unit path length for solute in the membrane. The change in concentration of the ultrafiltrate due to diffusion is equal to dn/dt (mols per unit time) divided by the filtration rate, Q (volume per unit time). The total sieving effect of ultrafiltration is:

$$c_2 = \left(\frac{A_s}{A_w} \right) c_1 + \frac{dn/dt}{Q} \quad (14).$$

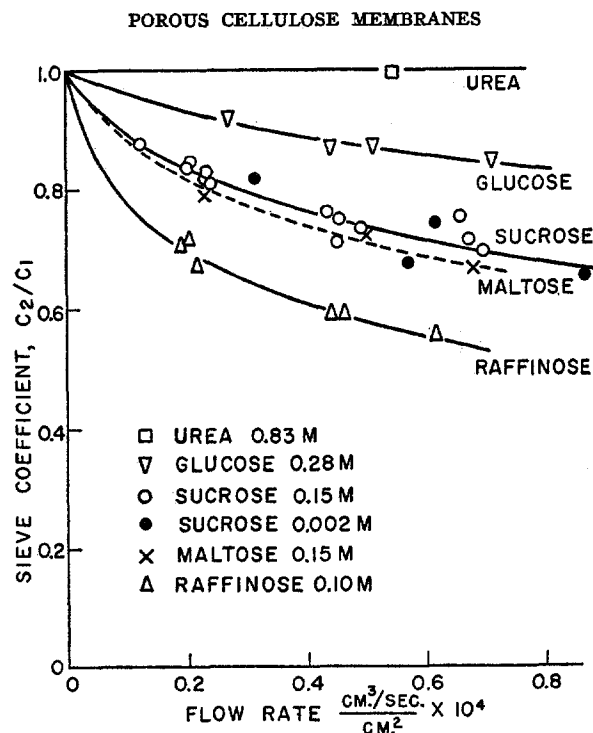


FIG. 4. Molecular sieving in ultrafiltration through Visking cellulose membranes.

Substitution for dn/dt by means of equation (13) leads to the following solution, given by Pappenheimer (2):

$$\frac{c_2}{c_1} = \frac{\left(\frac{A_z}{A_w} + \frac{D_z}{Q} \left(\frac{A}{\Delta x} \right)_z \right)}{1 + \frac{D_z}{Q} \left(\frac{A}{\Delta x} \right)_s} \quad (15).$$

A form more convenient for the present purpose is obtained by dividing numerator and denominator by (A_z/A_w) :

$$\frac{c_2}{c_1} = \frac{1 + \frac{D_z}{Q} \left(\frac{A}{\Delta x} \right)_w}{\frac{A_w}{A_z} + \frac{D_z}{Q} \left(\frac{A}{\Delta x} \right)_w} \quad (16).$$

since $(A/\Delta x)_w$, the effective diffusion area per unit path length for water, has been measured directly with tritium-labelled water, and is by definition equal to $(Aw/Ax) \times (A/\Delta x)_z$. A_w and A_z are individually computed from the respective molecular dimensions of solvent and solute molecules and the radius of the membrane pores by means of an equation similar to equation (11).

