Estimation of equivalent pore radii of pulmonary capillary and alveolar membranes

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Taylor, Aubrey E., and Kermit A. Gaar, Jr. Estimation of equivalent pore radii of pulmonary capillary and alveolar membranes. Am. J. Physiol. 218(4): 1133–1140. 1970.—The reflection coefficients (\( \sigma \)) of urea, glucose, and sucrose were estimated in an isolated dog lung. The \( \sigma \) was measured across the capillary for the three test substances were found to be 0.18 ± 0.003, 0.26 ± 0.002, and 0.044 ± 0.004, respectively. The same three test molecules were investigated in fluid-filled lobes and the \( \sigma \) was calculated to be 0.59, 0.72, and 0.81, respectively, for the alveolar membrane. Also, when flow was varied from ½ to 1½ times normal no difference was observed in the calculated \( \sigma \) for the capillary membrane. Antipyrine produced no osmotic transient for the pulmonary capillary membrane. When (1 − \( \sigma \)) was plotted as a function of time, a pore radius of 6 − 10 A was calculated for the alveolar membrane and one of 40 − 58 A was estimated for the pulmonary capillary membrane. Thus, the alveolar membrane represents a tight cellular-type structure, whereas the pulmonary capillary represents a highly permeable, porous structure.

osmotic transient; isolated dog lung; alveolar permeability coefficient

Starling (21) was the first to recognize that the hemodilution following the intravascular injection of a hypertonic salt solution was some function of the size of the salt molecule. It noted a relatively greater hemodilution with Na₂SO₄ than was observed with NaCl solution of equivalent osmolarity (21). Pappenheimer et al. (13) further investigated this observation in the measurement of osmotic transients in hindlimb capillaries for several small lipid-insoluble substances. Renkin (16) later demonstrated that lipid-soluble substances produced no observable osmotic transients in the isolated hindlimb. The results of these investigations led to the conclusion that the passive permeation characteristics of muscle capillaries were primarily dependent upon the relative molecular radius-to-membrane pore size ratio and the lipid solubility characteristics of the molecule in the membrane.

Staverman (22) originally formulated the theoretical basis for the relationship of osmotic transients to membrane characteristics. He related the derived Van't Hoff osmotic pressure for a particular molecular species to the observed osmotic pressure in a particular membrane system by defining a thermodynamic parameter, the reflection coefficient (\( \sigma \)). Any substance which is totally impermeable to the membrane has a \( \sigma = 0 \), and the observed osmotic pressure of that substance in the particular membrane system is exactly equal to the calculated Van't Hoff osmotic pressure. Any substance which is freely permeable to the membrane has a \( \sigma = 0 \) and is therefore incapable of exerting any osmotic pressure across the membrane. For intermediate molecules in a given membrane system, \( \sigma \) assumes values ranging between 0 and 1. Durbin (4) and Goldman and Solomon (9) have developed a theoretical relationship between "equivalent pore radius," as defined by Paganelli and Solomon (12), and the factor (1 − \( \sigma \)). Durbin (4) investigated the \( \sigma \) for several molecules in three artificial membranes and found good agreement between experimental results and the theory relating equivalent radius and 1 − \( \sigma \).

In previous study (24) using the isolated lung preparation we determined the permeability coefficients of several small lipid-insoluble substances across the pulmonary membrane (pulmonary capillary + alveolar membrane), and concluded from our results that the alveolar membrane was the limiting barrier between blood and alveoli. Also, other studies (23) from our laboratory involving measurements of filtration coefficients have indicated that the pulmonary capillary is relatively more porous than the alveolar membrane. In addition to our findings, Schneeberger-Keely and Karnovsky (18) have recently demonstrated that horseradish peroxidase (mol wt = 40,000) passed through the capillary wall but did not permeate the alveolar membrane. In contrast, studies by Chinard and Enns (2) have shown that only a small difference exists between the extraction of a blood indicator (albumin-¹³¹I) vs. ²²Na in a single pass through the pulmonary circulation, which finding could be attributed to either a very impermeable pulmonary capillary membrane or a very small extracellular volume for ²²Na.

