An Estimate of Reflection Coefficients for Rabbit Heart Capillaries

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ABSTRACT Isolated perfused rabbit hearts have been used to determine the reflection coefficients, σ, of the heart capillaries to certain lipoid-insoluble substances. This was done by initially perfusing the heart with a Ringer solution containing no test molecule and then suddenly switching to a solution which differed from the original only by containing a small amount of test substance. This produced a loss of weight of the heart which was continuously recorded as a function of time. Taking the "zero" time rate of weight change and using an equation given by Kedem and Katchalsky reflection coefficients for urea, sucrose, raffinose, and inulin were obtained. These turned out to be 0.1, 0.3, 0.38, and 0.69 respectively. Using the approach of Durbin and Solomon equivalent pore radii were estimated to be about 35 Angstroms.

INTRODUCTION

The structure of the capillary wall and the mechanisms of permeation through it have been controversial subjects among researchers in the field. While some postulate that the passage of water and lipoid-insoluble molecules is across aqueous channels or pores (1), others hold the view that this pathway is across the endothelial cells (2, 3).

The aim of this paper has been to obtain independent evidence about functional aspects of the wall. To this end the reflection coefficient, σ, has been determined for different lipoid-insoluble molecules. These experimental values of σ have been compared with the ones calculated from equations derived for porous membranes. The fact that the experimental values of σ follow the general shape of the curves derived from these equations seems to provide further evidence which supports the concept that the pathway for lipoid-insoluble molecules is a system of aqueous channels. The equivalent size of these pores was determined after the manner of Durbin (4) for artificial membranes, and of Solomon (5) for cell membranes.

METHOD OF APPROACH

Theoretical

Kedem and Katchalsky (6) have given an equation for volume flow across membranes, *i.e.*

$$J_v = L_p \Delta P - L_p \sigma RT \Delta C \tag{1}$$

where J_v = flow per unit area; L_p = filtration coefficient; ΔP = pressure difference across the membrane; RT = gas constant times absolute temperature; ΔC = concentration difference of the solute across the membrane.

This equation has been derived for the case of a bicomponent solution (*i.e.*, solvent and one solute). It may be used, however, in the case of multicomponent solutions when the composition and concentration are identical except for one component.

Equation (1) may be multiplied by the area becoming

$$\dot{Q} = K_f \Delta P - K_f \sigma RT \Delta C \tag{2}$$

where $\dot{Q} = J_v \times$ area and $K_f = L_p \times$ area. σ can be obtained from equation (2) for the case when $\Delta P = 0$.

$$\sigma = \frac{\dot{Q}/\Delta C}{K_f RT} \tag{3}$$

In this derivation ideal solution behavior has been assumed. This assumption seems to be reasonable in the present experiments in which very low concentrations of the osmotically active solute have been used.

Equation (3) is our working equation and we have devised experiments to obtain $\dot{Q}/\Delta C$ and K_f for the capillaries of the rabbit heart.

Experimental

ANIMALS, MATERIALS, AND TECHNIQUE

(*a*) *General* Rabbit hearts were suspended from a recording balance and perfused by means of a cannula tied into the aorta. The weight of the heart was continuously recorded on a twin channel recorder. Changes in the osmotic activity of the perfusion solution produced filtration or absorption of fluid to or from the heart. The rate of these processes was assumed to be equal to the weight change in time. This amounts to assuming that there was no accumulation of fluid in the heart chambers or vascular bed during the time of the transient. Accumulation in the heart chambers was prevented by letting the ventricles drain to the outside through plastic cannulae placed in the ventricles. Vascular volume changes were

assumed to be unimportant because of the minor changes in arterial pressure and arteriolar resistance during the course of the transient.

(*b*) *Detail*

1. *Animals and operative procedure* Rabbits (of both sexes) 2 to 3 kg in weight were anesthetized with 1.5 to 2 cc of 5 per cent nembutal injected into the lateral vein of the ear. This was followed by 2 cc of heparin (1,000 units/cc) put into the same vein for prevention of clotting.