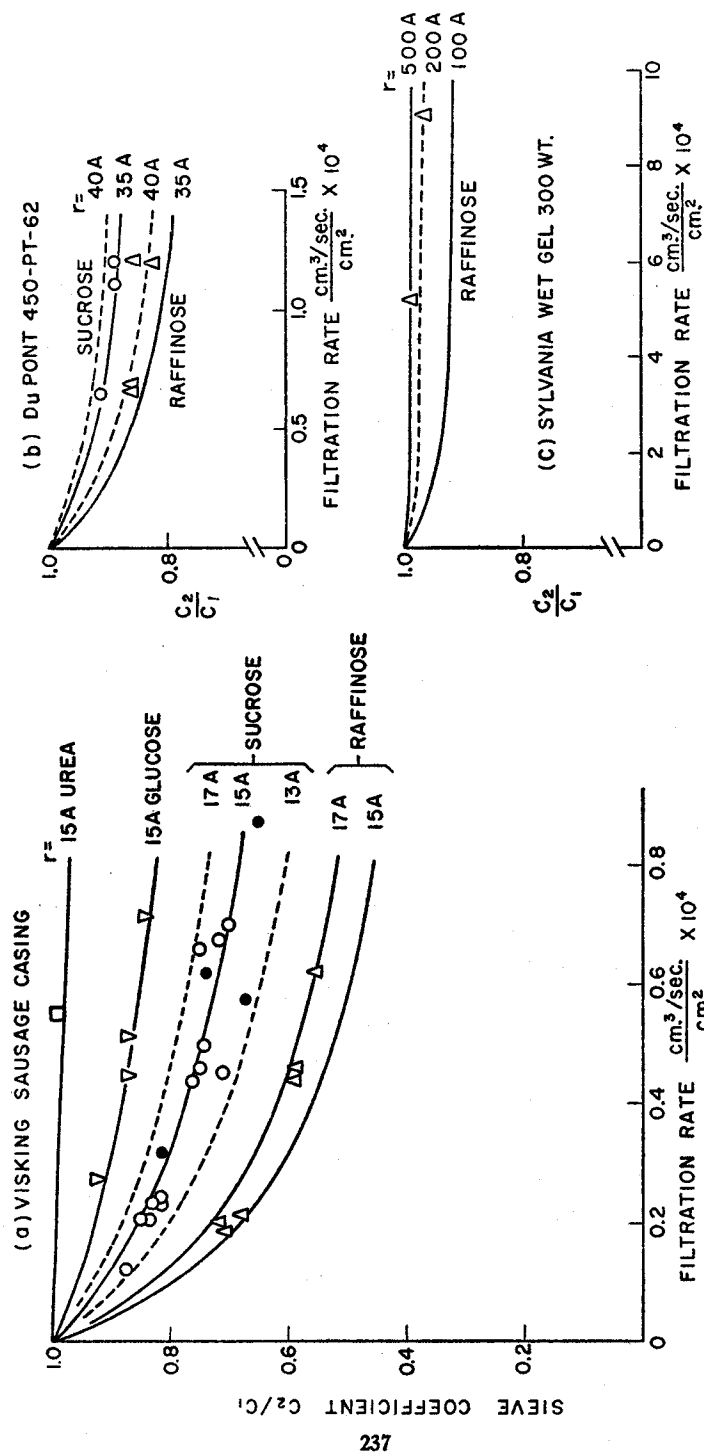


FIG. 5 a, b, and c. Estimation of average pore radius from molecular sieving data.

Equation (11) may not be used directly for the following reason. During laminar flow through a cylindrical pore, the velocity of flow varies with the distance from the axis, according to Poiseuille's equation:

$$v_r = v_0 \left(1 - \frac{\rho^2}{r^2} \right) \quad (17),$$

in which v_r is the velocity at distance ρ from the center, v_0 the velocity along the axis of the pore, and r the pore radius. Because of steric hindrance at the entrance to a pore, solute molecules of radius a may enter only if they fall within a cylinder of radius $r - a$. The mean velocity of flow within this cylinder is greater than the mean velocity through the entire pore. Consequently, a larger fraction of the solute enters the pore than in the absence of filtration. Ferry (21) has derived the following expression for steric hindrance at the entrance to the pores during ultrafiltration:

$$\left(\frac{A_s}{A_0} \right)_f = 2 \left(1 - \frac{a}{r} \right)^2 - \left(1 - \frac{a}{r} \right)^4 \quad (18).$$

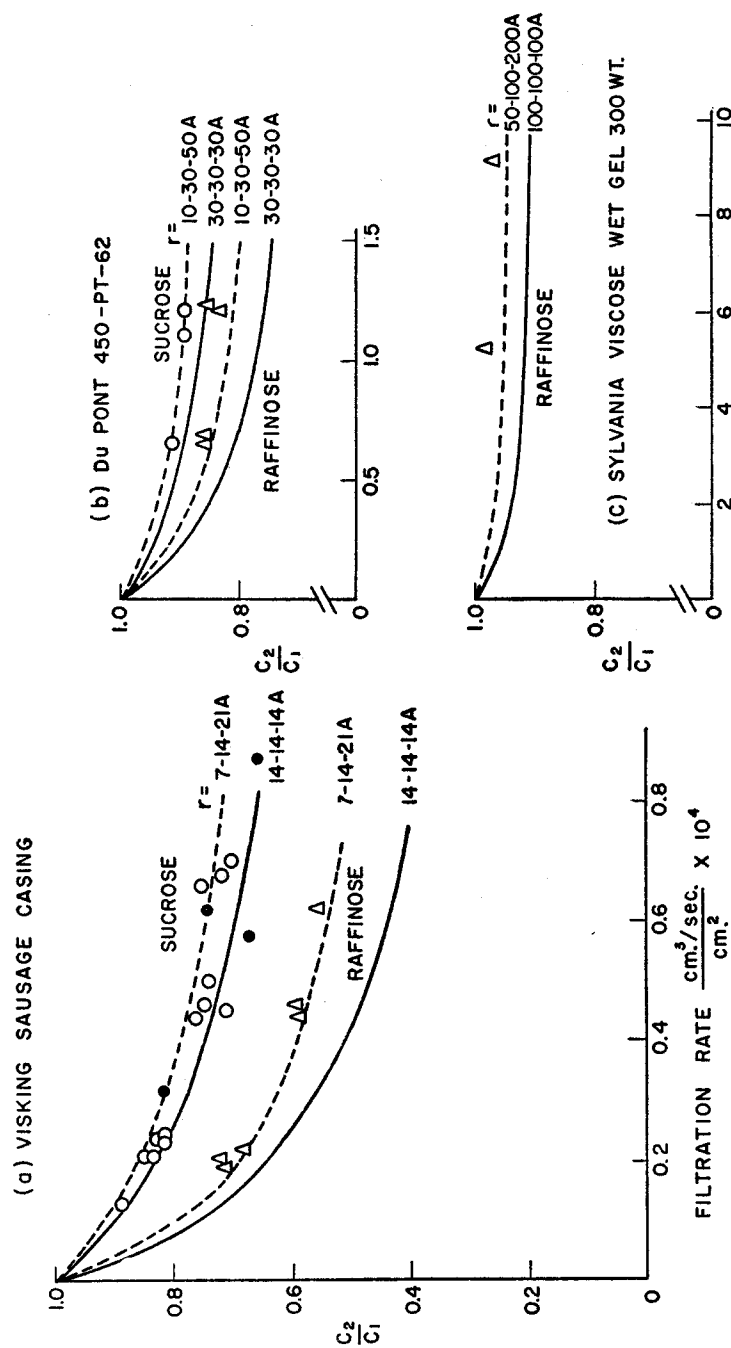
The frictional effect on solute molecules once they are within the pores is given by equation (10). The total restriction due to both factors is as follows:

$$\left(\frac{A_s}{A_0} \right)_f = \left[2 \left(1 - \frac{a}{r} \right)^2 - \left(1 - \frac{a}{r} \right)^4 \right] \left[1 - 2.104 \left(\frac{a}{r} \right) + 2.09 \left(\frac{a}{r} \right)^2 - 0.95 \left(\frac{a}{r} \right)^3 \right] \quad (19).$$

This equation differs slightly but significantly from equation (11). Both are compared in Fig. 2.

By means of equations (16) and (19), theoretical curves have been drawn predicting the variation of c_2/c_1 with filtration rate and molecular size in the three membranes studied. Pore radii were chosen in each case to provide the closest fit with the experimental data. The results of the curve-fitting process are shown in Fig. 5 *a, b, c*. For Visking cellulose membranes, the fit is very close and the effective pore radius determined by this method (r_f , see Table II) checks with the value independently estimated from restricted diffusion (r_d). In the case of du Pont cellophane, r_f is slightly larger than r_d ; and for Sylvania wet gel, r_f is considerably larger.

The deviations observed for the latter two membranes, as well as the very slight deviations discernible in the Visking data appear to be systematic. A possible explanation lies in the fact that the pores in each membrane may not all be of one size, and that individual pore sizes may extend over a wide range. This situation may be dealt with by applying equation (16) individually to each class of pores, determining the contribution of each class to the ultrafiltrate, and summing the contributions. In general, since most of the filtration takes place through the large pores ($Q \propto r^4$), the sieving produced by any distribution of pores will be less than for a homogeneous population of the average radius. Fig. 6 illustrates this effect. Since the calculation is so la-



borious, the pore populations were assumed to consist of only three classes. In each case, agreement between theory and experiment is improved, and the figures provide an estimate of the variation of pore radii in a given membrane. However, the errors introduced by assuming a uniform pore size are not much greater than the over-all experimental accuracy in the present case, and may be ignored in many applications. For molecular radii approaching mean pore radius, greater deviations are to be expected.

4. *Osmotic Pressure in Ultrafiltration.*—During molecular sieving, a steady-state solute concentration difference is maintained across the ultrafilter membrane. The osmotic pressure exerted by this concentration difference (P_π) may be estimated in the following manner. When a solution is filtered at a constant rate, the applied hydrostatic pressure is made up of three components:

$$P_{\text{tot.}} = P_f + P_d + P_\pi \quad (20).$$

P_f is the pressure required to overcome viscous friction in the membrane and is given by the expression

$$P_f = P_w(\eta_f/\eta_w) \quad (21),$$

in which P_w is the pressure required to filter water at the same rate and (η_f/η_w) the relative viscosity of the ultrafiltrate. P_d is the pressure required to do the work of diluting filtrand at c_1 to filtrate at c_2 . The *reversible* work of dilution (w) is given by the equation (reference 30):

$$w = P'_d v = nRT \left(\frac{c_1 - c_2}{c_2} - \ln \frac{c_1}{c_2} \right) \quad (22).$$

P'_d is the pressure required to do this work on volume v of filtrate containing n mols solute. It is a minimum estimate of P_d , since the filtration is done irreversibly. Setting $c_2 = n/v$, equation (22) may be solved for P'_d :

$$P'_d = RT \left[(c_1 - c_2) - 2.3c_2 \log \frac{c_1}{c_2} \right] \quad (23).$$

To obtain a maximum estimate of P_π , P_f and P_d are calculated from the experimental data and subtracted from the observed pressure ($P_{\text{tot.}}$). Table III lists experimental and calculated values for filtration of sucrose and raffinose solutions through Visking cellulose membranes. In other ultrafiltrations, the sieve coefficients were too small to permit sufficient accuracy. Listed in column 10 of the table is the ideal osmotic pressure across the membrane according to van't Hoff's law:

$$\pi = RT(c_1 - c_2) \quad (24),$$

and in column 11, the ratio P_π/π . Making due allowance for known sources of error, we may conclude that the osmotic pressure exerted by solutions of

sucrose and raffinose across membranes permeable to these solutes approaches closely to that predicted by van't Hoff's law.

