Filtration, Diffusion and Molecular Sieving Through Peripheral Capillary Membranes

A Contribution to the Pore Theory of Capillary Permeability[1]

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THE PENETRATION of capillary walls by water and dissolved substances appears to take place solely by processes which require no energy transformations on the part of the capillary endothelial cells. The rate of net fluid movement across the capillary wall has been shown to be simply proportional to the difference between hydrostatic and osmotic forces acting across the capillary membranes (1, 2). The chemical composition of ascitic fluid (3), edema fluid (4) or glomerular fluid (5) closely resembles that obtainable by filtration of plasma through inert artificial membranes of suitable porosities. Filtration through peripheral capillaries, like that through artificial membranes, varies inversely with the viscosity of the filtrate as this is altered by temperature (6).

These striking similarities between the permeability characteristics of living capillaries, on the one hand, and artificial porous membranes on the other, have given rise to the 'Pore Theory' of capillary exchange. In its simplest form, the pore theory supposes that the capillary walls are pierced with numerous ultramicroscopic openings which are in general too small to allow the passage of plasma protein molecules, but are of sufficient size and number to account for the observed rates of passage of water and nonprotein constituents of the plasma.

Many important questions arise when the pore theory of capillary exchange is examined in detail. The glomerular membranes allow molecules as large as inulin (effective diffusion diameter, d_e = 30 Å) to pass with no detectable hindrance. Egg albumin (d_e = 56 Å) passes rapidly through the glomerular membranes (7–9) and even hemoglobin (d_e = 62 Å) is believed to pass into the glomerular filtrate in appreciable concentration (7, 10). Theoretical and experimental studies on the sieving of molecules by ultrafiltration through porous membranes (11, 12) indicate that an effective pore diameter of at least 100 Å would be required to explain permeability of this magnitude. If this is the case, how are we to imagine the physical structure of the openings in the capillary membranes? Do pores of diameter 100 Å penetrate through the endothelial cells and their plasma membranes on both surfaces? Or are the openings confined to the narrow intercellular regions as postulated by Chambers and Zweifach (13)? In the latter case, is the small area available between cells sufficient to explain the observed filtration rates in such rapidly filtering systems as the kidney? If there is a distribution of pore sizes, or if water is free to

Received for publication May 2, 1951.

[1] This work has been supported by grants from the Life Insurance Medical Research Fund and from the Eugene Higgins Trust. It has also been aided by a three-year Pepsi-Cola Fellowship to E. M. Renkin and a Kellogg Foundation Fellowship to L. M. Borrero.

filter through regions inaccessible to larger molecules, one might suppose that glomerular filtrate would be diluted with respect to inulin, but this is contrary to fact, as indicated by analysis of glomerular fluid (14).

There are many indications that the capillaries in different regions of the body or in different species vary greatly in their permeability characteristics. Can these differences be evaluated quantitatively and simply explained in terms of a pore theory?

In this paper, we shall try to answer many of these questions. The paper is divided into three parts. PART I is concerned with filtration rates; data from several sources are employed to estimate and compare the filtration properties of *a*) the capillaries in the frog's mesentery, *b*) the capillaries in resting mammalian muscle *c*) the glomerular capillaries in the kidneys of frogs and mammals and *d*) collodion membranes of graded porosities. In PART II, a new method is described for determining diffusion rates of molecules through the capillary walls in resting muscle. The transcapillary diffusion rates of various lipoid insoluble molecules are described and the results employed to evaluate the area in the capillary walls which would be required for the transfer of these molecules by free diffusion. In PART III, filtration and diffusion data are combined to evaluate the effective pore dimensions which account, in large part, for the observed permeability of the capillaries to all lipoid-insoluble substances which have so far been studied. Finally, we shall discuss the relations between diffusion and ultrafiltration and show how, in the case of inulin, molecular sieving during ultrafiltration through muscle capillaries may be predicted from diffusion data.

PART I. FILTRATION CHARACTERISTICS OF CAPILLARY MEMBRANES

A. Definitions. Several different 'constants' or 'coefficients' have previously been employed to describe the filtration properties of living capillaries or of artificial porous membranes. Elford and Ferry (15) defined the filtration constant of a collodion membrane in terms of the flow of water (in ml/minute) across 1 cm.^{2} of membrane, 0.01 cm. thick, under a driving pressure of 100 cm. water. Landis (1), working with single mesenteric capillaries of the frog, defined the filtration constant in terms of $\mu^3/\text{sec}/\mu^2/\text{cm.}$ water filtration pressure. Pappenheimer and Soto-Rivera (2), working with the whole hindlimbs of cats, defined a filtration coefficient as the rate of filtration (ml/minute) per 1 mm. Hg pressure difference across the capillary walls of 100 gm. of tissue. A similar definition in terms of rise in venous pressure, rather than of capillary membrane pressure differential, has been used in connection with plethysmographic studies of fluid movement in human extremities (16).

All of these definitions derive from the following equation describing the viscous flow of fluids through inert porous membranes:

$$\dot{Q}_f = \frac{kA_m\Delta P}{\eta\Delta x} \qquad (1)$$

where $\dot{Q}_f$ = quantity filtered per unit time
k = specific filtration constant of the membrane (17)
A_m = area of membrane
ΔP = pressure difference across membrane
Δx = path length through membrane
η = viscosity of filtrate

When expressed in c.g.s. units, k has the dimensions of cm.[2] The physical significance of the specific filtration constant defined above may be simply explained by considering the case of a filter containing N cylindrical pores of uniform radius, r. In this case, the total cross-sectional pore area (A_p) is $N\pi r^2$ and by Poiseuille's Law:[2]

$$\dot{Q}_f = \frac{N\pi r^4 \Delta P}{8\eta\Delta x} = \frac{A_p r^2 \Delta P}{8\eta\Delta x} \qquad (2)$$

Combining *equations 1* and *2* and solving for k, we have

$$k = \frac{A_p}{A_m} \times \frac{r^2}{8} \qquad (3)$$

whence it is seen that k is an expression of the pore dimensions (r) and the fractional area of the membrane occupied by the pores ($A_p \div A_m$). For the case of a membrane containing long rectangular 'pores' of width w, the analogous expression is

$$k = \frac{A_p}{A_m} \times \frac{w^2}{12} \qquad (4)$$

Similar expressions, involving only pore dimensions and fractional pore area, may theoretically be derived for pores or channels of any given geometrical configuration. The full significance of the specific filtration constant for the study of porous membranes lies in its relation to the dimensions and fractional area of the pores as suggested by *equations 3* and *4*.