The purpose of the present study has been to determine experimentally 1) whether or not the pulmonary capillary membrane is permeable to small solutes, and 2) if the pulmonary capillary or alveolar membrane represents the primary barrier to diffusion of small molecules across the blood-alveolar barrier. Since the measurement of \( \sigma \) and equivalent pore radius has allowed membrane physiologists to compare different membranes, we believed that a measurement of osmotic transients across the pulmonary capillary in air-filled lungs as compared to osmotic transients in fluid-filled lungs would yield basic membrane parameters which would allow us to establish differences between the solute permeability characteristics of the two pulmonary membranes (25).

There is also the possibility of \( \sigma \) being negative, and in this case the solute is more permeable to the membrane than the solvent (10).
The isogravimetric method was used in this study in order to measure weight changes in an isolated lobe of the dog's lung as in previous studies (6, 7). Figure 1 is an illustration showing the experimental apparatus. The lung was suspended on one end of a sensitive beam-type balance in order to measure weight changes. A counterbalancing weight was placed on the opposite end of the balance to allow increased sensitivity in measuring changes in lung weight. A Grass type FT-10 force-displacement transducer was connected to the balance and the weight changes were recorded on a Grass polygraph. The lower left lobe of a dog's lung was perfused through the pulmonary artery, and the perfusate emptied into a venous reservoir. An especially designed (Mariotte) bottle containing the osmotically active substance to be tested was connected to the perfusion system at the input of the pump for the purpose of maintaining a constant infusion pressure. This allowed rapid changeover of the perfusing solutions without affecting the perfusion pressure or flow rate. The perfusate containing the osmotic substance was kept well mixed by a magnetic stirrer. The perfusing system was designed so that the outflow at the venous side could be drained into a container while the perfusing solutions were being exchanged. During the actual process of recording the osmotic transient special care was taken to prevent any abnormal pressure variations which might occur in the arterial and venous pressures as a result of changing the input pressure head to the perfusion pump. Flow was measured by counting volume calibrations on the perfusing bottle during the time that the perfusate was being changed. Arterial and venous pressures were recorded on the Grass polygraph using Statham pressure transducers.

Large mongrel dogs were anesthetized with 30 mg/kg of pentobarbital, and 10,000 units of heparin were given intravenously to each animal. A cannula was inserted into the femoral artery, and blood was collected rapidly from the cannula. The lower left lobe of the dog's lung was then excised and connected to the perfusing system after weighing.

In one series of experiments the blood collected from the animal was used as a perfusate. In another series the blood was centrifuged, and the plasma was collected. This plasma was mixed with an equal quantity of 6% dextran-Tyrode solution and used as the perfusate. In addition, prior to perfusion some lungs had been completely degassed, and the air side filled with 75 ml of Tyrode solution at a hydrostatic pressure equal to 1 cm H2O.

All experiments were carried out at room temperature (approximately 24 C). The perfusate was oxygenated throughout the course of the experiment by slowly bubbling a 95% O2-5% CO2 mixture through the perfusate in the venous reservoir. With the exception of lungs which were filled with Tyrode solution, the lung remained collapsed and at minimal gas volume with alveolar pressure equal to zero.

Once the lung had been adjusted to obtain an isogravimetric state, a filtration coefficient was obtained by the procedure previously described (23). The lung was then allowed to attain a new isogravimetric state. The homologous perfusate contained in the external infusion bottle was circulated through the lung several times and then returned to the bottle. This procedure insured that all perfusing solutions were homogenous and isosmotic in composition prior to the addition of the test substance for the purpose of obtaining an osmotic transient recording. An aliquot of the solution containing the osmotic test substance at a known concentration was then placed into the perfusate contained in the external infusion bottle. Then the interconnected tubing was opened and the test substance allowed to enter the lung perfusing system's arterial segment. After the test substance had arrived at the exchange vessels of the lung, an osmotic transient could be observed. Reagent grade urea, glucose, and sucrose were used to prepare stock solutions of the osmotic test substances.