The anesthetized animals were tied and the thorax was opened. After removing the thymus the pericardium was opened and a ligature passed around the aorta. The ligature was kept loose and gently pulled while an incision was made about 2 cm from the aortic valves. A cannula filled with Ringer's solution was inserted through the incision and the ligature was firmly tied around the cannula. Immediately after this the heart was removed from the thorax by severing its connections and perfusion was started. Through the pulmonary artery and through a slit cut in the left auricle two cannulae were passed into the ventricular chambers. The ventricular walls were perforated by the tips of these cannulae which were left as drains to prevent the accumulation of fluid inside the heart chambers. After this procedure was completed the heart was suspended from the arm of the balance by a string. Two platinum wires were then inserted into the heart, one to the aorta and the other into the ventricular wall. The platinum wires were connected to a Grass stimulator which kept the heart beating at a constant frequency of 1.5 cycles per second. 1 to 5 volts proved adequate to keep the heart electrically driven.

2. *Solutions* The Ringer solution contained per liter the following quantities: 149 mEq Na^+, 2.5 mEq K^+, 3.5 mEq Ca^{++}, 2.5 mEq Mg^{++}, 143 mEq Cl^-, 2.5 mEq $SO_4^=$, 12 mEq HCO_3^-, and 5.5 mM glucose. The solutions containing the test molecules were of the same composition as the Ringer solution except that a test molecule was added in the amount required by the particular experiment. The molecular species used were urea, sucrose, raffinose, and inulin.

Albumin solutions were prepared in the same way as the solutions containing test solutes. The concentration used was 4 gm per 100 cc of solution. The albumin used was bovine albumin in Ringer's solution from Mann Research Laboratory, Inc.

3. *Perfusion system* A Sigma pump was used to drive two separate perfusion systems in parallel. Each system consisted of a reservoir of 1 to 2 liters in volume connected to a filter by a rubber tube. Another tube emerged from the filter and passed through the bubble trap and the rest of its length was coiled inside a thermostated bath. The last part of the tube after leaving the bath was divided into two channels, one going to the cannula, the other back to the reservoir.

Any one of these channels could be opened while the other was simultaneously closed by the use of hemostats. In this way the solution flowing through one side of the system could be diverted to the cannula and into the heart while the solution in the parallel perfusion system was driven back to its reservoir.

The Sigma pump is designed to keep a constant flow in spite of resistance changes along the system. Thus, to increase the perfusion pressure the flow was increased.

4. *Flow through the heart* In our experiments an attempt was made to keep the flow high enough to prevent its limiting the rate of solute transfer across the capillary

wall. The flow which was to be used with each test solute was determined from experiments in which osmotic transients were obtained at different flows. The half-times of these transients were plotted against the flow. The results showed that in order not to influence the rate of the osmotically induced transients the flows should be higher than 60 ml/min. for molecules of the size of urea and higher than 30 ml/min. for inulin. Flow was measured by collecting the fluid coming out from the heart in a graduated cylinder for 60 seconds. Special care was taken that the flow be the same in both perfusion systems throughout the experiment.

5. *Pressure* The pressure in the system was recorded close to the aortic cannula. A needle connected to a strain gauge was inserted in the rubber tube which held the cannula. The strain gauge in turn was connected to an amplifier and paper recording system. At the end of each experiment a pressure calibration was made with a water-filled manometer connected to the aortic cannula. In most of the experiments the pressure was about 50 cm of water.

6. *Temperature* The temperature was kept constant at 27 ± 1°C in the perfusion system. For this purpose a container filled with distilled water could be heated at will. This water circulated between this container and the bath in which the tubes were immersed.

The immediate environment of the heart was kept at room temperature which varied between 24–26°C during the period of our experiments.

7. *Heart weight* The weight of the heart was continuously recorded. The balance consisted of a movable horizontal metallic bar, the center of which was attached to a Grass strain gauge. The movements of the bar were transduced into electrical signals by the strain gauge. A Sanborn twin viso recorder was used to record the signals.

The heart was suspended at one extreme of the bar while the other extreme was attached to a damping vane immersed in glycerin. During an experiment with the heart in position the balance was first brought to a null position by placing weights on either end of the bar and then the balance was calibrated by hanging weights of ¼, ½, and 1 gm at the end where the heart was suspended. This calibration was carried out several times during an experiment. The deflections recorded were linearly related to the weights. The absolute weight of the heart was obtained by weighing it on an analytical balance at the end of the experiment. Adding or subtracting any gains or losses during the experiment gave the weight at any time t.