These equations have been utilized by previous investigators to determine the effective pore diameters of collodion membranes in which the fractional pore area is simply determined from the wet and dry weights of the membranes (15, 18, 19). In PART III below, we shall show how equations related to *2* and *3* or *4* may be utilized in conjunction with diffusion data to evaluate the effective pore dimensions of the living capillaries in mammalian muscle. For the immediate purpose of comparing filtration constants, however, we shall follow Landis (1) in considering a *relative* filtration constant (k') in which the path length through the membrane (Δx) is included with the specific filtration constant. For this purpose *equation 1* becomes

$$\dot{Q}_f = \frac{k' A_m \Delta_p}{\eta} \qquad (1')$$

where $k' = k/\Delta x$ and has the dimensions of centimeters.

B. Relative Filtration Constant of Capillaries in Frog's Mesentery. Landis (1) found that on the average $0.0056\mu^3$/second filtered through $1\mu^2$ of capillary surface when the filtration pressure was 1 cm. H_2O. The viscosity of a protein-free filtrate of frog's plasma at 25° C. is 0.009 poises. When these values are converted to c.g.s.

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[2] The applicability of Poiseuille's Law to tubes of molecular dimensions has been discussed by Elford (12). The effective pore size of the hindlimb capillaries calculated in PART III below from the principle of *equation 2* and the assumption of Poiseuille's Law is about 30 water molecules in diameter. If the viscosity of water is altered during flow through such small channels, then pore dimensions estimated from *equation 2* will be altered in proportion to the square root of the change in viscosity (cf. also *eq. 6*).

units and substituted in *equation 1'*, they yield the following value for the relative filtration constant:

$$k' = \frac{\dot{Q}_f \eta}{A_m \Delta p} = \frac{0.0056 \times 10^{-12} \times 0.009}{10^{-8} \times 980} = 5.1 \times 10^{-12} \text{ cm.}$$

In table 4, this value is compared with similar values estimated for various membranes.

C. Relative Filtration Constant of Capillaries in Mammalian Muscle. Preliminary results for the filtration characteristics of capillaries in the perfused hindlimbs of cats were given by Pappenheimer and Soto-Rivera (2) in terms of the filtration

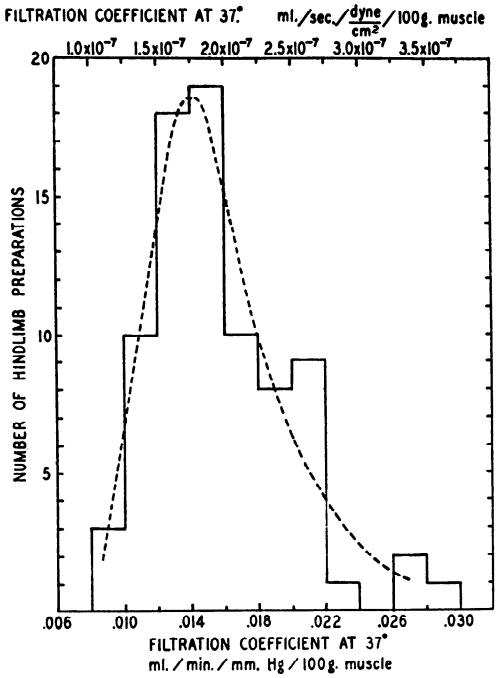


Fig. 1. CAPILLARY FILTRATION COEFFICIENTS in perfused hindlimbs of cats. Data from 81 hindlimbs in which there was no obvious sign of 'abnormality' at time of measurement. It is interesting to speculate upon the possible causes for this distribution curve. If the variations from one experiment to the next were a result of random variations in total capillary area (*not* pore size) the distribution should be a Gaussian one. If, on the other hand, the distribution is due to random variations in mean pore size, it should be skewed in favor of the larger filtration coefficients because filtration rate increases with the third or fourth power of the pore dimensions. The skewed curve of figure 1, therefore, indicates that in preparations with a high filtration coefficient the mean pore size may be higher than average. A filtration coefficient of 0.03 ml/minute/mm. Hg/100 gm. muscle is about the highest value consistent with retention of plasma protein in this preparation.

coefficient defined above. Figure 1 summarizes values obtained from 81 hindlimbs in experiments carried out in this laboratory during the past three years. The values are given per 100 gm. of muscle in the hindlimbs since almost the entire filtration surface is localized to the muscular tissue which comprises 70 per cent of the weight of the preparation (20).

The average value is 0.015 ml/min/1 mm. Hg difference of pressure across the capillary membranes in 100 gm. of muscle. The filtration coefficient defined in this way is proportional to the specific filtration constant and is useful for calculations relating to the fluid exchange in peripheral tissues. However, it cannot be compared with Landis' value for the frog's capillary nor utilized for analyzing the functional structure of the capillary wall without additional information concerning the capillary area. In the muscles of the perfused hindlimb, the capillary area is approximately

70 cm²/gm. of muscle.[3] The viscosity of a protein-free filtrate at 37° C. is 0.007 poises. The relative filtration constant calculated from *equation 1'* is then

$$k' = \frac{\dot{Q}_f \eta}{A_m \Delta p} = \frac{\frac{.015}{60} \times .007}{70 \times 100 \times 1.35 \times 980} = 0.19 \times 10^{-12} \text{ cm.}$$

In table 4, this value is compared with similar values estimated for various membranes.

D. Relative Filtration Constant of Glomerular Capillaries in Frog Kidney. In the case of the frog's mesenteric capillary and the capillaries in the hindlimb of the cat, all the primary quantities required for the calculation of relative filtration constant by *equation 1'* were obtained experimentally. In the case of the glomerular capillaries, similarly complete data do not exist and the filtration characteristics can

TABLE 1. DATA AND ASSUMPTIONS EMPLOYED FOR ESTIMATION OF RELATIVE FILTRATION CONSTANT OF GLOMERULAR CAPILLARIES IN FROG KIDNEY

QUANTITY	VALUE	REFERENCE
1. Diameter of glomerulus	160×10^{-4} cm.	(28)
2. Number of glomeruli in kidneys of 20-gm. frog	4000	(28, 29)
3. Dimensions of red cell	$22 \times 15 \cdot 10^{-4}$ cm.	(30)
4. Mean glomerular pressure	20 cm. H_2O	(31)
5. Protein osmotic pressure	8–13 (11 av.) cm. H_2O	(1, 32)
6. Filtration rate/20 gm. frog (high value)	2.2×10^{-4} ml/second	(28, 33)
Assumptions		
1. Glomerular capillaries occupy ½ the volume of the glomerulus		(34)
2. Diameter of capillaries is slightly larger than the small diameter of red cell (i.e. 16×10^{-4} cm.)		
3. Increase of protein pressure during filtration may be neglected		

only be estimated with the aid of certain reasonable assumptions. The data and assumptions which we have chosen for our estimation are summarized in table 1. *a*) From *quantities 1* and *2* and *assumptions 1* and *2*, the total surface area of the capillaries in the glomeruli of both kidneys is 10.4 cm.². *b*) From *quantities 4* and *5* and *assumption 3*, the pressure difference for filtration across the capillaries is 9 cm. H_2O or about 10^4 dynes/cm.². *c*) From *a* and *b* above and *quantity 6*, we have

$$k' = \frac{\dot{Q}_f \times \eta_{25°C.}}{A_m \times \Delta p} = \frac{2.2 \times 10^{-4} \times .009}{10.4 \times 10^4} = 19 \times 10^{-12} \text{ cm.}$$