Discussion.—In their studies on diffusion and filtration through the walls of living capillaries, Pappenheimer *et al.* (1) used van't Hoff's law to compute transcapillary concentration differences from osmotic pressures measured during solute diffusion. This procedure has been criticized by Grim (32) on the basis of derivations by Laidler and Shuler (33) showing that for membranes permeable to both solute and solvent, van't Hoff's law must be corrected by a factor dependent on the relative permeabilities of the membrane for both substances. Staverman (34, 35) independently reached the same conclusion, but presents a different correction factor. Grim tried to measure

TABLE III
Steady-State Osmotic Pressures during Molecular Sieving through Visking Cellulose Membranes
All pressures in dynes/cm.² $\times 10^{-6}$.

(1) Substance	(2) Filtration rate	(3) $c_1 - c_2$	(4) η/η_w (16, 31)	(5) P_w	(6) $P_{tot.}$	(7) $\frac{\eta}{\eta_w} \times P_w$	(8) P_d	(9) P_π	(10) π	(11) P_π/π
	ml./sec. $\times 10^4$	m/liter								
Sucrose $c_1 =$ 0.150 M/ liter	0.20	0.024	1.126	2.20	3.20	2.50	0.05	0.65	0.59	1.10
	0.40	0.036	1.113	4.25	6.00	4.75	0.10	1.15	0.89	1.29
	0.60	0.043	1.105	6.40	8.65	7.10	0.17	1.38	1.05	1.31
Raffinose $c_1 =$ 0.100 M/liter	0.20	0.030	1.105	2.20	3.25	2.40	0.12	0.73	0.74	0.99
	0.40	0.039	1.092	4.25	6.25	4.65	0.22	1.38	0.96	1.44
	0.60	0.045	1.083	6.40	9.20	6.95	0.30	1.95	1.11	1.76

the correction experimentally for glucose diffusing through the collodion membrane of an osmometer of conventional form. However, it seems doubtful whether the response of such an instrument is fast enough to follow the diminishing concentration gradient during diffusion of solute. A recent description of an osmometer of this type specially designed for the rapid measurement of protein osmotic pressure states that 3 to 8 hours were required to reach equilibrium with a non-diffusible solute (36). In Grim's experiment, P_π was decreasing exponentially with a half-time of less than $\frac{1}{2}$ hour, and the value of 0.0046 obtained from the ratio P_π/π must be considered an experimental artifact.

The experimental values of P_π/π listed in Table III were measured during maintenance of a steady-state concentration gradient and are not subject to such errors. Their approximation to unity indicates that Pappenheimer's method of computing transcapillary concentration differences is not greatly in error. Further evidence for the validity of Pappenheimer's method of measur-

ing capillary diffusion areas is provided by the almost identical relations obtained for diffusion area per unit path length as a function of molecular radius and pore radius by his method and by the present direct measurements on inert porous membranes.

SUMMARY AND CONCLUSIONS

1. A study has been made of the diffusion and filtration of a graded series of molecules (including tritium-labelled water, urea, glucose, antipyrine, sucrose, raffinose, and hemoglobin) in aqueous solution through porous cellulose membranes of three degrees of porosity.

2. Experimental results were in close agreement with predictions based on the membrane pore theory of Pappenheimer *et al.* (1, 2). Restriction to molecular diffusion is a function of pore radius and molecular radius described by equation (11) in the text. Molecular sieving during ultrafiltration is a function of total pore area per unit path length, pore radius, molecular radius, and filtration rate given by equations (16) and (19).

3. Estimates of average pore radius made by means of this theory were considerably larger than estimates made by the method of Elford and Ferry (3) (Table II). Sources of error in the latter method are discussed and a new method of membrane calibration is proposed in which the total cross-sectional area of the pores is measured by direct diffusion of isotope-labelled water.

4. Steady-state osmotic pressures of solutions of sucrose and raffinose measured during molecular sieving through cellulose membranes were found to be close to the "ideal" osmotic pressures calculated by van't Hoff's law. Thus the present experimental data support the methods used by Pappenheimer *et al.* in their studies on living capillary walls as well as their theory of membrane pore permeability.

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