Experimental Study of the Independence of Diffusion and Hydrodynamic Permeability Coefficients in Collodion Membranes

ELLIOTT ROBBINS and ALEXANDER MAURO

ABSTRACT   The two parameters usually invoked when discussing transport across membranes are the "diffusion permeability coefficient" and the "hydrodynamic permeability coefficient." In this study the magnitude of these two coefficients is established experimentally for collodion membranes of differing porosities. The hydrodynamic permeability is predominant while convergence of the two permeabilities tends to obtain as the membranes become less coarse. The flux data obtained are used to calculate "average pore diameter" and the meaningfulness of these calculations is interpreted. The relationship between the two coefficients and transport across membranes as treated by the system of irreversible thermodynamics is discussed.

It has been customary in recent years to invoke two permeability parameters in dealing with problems of transport across membranes. The first usually termed the "diffusion permeability coefficient" has been used to refer to the diffusion process across the membrane. The second, the "hydrodynamic permeability coefficient"[1] has been used to specify the non-diffusional flux that has been intuitively considered as a bulk or mass flow. If an ideal geometry of uniform cylindrical "pores" is assumed as a first approximation, and if the hydrodynamic flow is considered to be a Poiseuille flow, it is possible with these parameters to calculate an "average pore diameter" (6, 3, 9, 11). In an effort to gain further understanding of the nature of these two parameters experimental data were obtained on a simple inert collodion barrier which permit the separate evaluation of the diffusion and hydrodynamic coefficients showing how one can be changed independently of the other. With the aid of osmotic experiments it is seen that the average pore diameter calculated from such data can be useful in some cases but misleading in others.

[1] Also termed the "hydraulic conductivity," or "filtration coefficient" or "osmotic permeability coefficient." This last designation should only be used when the osmotic pressure is established by absolutely impermeant solutes.

EXPERIMENTAL

The collodion membranes used in these experiments were prepared according to the method of Pierce (12). Details may be found in a paper by Meschia (8). Varying amounts of ethylene glycol were used which resulted in membranes of different permeability; three groups of membranes were prepared as outlined in Table I.

A simple system was constructed following in general that used by Meschia (8). It consisted of two cylindrical lucite chambers, namely chamber A and chamber B, 3 inches in diameter and 1.5 inches long between which the collodion membrane, imbedded in a perforated steel plate was clamped (8). A graduated, calibrated glass tube with 1 mm. bore was led from chamber A to a mercury pool. The scale was graduated in millimeters and by means of a microscope 0.1 mm. could be read directly. The chambers were then filled with water as was the calibrated glass tube for one-half of its length. This resulted in a mercury-water interface which could be viewed with respect to the scale markings in the glass wall. When a hydrostatic pressure was applied to the phase in chamber A by raising the mercury pool, the

TABLE I

Group	Ethylene glycol	Nitrocellulose	Ethyl alcohol	Ether
	cc.	*gm.*	*cc.*	*cc.*
I	8	8	150	50
II	4	"	"	"
III	2	"	"	"

The evaporating time was 17 hours.

flux through the membrane could be directly read merely by noting the advancement of the mercury-water meniscus in the calibrated tube. To insure as much accuracy as possible readings were taken at intervals of 30 minutes (to several hours in the "tight" membranes) so that an appreciable volume of displaced water could be measured. Various hydrostatic pressures were applied and the flux recorded (Fig. 1). It was crucial that the system be leakproof and that a reasonable length of time elapse between the changing of the pressures and the taking of a reading in order that the system be in a steady state. Following this procedure the pressure was removed and chamber A was drained and refilled with water enriched with H_2O^{18} (1.3 per cent). Two glass beads were added to facilitate mixing. Chamber B was filled with a volume of distilled water equal to that of chamber A. The concentration of H_2O^{18} in distilled water is ordinarily 0.2 per cent. After the initial withdrawal of 0.05 cc. from both chambers the system was attached to a rocking device and shaken for 30 minutes. Two samples of 0.05 cc. were withdrawn from chamber B at intervals of 15 minutes. All samples were then analyzed for H_2O^{18} with the mass spectrometer. As will be shown below these procedures supplied the data necessary for determination of both the diffusion flux and the non-diffusional flux. The routine was carried out on each of the three groups of membranes.

Finally, measurements of osmotic pressure were made to obtain an estimate of the pore size of the membranes. These were merely qualitative experiments and will

not be described in any detail. With the membranes of group I a solution of hemo-globin was placed in chamber A and the hydrostatic pressure which exactly coun-terbalanced the osmotic flow was noted. Stable equilibrium conditions of zero flow were noted. Glucose was also tested on membranes of group I for osmotic effects.