[3] This estimate of total capillary surface was obtained by two independent methods: *a*) A hydrodynamic method based on the vascular volume and resistance to flow of the minute vessels in the perfused hindlimb; *b*) injection of dialyzed India Ink, followed by quick freezing and capillary counts in slices from the frozen tissue. Results from these two methods agree reasonably well. However, our estimate of 70 cm.²/gm. muscle is considerably lower than estimates ranging from 175 to 820 cm.²/gm. reported by previous workers (21–27). The details of our methods and reasons for the lower value will be presented elsewhere (20). It should be emphasized here, however, that the higher values estimated by previous workers would make the relative filtration constant and fractional pore area even smaller than our measurements indicate.

E. Relative Filtration Constant of Glomerular Capillaries in Mammalian Kidneys. The filtration characteristics of the mammalian kidney may be estimated from the data summarized in table 2. *a*) From *quantities 1* and *3*, it is found that *per glomerulus* the filtration rates are virtually the same in all species listed. Thus in man, dog, cat and rat, the values are respectively 0.83, 0.85, 0.75 and 0.88 $\times$ 10^{-6} ml/second/glomerulus (average 0.83 $\times$ 10^{-6} ml/second). *b*) From *quantities 4* and *5*, we have an effective filtration pressure difference of $70 - (28 + 10 + 5) = 27$ mm. Hg or 3.6 $\times$ 10^4 dynes/cm.^{2} *c*) From *a* and *b* and *quantity 2*, we have

$$k' = \frac{\dot{Q}_f \times n37°C.}{A_m \times \Delta p} = \frac{0.83 \times 10^{-6} \times .007}{0.75 \times 10^{-2} \times 3.6 \times 10^4} = 22 \times 10^{-12} \text{ cm.}$$

Verney and Rushton (42) have recently calculated a filtration coefficient of 4.6 $\times$ 10^{-4} ml/min/mm. Hg/cm.^{2} for the glomerular capillaries in the kidneys of the dog.

TABLE 2. DATA EMPLOYED FOR ESTIMATION OF RELATIVE FILTRATION CONSTANT OF GLOMERULAR CAPILLARIES IN MAMMALIAN KIDNEYS

QUANTITY	VALUE	REFERENCE
1. Number of glomeruli		
a) man (70 kg.)	2.4 $\times$ 10^6	(34)
b) dog (12 kg.)	1.0 $\times$ 10^6	
c) cat (3 kg.)	0.34 $\times$ 10^6	
d) rat (0.2 kg.)	0.067 $\times$ 10^6	
2. Surface area of capillaries per glomerulus	0.75 $\times$ 10^{-2} cm.^{2}	(34)
3. Total glomerular filtration rate		
a) man (65 kg.)	2.0 ml/second	(35)
b) dog (12 kg.)	0.85	(36)
c) cat (3 kg.)	0.33	(37)
d) rat (0.2 kg.)	0.05	(38)
4. Mean glomerular pressure	70 mm. Hg	(39)
5. Pressures opposing filtration		
a) mean protein	28 mm. Hg	
b) intra-renal	10	(40, 41)
c) pressure for urine flow	5	

When converted to c.g.s. units, this value corresponds with a relative filtration constant of 40 $\times$ 10^{-12} cm. This value is almost double that obtained by our method of estimation, a disagreement which is not surprising in view of the different methods and assumptions used for these calculations. The higher value would accentuate the large differences between the filtration characteristics of glomerular capillaries, as compared with muscle capillaries summarized in table 4.

F. Relative Filtration Constants of Collodion Membranes. In order to compare the filtration characteristics of collodion membranes with those of capillary membranes, it is necessary to assume a nominal value for membrane thickness. Microscopic examination of capillary walls would place their thickness somewhere between 0.1 and 1.0 $\times$ 10^{-4} cm., and we choose 0.3 $\times$ 10^{-4} cm. as a nominal figure which is likely to be correct within a factor of 3. Since this value leads to a relative filtration constant for albumin-impermeable collodion membranes, which is 4000-fold greater

than that of peripheral capillaries, a three-fold variation makes very little difference to the principal conclusions to be derived from the comparison.

Inspection of table 3 shows that collodion membranes with effective pore diameters of 100 to 120 Å are 99 to 95 per cent impermeable to serum albumin. It is this range of protein permeability which is of greatest interest in the comparison with living capillaries summarized in table 4.

TABLE 3. FILTRATION CHARACTERISTICS OF COLLODION MEMBRANES. VALUES CALCULATED[1] FOR MEMBRANE THICKNESS 0.3 × 10^{-4} CM. FROM THE DATA OF ELFORD AND FERRY (15) AND FERRY (43)

EFFECTIVE PORE DIAMETER, Å	SIEVE CONSTANT (φ) FOR SERUM ALBUMIN, $\varphi = \frac{\text{(Alb) FILTRATE}}{\text{(Alb) FILTRAND}}$	FRACTIONAL PORE AREA, A_p/A_m	RELATIVE FILT. CONSTANT × 10^{12}	EFFECTIVE PORE DIAMETER, Å	SIEVE CONSTANT (φ) FOR SERUM ALBUMIN, $\varphi = \frac{\text{(Alb) FILTRATE}}{\text{(Alb) FILTRAND}}$	FRACTIONAL PORE AREA, A_p/A_m	RELATIVE FILT. CONSTANT × 10^{12}
40		0.40	70	110	0.03	0.66	780
60		0.52	190	120	0.05	0.68	980
80		0.58	370	150	0.50	0.73	1700
100	0.01	0.64	630	200	0.70	0.78	3300

[1] Calculated on the assumption that relative filtration constant varies inversely with membrane thickness. The actual thicknesses used for these measurements were 50 μ or more.