8. *Measurement of osmotic pressure* The osmotic pressure developed by the albumin solutions was measured with two types of osmometers:

(*a*) *Capillary osmometer:* A collodion bag was tied to a stopper holding a capillary tube. The bag was filled with albumin solution and immersed in water. Attainment of equilibrium was accelerated by applying some pressure to the bag's contents with a syringe until the column in the capillary rose to the expected equilibrium level. Stabilization of the height of the column was obtained at the 2nd day of observation. These experiments were done at room temperature (25 ± 1°C).

(*b*) *Strain gauge osmometer:* A Statham strain gauge head was modified into a plastic cup. A collodion membrane was attached between the cup and a lower compartment, the bottom of which was formed by the metallic membrane of the

strain gauge. The strain gauge was connected to a Sanborn twin viso instrument for amplification and recording. To measure the osmotic pressure, the lower compartment was filled with Ringer's, then the membrane was put in place over a wire net which formed the roof of the lower compartment. The plastic cup was then tightly screwed and filled with the albumin solution. A negative pressure was then applied to the now sealed lower compartment. This negative pressure was made greater or about the same as the osmotic pressure developed by the albumin solution facilitating the attainment of equilibrium. After some 20 to 30 minutes the record was stable. At this point the membrane was punctured and the deflection caused in the record measured. In each experiment the strain gauge was calibrated with a water manometer. The measurements were made at room temperature which was approximately 24°C.

TABLE I

FILTRATION COEFFICIENT OF HEART CAPILLARIES

Heart weight	$\dot{Q}$	ΔP_0	K_f
gm	*cm³/sec.* × *10³*	*cm H₂O*	*cm³/sec./cm H₂O)* × *10⁴*
7.28	3.42	13.7	2.50
9.00	4.82	15.3	3.15
8.40	5.00	14.6	3.43
6.45	3.90	16.1	2.42
7.10	5.10	16.1	3.16
6.59	2.93	14.5	3.16
5.73	4.13	15.3	2.87
5.89	5.50	16.1	3.41
6.74	5.02	16.1	3.14
Mean 7.02	4.70	15.3	3.02

The results from the two osmometers agreed to within 3 per cent on the average.

Determination of K_f The hearts were perfused with a solution of albumin in Ringer's with a colloidal osmotic pressure of approximately 16 cm of water. The pressure of the system was raised to a level just enough to prevent filtration or absorption of fluid into or from the heart. After hydrostatic and osmotic pressures were equal the weight of the heart remained constant. At this point a switch was made from the albumin in Ringer's solution to albumin-free Ringer's while the pressure of the system was kept unchanged. As a result of this switch the hydrostatic pressure in the capillaries was essentially unchanged, but it was no longer balanced by an osmotic pressure and filtration into the extracellular space of the heart occurred. The rate of filtration at zero time was recorded as weight change per unit time. The initial hydrostatic pressure difference (ΔP_o in Table I) across the capillary wall was evaluated from the osmotic pressure of the albumin solution which had previously exactly opposed its action (*i.e.*, during the isogravimetric period).

Osmotic flows ($\dot{Q}$) The switch from the Ringer solution to the Ringer containing a test solute produced volume flow across the capillary walls due to the

hyperosmolarity of the testing solution. This osmotic flow was recorded as a weight change of the heart in time. The course of the weight change in time has been called an osmotic transient.

Records of the osmotic transients produced by urea, sucrose, raffinose, and inulin are shown in Fig. 1. The rate of volume change was plotted on semilog paper *versus* time (Fig. 2). The straight line joining the points was extrapolated to zero time.

At time zero the concentration difference across the capillary wall was assumed to be equal to the concentration of the test molecule in the perfusing fluid. This