The same observations were carried out with group III membranes using inulin as well. Osmotic experiments were not performed with the membranes in group II.
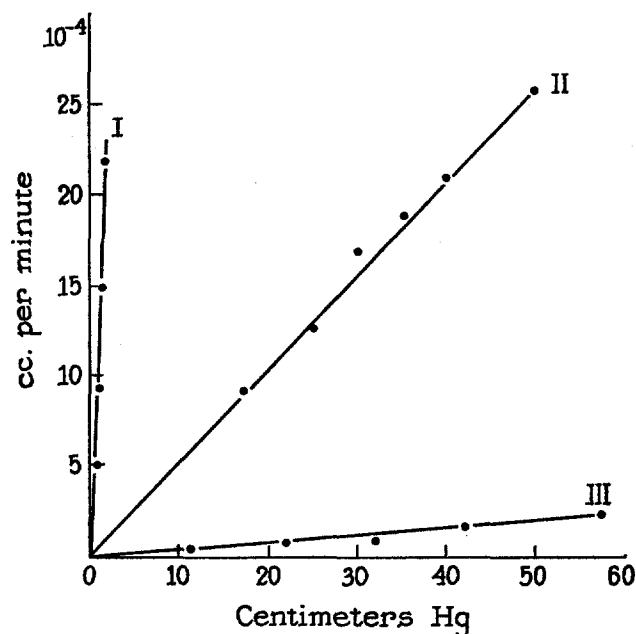

Figure 1.   The relationship between total flux and hydrostatic pressure and the effect of increased ethylene glycol in the membrane formula. I, II, III refer to the respective groups of membranes. A representative member of each group is plotted. The flux in (cc./min.)/cm. Hg can be converted to (moles/sec.)/(dyne/cm.^{2}) by dividing by 1.438×10^7.

RESULTS

In all membranes tested the flux was found to vary linearly with the applied hydrostatic pressure (Fig. 1). The results obtained within each of the three groups were quite consistent, varying at most by a factor of 2.6, in contrast with more marked differences between groups. Consequently the average of each of the groups is used in the following discussion. This in no way affects the conclusions drawn, and makes the line of reasoning followed more readily apparent.

The average flux per unit pressure difference for each of the three groups is listed in Table II.

It is noted that the flux decreases by a factor of 226 between groups I and III.

In order to estimate the component of transport that should arise from the process of diffusion, we can apply the general diffusion equation (1) to this barrier and then proceed to calculate the flux for a gradient of chemical potential. This will give the diffusion permeability coefficient. For convenience we have chosen to impose a gradient of chemical potential *via* the hydrostatic pressure, although a concentration gradient could also have been used. Thus:

$$\frac{dn}{dt} = \frac{DA}{RT} C \frac{d\mu}{dx} \qquad (1)$$

in which $d\mu/dx$ is the gradient of chemical potential, C is the concentration of the diffusing substance, D is the diffusion coefficient of the species, A is the area available for diffusion, and R is the gas constant.

Since we are dealing only with pressure, the differential of chemical potential in these experiments is simply

$$d\mu = \bar{V}dP \qquad (2)$$

in which $\bar{V}$ is the molar volume.

Since in our system consisting of one component, namely, water, $C\bar{V} = 1$, we have:

$$\frac{dn}{dt} = \frac{DA}{RT} \frac{dP}{dx} \qquad (3)$$

If the pressure gradient dP/dx is considered constant, (3) may be written

$$\frac{dn}{dt} = \frac{DA}{RT} \frac{\Delta P}{\Delta x} \qquad (4)$$

in which Δx is the thickness of the barrier across which ΔP is applied. Thus we arrive at an expression which allows us to determine the net flux due to diffusion when a ΔP is applied. For this calculation values of $DA/\Delta x$ for each membrane are needed. These can be obtained easily by invoking the more familiar form of the diffusion equation, namely, the Fick equation which results from using the concentration component of the chemical potential. That is

$$\mu = \mu^* + RT \ln C \text{ (for dilute solutions)}$$

and

$$d\mu = RT \frac{dC}{C} \qquad (5)$$

Whereupon substituting in (1) we have the Fick equation

$$\frac{dn}{dt} = DA \frac{dC}{dx} \qquad (6)$$

The factor $DA/\Delta x$ in equation (4) is a constant for any given membrane and may be evaluated by measuring the diffusion of H_2O^{18} across the membrane. Although the details of the calculations of $DA/\Delta x$ are found in