G. Conclusions and Discussion Relative to Part I. Table 4 summarizes the relative filtration constants which have been estimated in the foregoing paragraphs. From this table it is clear that membranes which have approximately the same permeability to albumin may have vastly different filtration characteristics. Thus the muscle capillaries are approximately 100-fold less permeable to water than glomerular capillaries and 4000-fold less permeable to water than a collodion membrane of comparable thickness and permeability to protein. On the pore theory of capillary permeability, this wide range of filtration constants could be explained if the fractional pore area available for water transport were smaller in the capillaries than in collodion membranes in inverse proportion to their respective filtration constants as required by *equation 3*. Thus, if the fractional pore area of the collodion membrane is 0.66 (table 3), the fractional pore area in glomerular capillaries would be 21 ÷ 780 × .66 = 0.017, and that in muscle capillaries would be only 0.00017.

TABLE 4

MEMBRANE	RELATIVE FILT. CONSTANT, CM. × 10^{12}
1. Muscle capillary, cat	0.2
2. Mesenteric capillaries, frog	5
3. Glomerular capillaries, frog	19
4. Glomerular capillaries, mammals	22
5. Collodion membrane, 0.3 × 10^{-4} cm. thick and of pore diameter (110 Å) sufficient to retain 95% of serum albumin	780

On the pore theory of capillary permeability, we therefore conclude that the pores involved in the filtration process occupy only a minute fraction of the total capillary surface. Strong evidence in support of this conclusion comes from the evaluation of free diffusion areas for small lipoid insoluble molecules, as described in PART II below.

Part II. Diffusion of Lipoid-Insoluble Molecules Through Capillary Membranes

If an inert lipoid-insoluble molecular species such as sucrose is suddenly added to arterial blood supplying a tissue there results an osmotic disturbance consisting of two related processes, *a*) the added molecules tend to diffuse from the plasma, across the capillary membranes, to the interstitial fluid and *b*) the concentration gradient of added molecules across the capillary membranes results in osmotic withdrawal of fluid from the interstitial compartment into the capillary blood. Both processes continue until the concentration difference across the capillary wall approaches zero as a result of loss of molecules from blood to extravascular fluid and dilution of the blood by the absorbed interstitial fluid. The rate at which these processes take place depends, in part, on the hindrance to diffusion of the added molecules through the capillary walls.

The nature of these processes was recognized by Starling (44) early in his researches on the fluid exchange and he demonstrated that the hemodilution which follows the injection of hypertonic salt solutions, depends on the size of the molecule injected. Thus Na_2SO_4 was found to produce greater hemodilution than an equimolecular dose of NaCl (45). Hypertonic solutions of glucose or sucrose are sometimes injected intravenously to bring about a reduction of cerebrospinal fluid pressure by osmotic withdrawal of fluid from the cerebrospinal system into the blood (46, 47).

In the isolated perfused hindlimb preparation the osmotic withdrawal of fluid during such an osmotic transient may be prevented by raising the mean hydrostatic pressure in the capillaries by known amounts just sufficient to maintain the limb at constant weight (2). Under these conditions the increment of mean capillary pressure is a measure of the partial osmotic pressure (or diffusion pressure) exerted by the added molecules across the capillary membranes.

A. Osmotic Transients, Magnitude and Time Course. Figure 2 shows the transient osmotic effects of adding urea, glucose or inulin to arterial blood supplying the perfused hindlimb of a cat. The experimental technique was as follows.

Before addition of each test substance, control measurements were made of isogravimetric capillary pressure and filtration coefficient exactly as described in ref. 2. In this experiment the control pC_i was 11.0 ± 1 mm. Hg as compared with a measured plasma protein osmotic pressure of 12.6 ± .3 mm. Hg. Each molecular species was added to the rapidly stirred blood in the perfusion reservoir: the circulating plasma volume was approximately 150 ml. The amounts added were: *1*) Urea, 5 ml. of 10 per cent leading to an initial plasma concentration of 0.053 M and a partial osmotic pressure (P_o) of 1000 mm. Hg; *2*) glucose, 5 ml. of 20 per cent leading to P_o = 750 mm. Hg; and *3*) inulin, 8 ml. of 20 per cent leading to P_o = 40 mm. Hg. About 1 minute was required for the reservoir blood to reach the hindlimb capillaries, at which time its osmotic activity produced a strong tendency to absorb fluid from interstitial fluid to blood capillary. This tendency may be counterbalanced by raising the venous pressure *before* the addition of the test molecules, thus producing rapid net filtration: the initial osmotic action of the test substances was then to reduce the rate of net filtration rather than to produce net absorption. With practice and experience with the substance under test, the investigator can usually anticipate the equilibrium pressure within 3 or 4 mm. Hg so that only minor readjustments of capillary pressure are necessary to reach the isogravimetric state following the addition of the test molecules. This procedure allows measurements to be made

much earlier in the transient than would otherwise be possible. If the test molecules are added during the isogravimetric state, then the large and sudden rise of venous pressure required to balance the initial osmotic effect causes *a*) a diminution of blood flow and *b*) an increase of vascular volume. Both these side effects increase the difficulty of obtaining reliable measurements during the initial phases of the transient. Once the initial adjustments have been made, the remainder of the diffusion process is followed by lowering the venous pressure in small steps to keep pace with the decreasing effective osmotic pressure in the capillaries. The blood flow is maintained constant throughout the transient by simultaneous adjustment of arterial pressure to compensate for the changes of venous pressure. Since an easily detectable rate of net filtration or absorption is produced if the hydrostatic pressure is 0.2 mm. Hg above or below the effective osmotic pressure considerable skill is often required to maintain both the limb weight and blood flow constant throughout osmotic and hydrostatic changes of 35 mm. Hg as shown in figure 2.

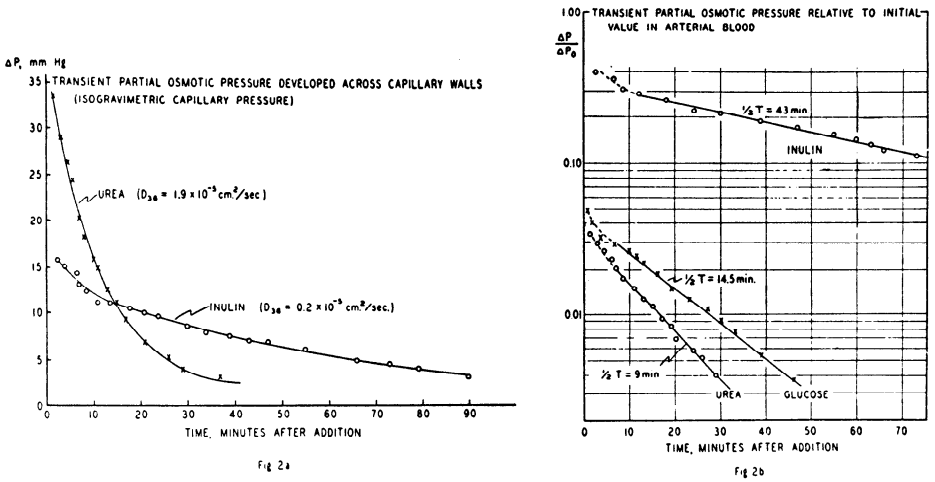


Fig. 2. OSMOTIC TRANSIENTS in capillary circulation. *Exper. 32-R*, cat hindlimb 330 gm. K_f = .017. T_A = 36° C. Figure *2b* shows exponential rates.