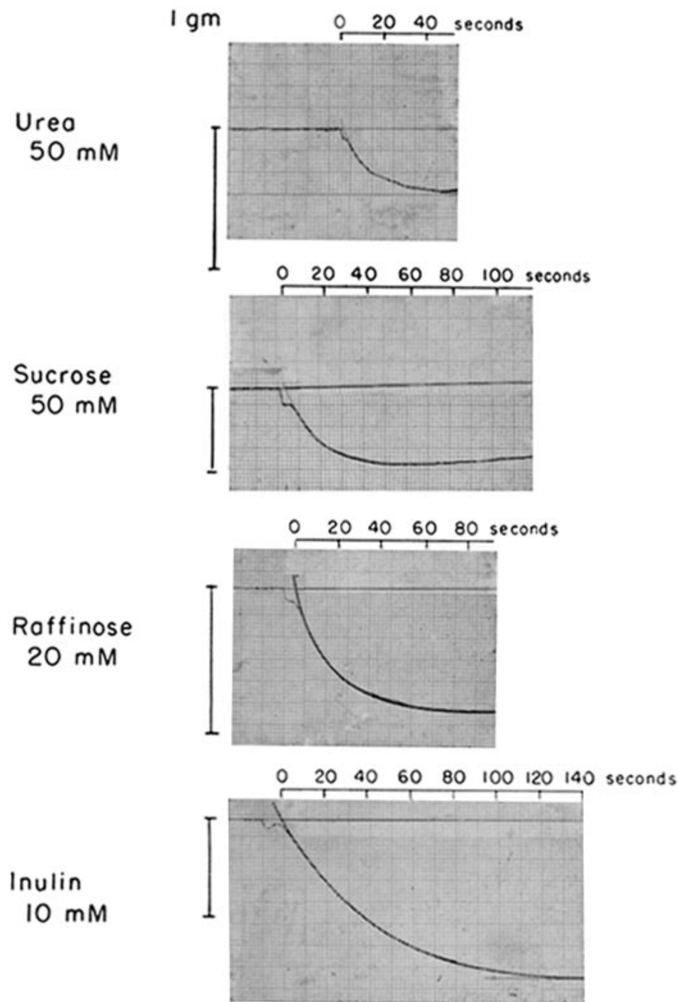

FIGURE 1. Records of the osmotic transients obtained for different substances. The magnitude of 1 gm for each record is indicated at the left by a vertical bar. Time is indicated in seconds. The initial irregularities on the record are switching artifacts. The millimolar concentration of each substance used for the recorded transient is indicated.

assumption is made more plausible because of the high perfusion rates employed. It should be emphasized that extrapolation to zero time, besides enabling one to obtain the initial difference in concentration across the capillary, also permitted the use of equation (3) which has no pressure term in it. At zero time no pressure difference across the capillary existed because the heart was previously in the isogravimetric state.

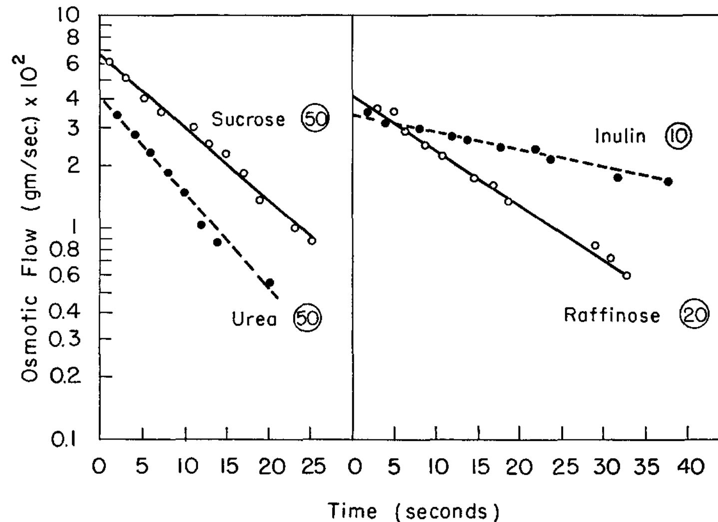

FIGURE 2. A semilogarithmic plot of the rate of weight change as a function of time. The concentration of each substance in millimoles per liter is indicated by the circled number near the corresponding name.

RESULTS

The results are given in terms of the variables which appear in equation (3).

The Filtration Coefficient

The values of the filtration coefficient K_f are shown in Table I. The average was $3.08 \pm 0.12 \times 10^{-7}$ ($\pm$SEM) cm^5 sec.$^{-1}$ dyne^{-1}.