Northrop and Anson (10) they are included in the Appendix, because some modifications have been made. The equation arrived at is:

$$\frac{DA}{\Delta x} = \frac{V2.3}{2\Delta t} \log \frac{C' + C'' - 2C''_{t_0}}{C' + C'' - 2C''_{t_0+\Delta t}} \tag{7}$$

C' is the initial concentration in chamber A at time $t = 0$

C'' is the initial concentration in chamber B at time $t = 0$

C''_{t_0} is the concentration in chamber B at time $t = t_0$

$C''_{t_0+\Delta t}$ is the concentration in the chamber B at time $t = t_0 + \Delta t$

It should be noted that t_0 is not zero time but rather that point at which the initial sample is withdrawn for analysis, whereas Δt is the length of time that has elapsed between t_0 and $t_0 + \Delta t$. When the values of the above defined concentrations are determined by mass spectrometric analysis for a Δt of 30

TABLE II

Group	Flux
	moles/sec. per dyne/cm.²
I	8.8×10^{-11} (3 membranes)
II	3.1×10^{-12} (5 ")
III	3.9×10^{-13} (3 ")

TABLE III

Group	DA/Δx
I	3.0×10^{-3}
II	1.4×10^{-3}
III	2.7×10^{-4}

TABLE IV

Group	Diffusion flux from (4)	Total observed flux	Ratio diffusion/total
I	1.2×10^{-13}	8.8×10^{-11}	1/730
II	5.6×10^{-14}	3.1×10^{-12}	1/55
III	1.1×10^{-14}	3.9×10^{-13}	1/36

minutes and are then inserted in (7), $DA/\Delta x$ for the three groups of membranes is found to be equal to the values listed in Table III.

Having established a factor $DA/\Delta x$ the diffusion flux, dn/dt, can be evaluated by means of (4) for a ΔP of 1 dyne/cm.². The value of 1 dyne/cm.² is chosen in order that the result be comparable to the total observed flux which is given in moles/sec. per dyne/cm.² The data are given in Table IV.

DISCUSSION

Several conclusions are suggested by these results: First, it is noted that the flux due to diffusion is a very small part of the total flux in all three groups, especially group I, and therefore we must recognize the predominance of a non-diffusional transport. The importance of this point may be further emphasized by stating that for these membranes the proportionality constant connecting observed flow and applied hydrostatic pressure cannot be accounted for in general, by the use of diffusion theory.[2] This has been the assumption used by several investigators (1, 15). Second, it is seen that as the membranes become "tighter" the change in the diffusional component of flux is less than the non-diffusional component. For example, comparing group I and group III we see that while the total flux decreases by a factor of 226, the diffusional component drops by only a factor of 11.1. Since we have already shown that the total flux is almost solely non-diffusional, this factor of 226 is largely due to the decrease in non-diffusional flux.

These data are in agreement with the view that the non-diffusional component is related to a Poiseuille type flow (3, 6, 11, 16) (*i.e.* a function that varies r^4 in which r is the "average pore radius") while the diffusional component is determined by the total area (*i.e.* a function of r^2) so long as the pores are larger than the diffusing molecule. If it is imagined that the barrier consists of uniform cylindrical pores, the average pore radius may be calculated as follows:—

$$\Phi_P = \frac{n\pi r^4 \Delta P}{8\eta \bar{V} \Delta x}$$

in which Φ_P is the Poiseuille flow and n is the total number of pores and η the coefficient of viscosity. $\bar{V}$ is used to convert the flow to moles

$$\Phi_P = \frac{r^2 A \Delta P}{8\eta \bar{V} \Delta x}$$

since $A = n\pi r^2$ in which A is the total area available for flow

$$\Phi_D = \frac{DA \, \Delta P}{RT \, \Delta x}$$

in which Φ_D is the diffusion flux, dn/dt

$$\frac{\Phi_P}{\Phi_D} = \frac{r^2 RT}{8\eta D \bar{V}} \tag{8}$$

[2] Membranes of this type have been used previously to elucidate the nature of osmotic flow (7).

Thus r is the only unknown. Since Φ_D is very small with respect to Φ_P, the total measured flux may be used in (8) with small error as mentioned above. Thus if the flows obey the above laws, their ratio is proportional to the square of the pore radius. The tendency for the magnitude of the mass flow and diffusional flow to converge is seen in the data, which suggests that the non-diffusional component is determined by a quasi-Poiseuille conductance. It is important to emphasize that the assumption of a quasi-Poiseuille flow should be considered as a first order approximation.