Figure *2a* shows the time course of such osmotic transients for urea and for inulin diffusing across the capillary membranes in the hindlimb of a cat. Throughout each transient the mean capillary pressure was continually adjusted by varying the venous pressure so that the leg remained at constant weight (isogravimetric state). It is evident from figure *2a* that the osmotic transient declines much more rapidly for urea than it does for inulin. The curves shown in figure *2a* are logarithmic over most of their range, as shown in figure *2b*, and we soon found that, in general, the logarithmic slopes increased with the diffusion coefficient of the molecule being tested. Table 5 summarizes values obtained for the 'half-times' (the half-time is reciprocally related to the logarithmic slope. $\frac{1}{2}T$ = .301/slope) of osmotic transients produced by various molecules at 37 ± 2° C. and at 10 ± 2° C.

In carrying out the experiments at low temperature the arterial blood was cooled to about 8° C. just prior to its entrance into the arterial cannula. Care was taken to allow the tissue to reach a steady state of heat exchange as indicated by a constant arteriovenous temperature difference recorded from thermocouples inserted in the arterial and venous cannulae. Cooling the arterial blood to 10 ± 2° C. pro-

duces a profound dilatation of the arterioles (48) without appreciable alteration in the filtering area of the capillaries (6).

Examination of table 5 shows that the half-times for diffusion are inversely related to the free diffusion coefficients. For inulin, raffinose, glucose, urea and NaCl the product $D \times \frac{1}{2}T$ lies within the range 7 to 10.6 $\times$ 10^{-5} despite a 20 to 1 distribution of diffusion coefficients. The osmotic transient for sucrose proceeded slightly more rapidly than would be predicted from its free diffusion coefficient in water. The effects of temperature are of special significance, for if the capillary transfer involved some process other than diffusion, such as membrane solubility or a metabolic process, then a change from 37° to 10° C. might be expected to produce a very large reduction in rate of transfer. For the molecular species shown in table 5, however, the changes of transfer rate with temperature are approximately proportional to the temperature coefficient of diffusion in water.

TABLE 5. OSMOTIC TRANSIENTS IN HINDLIMBS OF CATS

MOLECULARS SPECIES	NO. OF EXPER.	TEMP. °C. ± 2	FREE DIFF. COEFF., D CM.² SEC. × 10^5	HALF-TIME OF OSMOTIC TRANSIENT, $\frac{1}{2}T$ MIN.	$D \times \frac{1}{2} T \times 10^5$
Inulin	2	10	0.11	95±20	10±2
Inulin	7	37	0.21	33±7	7±1.5
Sucrose	3	10	0.37	12±3	4.5±1
Raffinose	1	37	0.64	11.5	7.4
Sucrose	6	37	0.75	7.3±1.5	5.4±1
Glucose	6	37	0.90	9.3±3	8.4±2.5
Urea	2	10	0.94	9.0±2	8.5±2
NaCl	1	10	1.03[1]	9.7	10.0
Urea[2]	5	37	1.75	5.8±1	10.2±3
NaCl[2]	3	37	2.03[1]	5.3	10.6±3

[1] For NaCl in 0.15 M solution. [2] For these rapidly diffusing molecules the slope of the osmotic transient may be limited by blood flow as described in text p. 27. The data given refer to plasma flows greater than 8 ml/minute/100 gm. tissue.

The simple exponential nature of most of the osmotic transients (fig. 2*b*) and the regularity of the results, obtained with different molecular species and at different temperatures, encouraged us to believe that analysis of the osmotic transient curves would yield important information concerning the structural properties of the capillary wall considered as a barrier to diffusion. A constant product of half-time and free diffusion coefficient is the result to be expected in simple two compartment diffusion systems such as those employed for the determination of molecular diffusion coefficient (49). In a system of two compartments of known volumes separated by a membrane of pore area A and thickness Δx, and in which each compartment is well stirred, the pore area per unit path length through the membrane may be easily evaluated from the product of diffusion half-time and diffusion constant. We therefore began our analysis by assuming that the capillary exchange could be considered in terms of a simple two compartment system in which *a*) the concentration of the test molecules in the capillary 'compartment' was uniform and equal to that