Osmotic Flows

The flow across the capillary wall, $\dot{Q}$, produced by hyperosmotic solution of the test molecules was measured at different concentrations of each test molecule with the exception of urea which was studied only at a concentration of 50 mM. The flow per unit concentration difference was plotted against the concentration. The points fell on a straight line which was calculated by means of the least squares method (Fig. 3). The intercept of each line corresponds to the flow per unit concentration difference at zero concentration. As the test molecules were dissolved in Ringer's the $\dot{Q}/\Delta C$ determined in this

way corresponds to a fluid having the same viscosity as the Ringer solution used for the determination of K_f. As σ is to be obtained from the ratio of two flows, *i.e.* $\dot{Q}/\Delta C$ and $K_f RT$, both must be measured for the same fluid. Other-

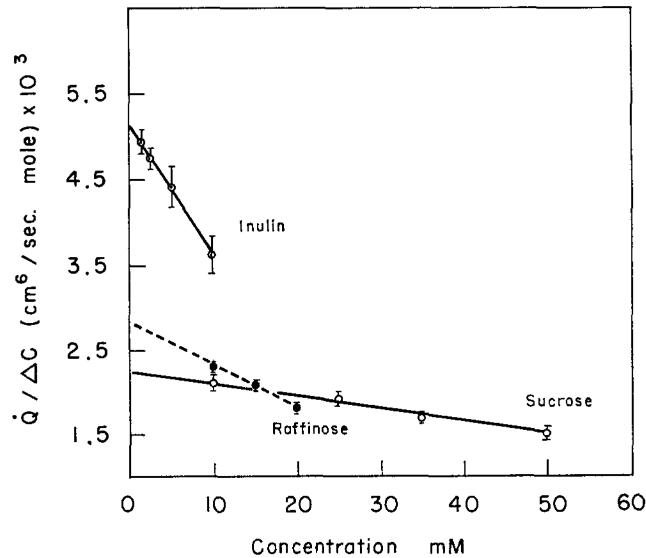

FIGURE 3. Osmotically induced flows as a function of solute concentration. The vertical bars represent a magnitude equal to twice the SEM.

TABLE II
TRANSCAPILLARY FLUID FLOW PER UNIT CONCENTRATION DIFFERENCE

Molecule	$\dot{Q}/\Delta C_o$	Standard error of the mean
	cm^6/sec./mole $\times 10^{-3}$	
Urea	0.74	0.03
Sucrose	2.25	0.01
Raffinose	2.82	0.03
Inulin	5.13	0.03

wise the resistance of the membrane will be different for both flows and an error would be introduced in the evaluation of σ.

The values of $\dot{Q}/\Delta C$ for the four molecules used are listed in Table II.

The Reflection Coefficient

Using equation (3) and the values of K_f and $\dot{Q}/\Delta C$ the magnitude of σ was determined for each of the four solutes. The values are listed in Table III. It can be seen in this table that σ increased as a function of the molecular radius.

An equation has been given by Durbin *et al.* (9) for σ as a function of the areas for filtration of solute, A_{sf} and water, A_{wf}.

$$\sigma = 1 - \frac{A_{sf}}{A_{wf}} \tag{4}$$

TABLE III

Molecule	Molecular radius*	$\sigma \pm$ SEM
	A	
Urea	1.7–2.6	0.10±0.01
Sucrose	5.2	0.30±0.01
Raffinose	6.0	0.38±0.02
Inulin	10.7–15.2	0.69±0.03

* The molecular radii for urea, sucrose, and raffinose were obtained from the paper by Schultz and Solomon (7). The values used for urea were the minimum and maximum values reported in Table III of the paper of Schultz and Solomon (7). For inulin the value of 10.7 was obtained from density and molecular weight determinations carried out on Difco inulin in this laboratory. The molecular weight determinations were made by three different techniques: freezing point determinations, vapor tension determinations, and sedimentation velocity measurements in an ultracentrifuge. These techniques gave an average value of 4600 with a range of 4200 to 5400. The value of 15.2 was taken from the data given by Pappenheimer *et al.* (8) which was said to have been obtained from hydrodynamic data.