RESULTS

Pulmonary Capillary Reflection Coefficients

The equation relating volume flow across the capillary (Jv,c) to the hydrostatic pressure (ΔP̄) and colloidal osmotic pressure difference (Δπi) across the capillary is:

\[
J_{v,c} = L_{p,c}(\Delta P - \sigma_i \Delta \pi_i) - L_{p,ci} \Delta \pi_i
\]

where \(L_{p,c}\) is the hydraulic conductivity of the pulmonary capillary membrane and \(\sigma_i\) is the reflection coefficient of the plasma colloids (3). When a diffusible substance is added to the system equation 1 expands to:

\[
J_{v,c} = L_{p,c}(\Delta P - \sigma_i \Delta \pi_i) - L_{p,ci} \Delta \pi_i
\]

where the subscript \(i\) refers to the test substance. When the lung is in an isogravimetric condition (\(\Delta P = \sigma_i \Delta \pi_i\)) is equal to 0, and equation 2 reduces to:

\[
J_{v,c} = -L_{p,ci} \Delta \pi_i
\]

so that the reflection coefficient of the substance \(i\) is related to the volume flow and osmotic pressure by rearranging equation 3:

\[
\sigma_i = J_{v,c}/L_{p,ci} \Delta \pi_i
\]

Figure 2 illustrates the osmotic transients obtained in an isolated lung for urea, glucose, and sucrose for the concentration of each test species indicated. Equation 4 is a stationary-state equation which describes only the initial change. In order to calculate the reflection coefficients by this equation and also to know \(\Delta \pi_i\), the curves shown in Fig. 2 must be extrapolated to zero time. This can be ac-
FIG. 2. Osmotic transients recorded for urea, glucose, and sucrose across pulmonary capillary. Curves differ in their initial slopes and duration. \( \sigma \) was calculated from each transient and are shown above each respective curve. Note particularly that as \( L_p \) decreases, \( \sigma \) increases.

FIG. 3. Successive determinations of glucose transients in same lung. Initial slopes do not vary from one transient to another by more than 10%.

Flow rates were relatively high (150–400 ml/min) in all experiments, and this necessitated reusing all the perfusate upon completion of each osmotic transient. Prior to initiating another osmotic transient the entire perfusate was thoroughly remixed several times to insure osmotic equilibrium. The next test substance could then be added to the perfusate and another osmotic transient recorded. Any experiment that demonstrated a change in arterial or venous pressure during the course of an osmotic transient was discarded since this indicated that the initial isogravimetric state of the lung had been lost.

It is especially important to note that the osmotic transients in Fig. 2 persisted for only a very short time interval, indicating that the extravascular space equilibrates very rapidly with the test molecules. The osmotic transients measured by Pappenheimer et al. (13) in skeletal muscle were of a much longer duration, reflecting the larger extravascular distribution of their test molecules in skeletal muscle relative to lung tissue.

Reproducibility of \( \sigma \) calculations. The same test molecule was used repeatedly in several experiments in order to determine the reproducibility of the measurements. Figure 3 shows one such determination for glucose. All four initial slopes yield similar values for \( J_{v,c} \). At least two different test molecules were always observed in the same lung, and in no instance was any deviation found from the sequence \( \sigma_U < \sigma_G < \sigma_S \). Experiments which lasted for more than 3 hr finally began to show some changes in all osmotic transients. However, Fig. 2 was chosen to illustrate that from lung to lung, the \( \sigma \) values would vary with the \( L_{p,e} \), i.e., small \( L_{p,e} \) would yield higher \( \sigma \), and vice versa. The \( \sigma \) values for the three molecules are larger than the average values given in the following section because of the smaller \( L_p \) for this particular lung.

\( \sigma \) values for pulmonary capillary membrane. In 22 lungs \( \sigma \) for urea, glucose, and sucrose was measured for the pulmonary capillary membrane using a plasma-dextran perfusate. The average values \( \pm \text{SEM} \) for each substance were .018 \( \pm .003 \), .026 \( \pm .002 \), and .044 \( \pm .004 \), respectively. This indicates that these substances exert only 2–5% of the calculated Van't Hoff osmotic pressure across this membrane.

In another series of experiments the \( \sigma \) values for urea and glucose were investigated in eighteen lungs that were perfused with whole blood. The \( \sigma \) values found for urea and glucose were .019 \( \pm .002 \) and .025 \( \pm .003 \), respectively.
There was no statistical difference found for the calculated $\sigma$s between plasma-dextran and whole-blood perfusates.