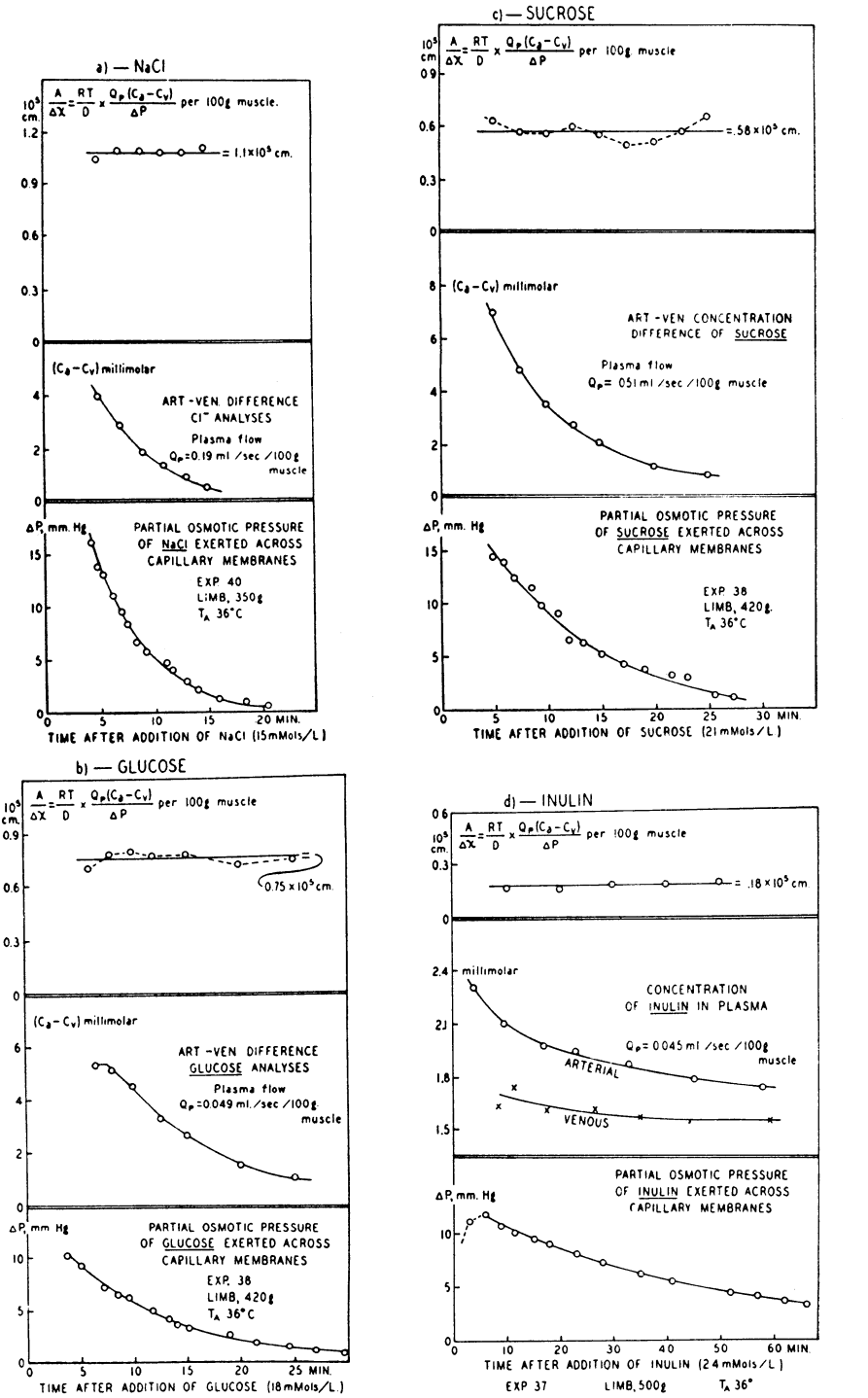


Fig. 3. FOUR EXAMPLES showing that the ratio of arteriovenous difference in concentration to the effective osmotic pressure is independent of time.

in arterial blood and *b*) free diffusion throughout the interstitial fluid was so rapid that the concentration in this 'compartment' could be considered uniform. If these assumptions were correct, then we should expect that extrapolation of the logarithmic curves to zero time would yield an osmotic pressure corresponding to the initial concentration of the added molecules in arterial plasma. A glance at figure 2*b* shows that this is far from the truth. The initial osmotic pressures obtained by extrapolation to zero time were only a small fraction (urea 4%, glucose 6%, and inulin 40–50%) of the osmotic pressure (P_o) corresponding to the concentration of the test molecules in arterial blood.

We emphasize the failure of these assumptions to explain the facts because these assumptions are implicit in equations which have been widely used to calculate transcapillary exchange rates from the 'arterial disappearance curves' of tracer molecules injected into the circulation (50–56).

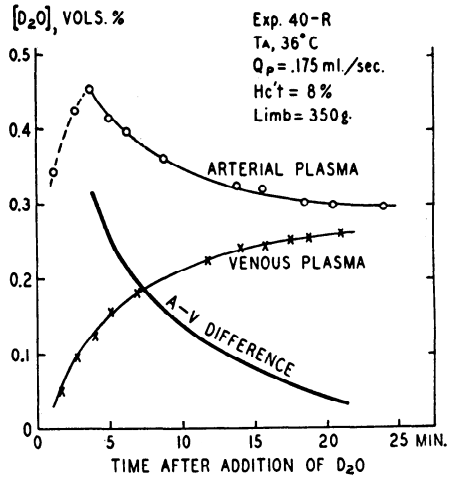


Fig. 4. TIME COURSE OF DISTRIBUTION of D_2O in perfused cat limb. This figure is included to show that molecules introduced into the system in 'tracer' quantities also have a large arteriovenous difference in the initial phase of the diffusion process. The mean capillary concentration of D_2O is initially only a small fraction of that in arterial plasma. 1.29 ml. of D_2O were added to about 270 ml. circulating blood volume in which the hematocrit had been previously reduced to 8 per cent by adding plasma and Ringer's solution. The low hematocrit minimized complications arising from the distribution of D_2O between cells and plasma. D_2O was analyzed in a mass spectrometer (58). Most of the distribution time of D_2O is concerned with the passage of D_2O into the muscle cells rather than across the capillary walls.

The reason for the inequality between concentration in arterial blood and that of capillary blood is shown in figures 3*a*, *b*, *c*, *d* and figures 4 and 5. Early in the diffusion process, when diffusion rate is large, there is a large arteriovenous concentration difference and the mean capillary concentration is very much less than that in arterial blood. As the outside compartment becomes saturated the diffusion rate slows, the arteriovenous difference progressively diminishes, and the mean capillary concentration only gradually approaches the concentration in arterial blood.

A similarly large arteriovenous difference also occurs when tracer molecules are injected into the circulation, in man (57) dog (59) and in the perfused hindlimb. Figure 4 shows the time course in arteriovenous difference of D_2O following its addition to the perfused hindlimb preparation.[4] The implications of these findings for the analysis of transcapillary exchange rates from 'arterial disappearance curves' will be taken up in the DISCUSSION at the end of this paper.

It is clear from our experiments that the diffusion process involves a concentra-

[4] It is a pleasure to acknowledge the collaboration of Dr. I. S. Edelman in performing this experiment with its associated analyses for D_2O.

tion gradient along the length of the capillary as well as a concentration gradient across the capillary wall. The mathematical analysis of diffusion in this system involves cylindrical diffusion from a capillary with a variable concentration gradient along its length. The solution of the diffusion equations under these circumstances is complex and the numerical results depend greatly on the geometry assumed for the interstitial space. We[5] were unable to derive an equation which would relate the observed diffusion rates to the area for free diffusion through the capillary walls. When, however, both the osmotic transients and the arteriovenous difference curves