In this connection it is pertinent that the calculation of average pore radius by the method just described gives a value of 97 A for group I. Clearly this value is inadmissible since these membranes were impermeable to hemoglobin whose radius is 40 A.[3] However, a similar calculation for membranes of group III gives a pore radius of 21 A which has more physical significance as can be seen by the following observation: the flux of water through a membrane due to the mechanism of osmosis rises abruptly as the radius of the solute molecule used to establish the mole fraction difference of solvent across the membrane approaches the pore size of the barrier (2, 4, 13, 14). This behavior can be used as an independent method for *estimating* the pore size of a membrane. In our experiments glucose and inulin were used as solutes to establish a mole fraction difference of water, whereupon the osmotic effect observed was 15 and 62 per cent respectively, of the theoretical maximum. That is to say, at zero time, before appreciable solute diffusion had occurred, the hydrostatic pressure required to maintain a stationary meniscus was 15 and 62 per cent of the theoretical value that would have been necessary to establish thermodynamic equilibrium across a strictly semipermeable membrane. Since the Stokes-Einstein radii of glucose and inulin are approximately 4.0 A and 15.0 A respectively, it seems reasonable to conclude from the discussion above that the pore radius is in the neighborhood of 15.0 A. Thus the calculated value of 21 A is physically significant.

At first glance it seems inconsistent that the calculations should give more reasonable results as the pore radius becomes smaller. If a true Poiseuille conductance were indeed the dominant mode of transport in the above experiments, it would be expected that just the opposite would obtain, namely, membranes with pores of larger radius would give results more closely in accord with theoretical predictions based upon Poiseuille's law. That this does not turn out to be the case is not altogether surprising since aside from other factors, such as the validity of laminar flow in this range of pore size, the ideal geometry of uniform cylindrical tubes passing through the membrane is almost certainly not realized.

[3] That the membranes of group I are absolutely impermeable to hemoglobin is evidenced by the completely stable conditions of zero flow encountered in osmotic experiments over periods of 24 hours. Even slightly permeable membranes would display markedly different behavior.

It should be noted in passing that attempts to obtain membranes tighter than group III were unsuccessful. That is, by using smaller concentrations of ethylene glycol both permeabilities abruptly fell to zero implying complete solidification of the barrier as the end result of the pores becoming smaller in size.[4]

With regard to this tendency towards solidification, it is possible with the data obtained to calculate the relative change in effective area, A, which resulted from the change in glycol concentration in the membrane preparation. If the assumption is made that the thickness, Δx, is reasonably constant for the three groups, then the ratio of the $DA/\Delta x$ values should give the ratio of the areas since the other terms cancel. Thus if one considers the area, A, of group I as 100 per cent, the relative areas of group II and group III are 46 and 9 per cent, respectively.

To avoid possible confusion it should be noted that in the references in which the ratio, Φ_P/Φ_D, and pore diameter are evaluated, the "mass flow" has been established by means of the osmotic mechanism. That is, a mole fraction difference of the solvent has been established across the membrane by using impermeant solutes. As is well known in the case of impermeant solutes the osmotic pressure difference can be equated to hydrostatic pressure difference both thermodynamically and experimentally. The difference in chemical potential of the solvent that arises from the introduction of an impermeant solute is the same as that which occurs upon the application of a hydrostatic pressure and the flow of solvent occurs in the same way in both cases. The osmotic mechanism is the only feasible one when the barrier is deformable such as in biological cells. In our experiments with a rigid membrane we found the application of pressure more convenient.

An analysis of membrane transport by means of the formalism of irreversible thermodynamics has recently appeared (5). This treatment states that only certain phenomenological coefficients are necessary and sufficient to describe transport phenomena across a barrier. In the light of our discussion only one of the phenomenological coefficients will be commented on, namely, the "filtration coefficient," L_p.[5] This is the proportionality coefficient between total flux or "volume flow" and hydrostatic pressure. In keeping with the generalized nature of irreversible thermodynamics no attempt is made in (14) to discuss the kinetic mechanism of the volume flow. It is the desire to elucidate the nature of the constant L_p that warrants our attempt to resort to

[4] Absence of a measurable flow was observed in the restricted pressure range used in the experimental procedure described here and at the limited sensitivity of our volumetric readings. Observations were not made at high pressures. Diffusion studies with H_2O^{18} were restricted to the usual time as for the previous membranes.