Effect of blood flow on calculated $\sigma$ values. Since the rate of pulmonary blood flow could possibly affect the $\sigma$ calculations, $\sigma$ vs. flow was plotted for all experiments. It was found that there was no discernible tendency for the $\sigma$s to be either smaller or larger for flows greater than 150 ml/min, which was the lowest flow in this series of lungs. In fact, in several experiments in which $\sigma$ was determined for the same molecule and flow was altered, the variability was such that the lower flows sometimes yielded higher $\sigma$s.

Lipid-soluble substances and osmotic transients. In five experiments 20 mM antipyrine was added to the perfusing medium and no osmotic transient was observed. In this series we utilized Renkin's experimental method (16) (for antipyrine in a hindlimb preparation). For example, we first obtained a urea transient and then placed antipyrine into the perfusing medium and no osmotic transient was observed. Next both urea and antipyrine were simultaneously added to the perfusing medium, and an osmotic transient occurred which was very similar to the first urea osmotic transient observed. In one experiment an increase in arterial pressure was observed following addition of antipyrine; however, in the other four experiments no such pressure variation occurred. The fact that no osmotic transient was observed with antipyrine indicates that the capillary membrane possesses the fat-soluble nature found in most membrane systems.

Osmotic transients of larger molecules. In several experiments we attempted to obtain osmotic transients which should be produced by larger-type molecules, such as polyethylene glycols and albumin, across the pulmonary capillary membrane. However, when any substance capable of exerting an appreciable osmotic pressure was added to the perfusate, the arterial pressure always became elevated. This effect is unexplained; however, other investigators (5, 15) have observed a similar effect on pulmonary arterial pressures when using hyperosmotic solutions in the presence of red cells. We observed this effect with a plasma-dextran perfusate which contained only a small quantity (Hct < 5.0) of red cells in the perfusate. From our own observations it appears that the most important factor which could cause this change in arterial pressure is how much fluid an osmotically active substance is able to pull, i.e., the effective osmotic pressure exerted at some point in the pulmonary circulation rather than the calculated osmotic pressure. Probably the reason that such a large amount (5%) of NaCl is required to initiate this response is because of the low $\sigma$ for NaCl across the pulmonary capillary, which we found to be $0.023 \pm 0.004$ SEM. However, we did not use the results obtained with NaCl for the subsequent determination of equivalent pore dimensions because the analysis presented is only valid for uncharged molecules.

Alveolar Membrane Reflection Coefficients

In one series of experiments the alveolar side of the lung was completely filled with Tyrode solution after complete degassing had been produced using the oxygen method described by Kylstra (11). In the fluid-filled lung the osmotically active molecules must diffuse across both the capillary and alveolar membranes, a fact which makes the actual system analysis much too complex to obtain any experimental information. When the osmotically active substance reaches the capillary exchange vessels of the lung an initial weight change is observed which results from a pull of fluid out of the interstitial spaces. This is quickly followed by a much larger weight change resulting from a pull of fluid from the fluid-filled alveoli. To facilitate analysis of the data the capillary transient curve was subtracted from the alveolar osmotic transient curve, and the difference curve was used to calculate $\sigma$.

The upper series of curves in Fig. 4 are plots of weight per unit osmotic pressure per 100 g of lung tissue observed in six fluid-filled lungs (solid lines), and the lower curves (dotted lines) represent the average of all osmotic transients previously observed in lungs that were not fluid filled. The respective curves for urea, glucose, and sucrose can be seen to have a relatively constant initial slope for about .5 min. We assumed that this initial slope represented the maximum volume pull from the alveolar fluid which occurred when the difference in osmotic pressure between blood and pulmonary interstitial fluid and alveolar fluid ($\Delta\pi_{alv}$) is equal to the

![Image](https://via.placeholder.com/150)