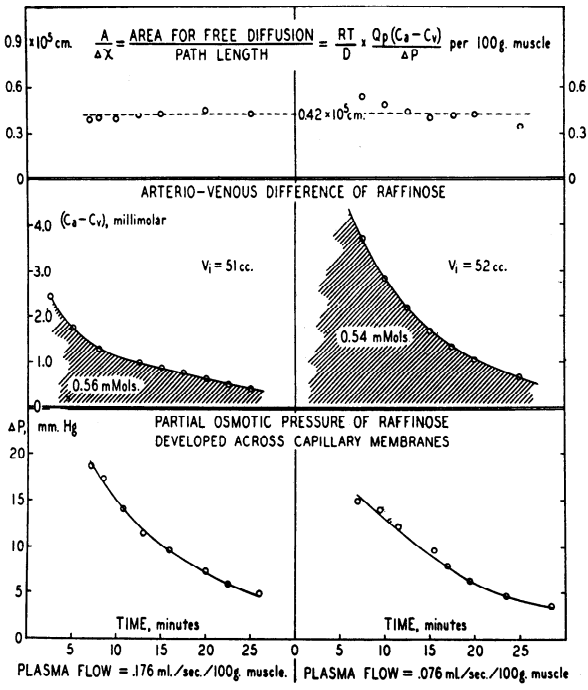


Fig. 5. DIFFUSION OF RAFFINOSE at two blood flows from capillaries in hindlimb of cat. *Exper. 41*, limb weight 270 gm., T = 36° C. K_f = .014 cc/minute/mm. Hg/100 gm. muscle. The blood flow was altered by varying the arterial perfusion pressure. The same quantity of raffinose was added to the perfusion reservoir at each of the two flows. Note that the time course of the osmotic transient was relatively unaffected by the flow rate (cf. also fig. 6), whereas the arteriovenous difference was increased at low flow and by an amount such that the calculated area per unit path length was unaltered as would be expected of a geometrical property of the capillary wall.

The area under each arteriovenous difference curve multiplied by the plasma flow represents the quantity of raffinose which diffused into the interstitial space: Within the error of our methods it was the same at both flows (0.56 and 0.54 mM). The volume of fluid into which the raffinose diffused (V_r) is given by $V_r = M_r/C_{eq}$, where M_r is the amount which diffused and C_{eq} is the measured equilibrium concentration. It was 51 ± 1 ml. or 19 per cent of the limb weight at both flows.

are considered together, a simple solution for capillary pore area may be derived which is independent of the time course of the diffusion process.

B. Determination of Diffusion Area per Unit Path Length Through the Capillary Walls in the Hindlimb Muscles of the Cat. *1*) Definition of symbols, c.g.s. units:

dm/dt—rate of net diffusion of test molecules across the capillary walls at any time, t

A—area in the capillary walls available for free diffusion of the test molecule; as will be shown below this is a *virtual* area which varies with molecular size

Δx—path length for diffusion through the membrane

Δc—mean concentration difference at time, t, across the capillary membranes which is effective in causing net diffusion and osmotic pressure

[5] During this phase of the investigation Prof. J. Wyman, Jr. spent many hours working on possible solutions to the problem and we wish to express our gratitude to him.

c_a, c_v—concentration of the test molecules in arterial and venous blood (or plasma) respectively at time, t

ΔP—partial osmotic pressure exerted by the test molecules across the capillary membranes at time, t

$\dot{Q}_b, \dot{Q}_p$—flow of blood or plasma

D—free diffusion coefficient of the molecule

R—the gas constant

T—the absolute temperature

2) The rate at which molecules leave the vascular system at any time during the transient is

$$dm/dt = \dot{Q}_b(c_a - c_v) \qquad (i)$$

If the molecular species does not penetrate the red cell it is convenient to use $\dot{Q}_p$ rather than $\dot{Q}_b$

3) From Fick's Law of Diffusion

$$dm/dt = DA \cdot \Delta\bar{c}/\Delta x \qquad (ii)$$

In applying this equation it is important to make clear that a) A is a virtual area corresponding to the area which would be required for the observed transfer rate by free diffusion in water. b) $\Delta\bar{c}$ is that concentration difference across the membranes which, when substituted in *equations ii* and *iii* would give rise to the observed rate of diffusion and the observed osmotic pressure. Its value may be different in different capillaries and it varies with time. The important point is that *the same concentration difference which is effective in causing diffusion also gives rise to the observed osmotic pressure.*

4) From Van't Hoff's Law,

$$\Delta P = \Delta\bar{c}RT \qquad (iii)$$

For precise formulation $\Delta\bar{c}$ should be multiplied by the activity coefficient of the test molecule. However, a similar correction should also be applied to the diffusion coefficient and since these two quantities appear as a ratio in the final *equation 5* we shall employ concentrations rather than activities.

5) Eliminating dm/dt and $\Delta\bar{c}$ from *equations i*, *ii* and *iii* and solving for $A/\Delta x$, we have finally

$$\frac{A}{\Delta x} = \frac{\dot{Q}_b(c_a - c_v)RT}{D \cdot \Delta P} \qquad (5)$$

All quantities on the right are measurable experimentally; if our *equation 5* is correct we should now be able to determine for any test molecular species the area per unit path length in the capillary walls required to explain the observed transfer rate of the molecules by free diffusion. This area should be independent of time and we should therefore predict from *equation 5* that at constant blood flow and temperature the ratio of arteriovenous concentration difference to osmotic pressure would be constant throughout the osmotic transient. Experimental confirmation of this prediction is shown for four molecular species in figure *3a*, *b*, *c* and *d*. In each case the arteriovenous concentration difference, as determined by chemical analysis of serial samples of arterial and venous blood, bore a fixed ratio to the partial osmotic

pressure as determined from the blood flow, venous pressure and post-capillary resistance to blood flow. A detailed account of figure 3 is given below.

In figure 3 are given four examples showing that the ratio of arteriovenous difference in concentration to the effective osmotic pressure is independent of time. The osmotic transients were measured as described in Section **A** above. The arteriovenous differences were determined from analysis of serial samples of arterial and venous blood drawn from the arterial and venous cannulae during the osmotic transient. Approximately 6 arterial samples and 10 venous samples were drawn during the course of each run. Inulin and sucrose were hydrolyzed with HCl and analyzed colorimetrically after reaction with diphenylamine according to the method of Harrison (60). For glucose, the same procedure was used, with the hydrolysis omitted. Chloride was titrated electrometrically with $AgNO_3$. Smooth curves were drawn through successive points for arterial and venous bloods as illustrated for the case of inulin in figure 4*d*. The arteriovenous difference curves were obtained by subtraction of the venous curve from the arterial curve. In the case of glucose a small blank correction was required since venous blood contained slightly less glucose than arterial blood during the control period owing to utilization of glucose by the tissues.

It should be noted that the area per unit path length for diffusion through the capillary walls decreased progressively with increasing molecular size: but for each molecular species it was independent of time as would be expected of a geometrical property of the capillary wall.

For any given molecular species the (virtual) area for free diffusion through the capillary walls should be independent of the blood flow. Figure 5 illustrates the effects of varying the blood flow (by varying the arterial perfusion pressure) on the arteriovenous concentration difference and osmotic transient in the case of raffinose. At low flow the arteriovenous difference is larger than at high flow and by an amount just sufficient to maintain constant the ratio $\dot{Q}_p(c_v - c_v)/\Delta P$. The calculated diffusion area per unit path length is therefore independent of the flow over this range. Almost the entire effect of a change of flow is found in the arteriovenous concentration difference; the osmotic transient is relatively unaffected. Figure 6 shows a more detailed analysis of the effects of flow on the osmotic transient alone for sucrose. It is clear that variations of flow in the range shown have very little effect on the development of osmotic pressure. At very low flows and with more rapidly diffusing molecules such as NaCl or urea the flow may limit the diffusion rate, but even this limitation does not alter the calculated value of diffusion area per unit path length as shown in table 6.