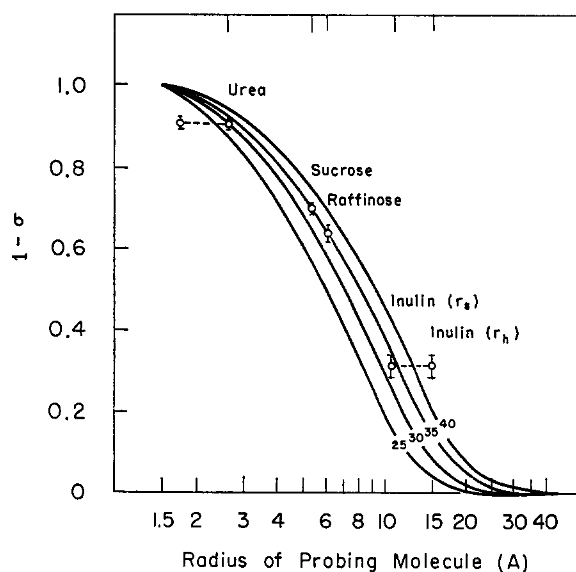

FIGURE 4. Values for $1 - \sigma$ are plotted against the radius of the test molecules. The curves represent solutions of equations (4) and (5) in the text for different values of the pore radii. The bars represent a magnitude of twice the SEM.

Renkin (10) has derived an equation for the restricted area for filtration across a porous membrane.

$$A_f = A_o\left[2\left(1-\frac{a}{r}\right)^2-\left(1-\frac{a}{r}\right)^4\right]\left[1-2.104\left(\frac{a}{r}\right)+2.09\left(\frac{a}{r}\right)^3-0.95\left(\frac{a}{r}\right)^5\right] \tag{5}$$

where A_f is the area for filtration of the test molecule and A_o is the geometric area; a is the molecular radius and r is the radius of the pore. From equations (4) and (5), $(1 - \sigma)$ was calculated for four different pore radii. This was done by taking A_{sf}/A_o and dividing this ratio by A_{wf}/A_o. For a fixed radius of the pore and of the water molecule $(1 - \sigma)$ becomes a function only of the radius of the solute particle. From these calculations curves were constructed for 25, 30, 35, and 40 A radii. As shown in Fig. 4, the curve calculated for a 35 A radius was the one which appeared to give the best fit of the experimental values of σ.

DISCUSSION

The values of σ were obtained for thermodynamic relations and experiments which did not require any assumption regarding the structure of the membrane. The fact that the results fit a curve derived for a porous model provides evidence consistent with the hypothesis that the capillary wall is pierced by aqueous channels. It is possible, of course, that each channel has only a transitory lifetime.

The equivalent pore radius here given corresponds to an idealized model of a membrane having pores of uniform size and cylindrical shape. It seems difficult at the present time to give a detailed morphological interpretation of the results obtained in transport experiments. The equivalent pore radius is a magnitude which accounts for the values of σ found for the solutes tested. In this sense it is more a functional parameter than a morphological entity.

The pore radius here given is in the range of 30 to 45 A given by Pappenheimer *et al.* (8) for skeletal muscle capillaries. However, this agreement should not be stressed because Pappenheimer's values were obtained without the correction suggested by Ussing (11), Grim (12), and Kedem and Katchalsky (6). This point is difficult to resolve because we have no good reflection coefficient to use for this correction. Values obtained by Kedem and Katchalsky from Pappenheimer's data may be in error because Pappenheimer's experiments were not designed to obtain the necessary values of concentration difference and hydrostatic pressure difference across the capillary wall at zero time. If the reflection coefficients calculated by Kedem and Katchalsky are applied to Pappenheimer's data for $A_p/\Delta x$ they produce the

rather absurd result that this ratio (*i.e.*, the ratio of pore area to path length) is over two times larger for inulin than for glucose and sucrose, which have identical values.

It has been claimed by Crone (13) that the capillary wall is not a porous barrier. This claim is based on his finding that the ratio of permeability coefficients of sucrose and inulin is similar to the ratio of the free diffusion coefficients of these same molecules. It is possible that Crone's results reflect the limitations to solute transfer due to low blood flow rather than restrictions at the capillary wall. In the case of low blood flow back diffusion from tissue to capillary becomes of great importance and this has been neglected in Crone's treatment. It has been shown by Johnson *et al.* (14) that flow is a rate-limiting process for the transfer of small molecules like D_2O across both capillary walls and cell walls. Schafer and Johnson (15) have shown that in the case of larger molecules like sucrose flow, if low enough, may become an important factor in determining the over-all rate of transfer.

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