**Fig. 4.** A plot of unit weight gain as a function of time in fluid-filled lungs for urea, sucrose, and glucose. Osmotic transient measured in fluid-filled lungs was corrected for difference in surface area per gram of lung tissue so that this transient could be compared with capillary transient (700 cm$^2$/g for fluid-filled, 480 cm$^2$/g for non-fluid-filled) (26). This tends to make calculated $\sigma$s approach a minimum value. Solid line represents average osmotic transient for non-fluid-filled lungs. Dashed line results from subtraction of capillary curve from curve representing fluid-filled lungs.
The permeability coefficient of sucrose was calculated by this equation for urea, glucose, and sucrose and found to be .55 ± .05 for sucrose were followed for 1 or 2 hr by allowing the alveolar fluid to accumulate only 8-10% of the initial radioactivity of the blood, indicating that the alveolar membrane is only slightly permeable to sucrose. This was further investigated by the addition of radioactive sucrose (14C labeled) to the perfusing medium of three fluid-filled lungs in order to measure the permeability coefficient of this molecule across the alveolar membrane. After 2 hr it was found that the alveolar fluid had accumulated only 8-10% of the initial radioactivity of the blood, indicating that the alveolar membrane is only slightly permeable to sucrose. The permeability coefficient of sucrose was calculated from this data and found to be .55 ± .05 SEM × 10^{-7} cm/sec. This approach is oversimplified because the molecules begin to exert their osmotic pressure across the alveolar membrane immediately on crossing the capillary barrier, and the concentration gradient at this time is unknown. However, at least four experimental findings indicate that the approach is still useful for the estimation of the solute radius for all three molecules tested, as shown in Fig. 5A for urea, glucose, and sucrose. The data is best described by the curves shown for a pore radius of 8-10 A. (SEM are shown for 1 - σ and were calculated from the curves of Fig. 4.)

Figure 5B is a replotted of Durbin's data (4) for three membranes in which he calculated pore radius and measured σ for several molecular species. It should be emphasized here that we did not plot theoretical curves in the same manner as described above for the estimation of the equivalent pore radius of the alveolar membrane. The reason for this is discussed later. When the data is plotted on the curves obtained by Durbin a pore size of 40-80 A can be estimated for the pulmonary capillary membrane.

DISCUSSION

One of the strongest arguments for using the nonequilibrium thermodynamic approach to measure various membrane parameters is the fact that a model need not be assumed for the membrane. This is adequate for well-stirred artificial membranes; however, when one then uses σ to estimate equivalent pore radius in a flowing, nonhomogeneous membrane system several important physical and physiological factors need to be considered.

Concentration Gradient Down Capillary

When σ is calculated the diffusion force acting across the capillary to cause fluid movement must be known. The only force known in our study is the initial osmotic pressure of the perfusate. If the molecules tend to diffuse out at the arterial end of the capillary so that the venous end has a relatively low concentration of the test substance, then the calculation of sigma would be much too small. We varied the flow through the lung to test this possibility and did not see any consistent changes in the osmotic transients even with large flow rates. This finding could be attributed to at least two things: 1) The test substance is exerting its full osmotic pressure across the capillary, or 2) the substance always has a large gradient along the capillary, and even at large flow rates a state cannot be reached in which no difference in the gradient exists along the capillary.

Vargas and Johnson (26) computed equivalent pore radius in the isolated heart using the thermodynamic approach. Their porc calculation was similar in magnitude to the value obtained by Pappenheimer in the hindlimb. Kedem and Katchalsky (10) computed σ from the hindlimb data of Pappenheimer which were very small (Table 1); in fact, if these σ were used to compute equivalent pore radius then a pore size of 140 A would be calculated. This is more than 3 times that calculated by Pappenheimer using a different computational procedure. Pappenheimer did show differences in osmotic transients at different flow rates for continuous recirculation of the perfusate. During this entire time the lung continued to gradually lose weight.

Calculation of Equivalent Pore Radii

The factor 1 - σ for the alveolar σ was plotted as a function of the solute radius for all three molecules tested, as shown in Fig. 5A. This gives an estimation of the equivalent pore radius for each membrane. The theoretical curves were calculated using Renkin's equation (17) for a pore radius of 6, 8, and 10 A. The data is best described by the curves shown for a pore radius of 8-10 A. (SEM are shown for 1 - σ and were calculated from the curves of Fig. 4.)
Calculation of Equivalent Pore Radius of Capillary

Durbin found that $1 - \sigma$ was equivalent to area available to solute/area available to water ($A_s/A_w$) in several artificial membrane systems. Recently, Solomon (20) has reviewed the computation of equivalent pore radius by this procedure and investigated the term neglected in assuming that $1 - \sigma$ is equivalent to $A_s/A_w$. For the dialysis tubing data of Durbin (4), it was found that the calculated pore size was only slightly affected by neglecting this term. However, for larger pores this term could become more important. Also, there always exists an unstirred layer effect when studying diffusion of molecules across a barrier. This certainly affects calculations made across highly porous structures.