[5] It is to be kept in mind that we are considering the special case in which water is the *only* permeant species and the single solute is absolutely, impermeant. Thus L_p refers to the solvent, namely, water and not a solution.

other methods; *i.e.*, the splitting up of the volume flow into components by the arguments pursued above.

We have thus tried to demonstrate that for collodion membranes the "filtration coefficient" L_p is not a Northrop-Anson diffusion permeability coefficient; *i.e.*, $DA/\Delta x$. Moreover to assume that it can be treated as a Poiseuille conductance as we have seen is also fraught with difficulties. The concept of pores and the concept of mass flow obeying Poiseuille's law are only first approximations. We suggest in keeping with a consistent phenomenological approach that the terms mass flow, "bulk flow," and "Poiseuille flow" in contrast to diffusion flow be substituted by the term non-diffusional flow. A non-committal way to stress the general nature of the coefficient L_p would be to use the term, "hydraulic conductivity."

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APPENDIX

Derivation of the Northrop-Anson equation. The following symbols are used:

D, diffusion coefficient
S, total solute at beginning of experiment (H_2O^{18} in this case)
v_1, volume of concentrated solution (chamber A)
v_2, volume of dilute solution (chamber B)
n, number of moles of H_2O^{18} in dilute solution $= c''v_2$; c'' is the concentration
$s - n$, number of moles of H_2O^{18} in concentrated solution $= c'v_1$
$dn/dt = (+DA/\Delta x)/(c' - c'')$, Fick's equation assuming a linear concentration gradient.
Substituting for c' and c'' gives:

$$\frac{dn}{dt} = \frac{+DA}{\Delta x}\left(\frac{s-n}{v_1} - \frac{n}{v_2}\right) = \frac{+DA}{\Delta x v_1 v_2}[v_2 s - (v_1 + v_2)n]$$

This may be integrated to give:

$$-\frac{1}{v_1 + v_2}\ln[v_2 s - (v_2 + v_1)n]\Big]_{n_0}^{n} = \frac{DAt}{\Delta x v_1 v_2}\Big]_{t_0}^{t}$$

If $v_1 = v_2 = v =$ a constant, then

$$\frac{DA}{\Delta x} = \frac{v2.3}{2\Delta t}\log\frac{s - 2n_0}{s - 2n}$$

in which the change from natural logarithms has been made. Note that n_0 is the number of moles of H_2O^{18} in the dilute solution at t_0. If both numerator and denominator of the logarithm are divided by v, the result is our equation (9).

$$\frac{DA}{\Delta x} = \frac{v2.3}{2\Delta t}\log\frac{C' + C'' - 2C''_{t_0}}{C' + C'' - 2C''_{t_0 + \Delta t}}$$

REFERENCES

1. CHINARD, F., *Am. J. Physiol.*, 1952, **171**, 578.
2. DURBIN, R. P., National Biophysics Conference (Abstract) 1957, 27.
3. DURBIN, R. P., FRANK, H., and SOLOMON, A. K., *J. Gen. Physiol.*, 1956, **39**,
4. GRIM, E., *Proc. Soc. Exp. Biol. and Med.*, 1953, **83**, 195.
5. KEDEM, O., and KATCHALSKY, A., *Biophysica and Biochem. Acta*, 1958, **27**, 229.
6. KOEFOED-JOHNSEN, V., and USSING, H., *Acta Physiol. Scand.*, 1953, **28**, 60.
7. MAURO, A., *Science*, 1957, **126**, 252.
8. MESCHIA, G., *Yale J. Biol. and Med.* 1954, **27**, 206.
9. NEVIS, A. H., *J. Gen. Physiol.*, 1958, **41**, 927.
10. NORTHROP, J., and ANSON, M., *J. Gen. Physiol.*, 1929, **12**, 543.
11. PAPPENHEIMER, J., *Physiol. Rev.*, 1953, **33**, 387.
12. PIERCE, H. F., *J. Biol. Chem.*, 1927, **75**, 795.
13. RENKIN, E. M., *J. Gen. Physiol.*, 1954, **38**, 225.
14. STAVERMAN, A. J., *Rec. trav. chim. Pays-bas*, 1951, **70**, 344.
15. TUNG, L. H., *J. Poly. Sc.*, 1958, **32**, 477.
16. ZEUTHEN, E., and PRESCOTT, D., *Acta Physiol. Scand.*, 1953, **28**, 77.