In deriving $A_s/A_w$ the assumption is made that the solute and water diffuse through the same area. Now, if $A_w$ is actually larger than $A_s$, then a plot of $(1 - \sigma)$ using the Renkin equation (16) for the reference plot will be in error. Yudilevich (27) has recently demonstrated that water passes through the capillary endothelial cells; therefore, the approximation of $1 - \sigma$ to $A_s/A_w$ as defined by Renkin is questionable in capillaries.

Obviously, we can calculate only an approximate pore size for the pulmonary capillary membrane and, as an alternative, we have plotted our data on the curve obtained by Durbin (4) in artificial membranes. One reason for choosing this approach is because we are actually plotting the ratio of bulk flow caused by an osmotic gradient to that caused by a hydrostatic gradient and comparing our results to those of Durbin's. This allows us to at least approximate the range of pore sizes in the capillary.

The pore radius as approximated by this method lies between 40-80 Å. A pore size is also computed in the APPENDIX using the single-pass data of Chinard (2) for water. This later pore calculation is obviously a maximum one since the unidirectional flux of water across the pulmonary capillary by this simple calculation is underestimated. Therefore, the pore range is most likely between 40-58 Å. However, the possibility that the pulmonary capillary has a larger equivalent pore radius cannot be ruled out in view of the recent data of Boyd et al. (1), who calculated a pore radius greater than 100 Å for the pulmonary capillaries of sheep.

Calculation of Equivalent Pore Radius of Alveolar Membrane

The calculation of the equivalent pore radius of the alveolar membrane is not necessarily subject to the same theoretical arguments presented for the capillary. The osmotic forces in the interstitial lung tissue during an osmotic transit are entirely unknown; however, the pore radii probably are not too different from the calculated estimate because the $\sigma$s are approaching unity. The low permeability of sucrose across the double-membrane system further substantiates this concept.

In addition, a previous study (23) has established that a large difference exists between the conductances of the alveolar and capillary membranes. Table 1 lists the conductances, permeability coefficients, and $\sigma$s obtained across fluid-filled lungs. The alveolar membrane has permeability characteristics which are similar in magnitude to those de-
termed in cellular membranes, whereas the pulmonary capillary has characteristics of other capillary beds.

The recent data of Schneebeger Keely and Karnovsky (18) indicate that the major barrier to diffusion across the blood-air barrier is the junction between alveolar lining cells. One would expect this barrier to exhibit passive transport characteristics similar to other organs which are lined with epithelial cells and possessing equivalent pore radii in the range of 4–10 Å.

It has not been possible in the present study to establish precise pore sizes for the pulmonary capillary membrane; however, there is no doubt that small lipid and lipid-insoluble molecules leave the pulmonary circulation very easily, and that the alveolar membrane represents the major barrier to the diffusion of small lipid-insoluble substances across the alveolar-capillary membrane.

APPENDIX

Calculations of pore radius.

According to Renkin (17):

\[
r = \left( \frac{8Lp\eta}{A_w \Delta x} \right)^{1/2}
\]

where

\[
A_w = \frac{\bar{V}_w}{\Delta x} = \frac{D_w}{\eta}
\]

where \( \bar{V}_w \) is the unidirectional flux of D₂O moles/sec·cm²

\( \Delta x \) is the partial mole volume of water cm³/mole

\( D_w \) is the diffusion coefficient of D₂O in free solution (2.3 × 10⁻⁶ cm²/sec)

and

\( L_p \) is the hydraulic conductivity of the pulmonary capillary

\( \eta \) is the viscosity of water (1.0 × 10⁻⁵ dyne·sec/cm²)

REFERENCES