The fact that $A/\Delta x$ is independent of blood flow (fig. 5, table 6) indicates that the rate of filtration and absorption at the arterial and venous ends of the capillaries plays a negligible role in determining the rates of transfer of the test molecules. The pressure drop along the capillary length, and hence the rate of filtration and absorption, varies proportionally with the blood flow. However, the 2.5-fold variations of flow shown in figure 5 and table 6 had no detectable effects on the values of $A/\Delta x$. In PART III below we shall show that diffusion velocities of small molecules through the capillary walls are overwhelmingly greater than filtration velocity, even when rapid net filtration along the entire capillary length is produced by raising the venous pressure above the protein osmotic pressure.

The results illustrated in figures 3 and 5 demonstrate three principal points.

1) For each molecular species the ratio of arteriovenous concentration difference to the partial osmotic pressure developed across the capillary membranes is independent of time. When multiplied by the flow and RT/D it should be equal to the

area per unit path length for free diffusion through the capillary walls as stated in *equation 5*. The value so obtained is independent of the blood flow in the range studied.

2) The area per unit path length is largest for small molecules and diminishes progressively with increasing molecular size. This is shown in detail in figure 7, in which the diffusion areas per unit path length are plotted as a function of molecular weight for six molecular species. Since the path length is presumably the same for each species it follows that the area for 'free' diffusion becomes progressively restricted with increasing molecular weight. On the pore theory of capillary permeability these results might be interpreted in two ways or their combination. *a*) If there were a distribution of pore sizes the large molecules might be barred from areas available for the diffusion of the smaller molecules. *b*) The progressive decrease in area might

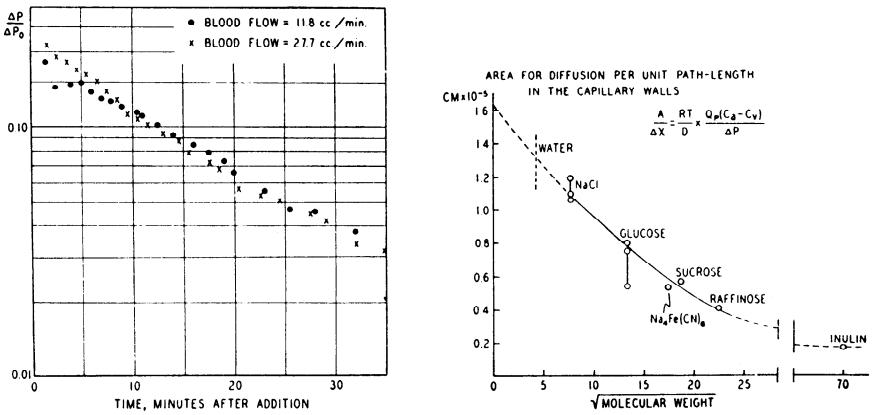


Fig. 6 (*left*). EFFECT OF BLOOD FLOW on diffusion of sucrose. *Exper. 35-R*, cat hindlimb 350 gm., temp. 9° C. K_f = .011. For molecules the size of sucrose or larger the osmotic transient is relatively unaffected by the blood supply over a wide range of flow rates. For smaller, more rapidly diffusing molecules the osmotic diffusion transient may be limited by the flow as shown for NaCl in table 6.

Fig. 7 (*right*). The area per unit path length available for diffusion through the capillaries is progressively diminished with increasing molecular size. The experimental values for $A/\Delta x$ were determined as shown in figures 3 and 5. These relations between molecular size and restriction to diffusion may be predicted theoretically as described in PART III, *section B* and figure 10.

be a virtual one resulting from steric or other factors impeding diffusion through pores of uniform size. In PART III below evidence will be given to show that the second of these two factors is the predominate one. In the meantime it is useful to consider the area available to a molecule the size of water and to express the diffusion areas of the solute molecules relative to this value. Extrapolation of the results of figure 7 to a molecular weight of 18 (equivalent to that of water) yields a diffusion area per unit path length of 1.3×10^5 cm. in the capillaries of 100 gm. of muscle. Table 7 summarizes the effective diffusion areas available to the various molecular species so far studied relative to the area for a molecule the size of water. The values are taken from the smooth curve of figure 7.

3) An estimate can now be given for the maximum area for diffusion of water and lipoid insoluble solutes through the capillary membranes of the perfused hind-

limb muscles. The (extrapolated) value for water is $A_w = 1.3 \times 10^5 \Delta x$ cm.^{2}/100 gm. muscle. The path length (Δx) through the capillary wall is not likely to be more than 1 μ, so that $A_w < 1.3 \times 10^5 \times 1 \times 10^{-4} = 13$ cm.^{2} This may be compared with a value of 7000 cm.^{2} estimated for the histological surface area of the capillaries in 100 gm. of muscle (20). In terms of the fractional area of the capillary wall available for the diffusion of a molecule the size of water, we have 13 ÷ 7000 or less than 0.2 per cent. The fractional areas available to various molecular species listed in table 7 are less than this in proportion to their relative diffusion areas.

On the pore theory of capillary exchange we therefore conclude that only a minute fraction of the capillary wall is involved in the exchange of these substances—a conclusion which we have already predicted from the consideration of filtration rates in the last paragraph of PART I above.

TABLE 6. EFFECT OF BLOOD FLOW ON AREA FOR FREE DIFFUSION PER UNIT PATH LENGTH FOR A RAPIDLY DIFFUSING MOLECULE (NaCl)

PLASMA FLOW *ml/min.*	HALF-TIME OF OSMOTIC TRANSIENT *min.*	$A/\Delta x$(NaCl) *cm/100 gm. muscle*
27.6	3.5	1.1×10^5
10.0	9.5	$1.0 \pm .2 \times 10^5$

TABLE 7. EFFECTIVE DIFFUSION AREAS RELATIVE TO THAT FOR WATER IN CAPILLARIES OF PERFUSED HINDLIMB

MOL. SPECIES	H_2O	NaCl	GLUCOSE	SUCROSE	$Na_4Fe(CN)_6$	RAFFINOSE	INULIN
A/A_w	1.00	0.84	0.59	0.44	0.40	0.32	0.14

PART III

A. Combination of Filtration Data with Diffusion Data to Evaluate Effective Pore Size. The data for filtration and diffusion given in PARTS I and II above may be utilized to determine the dimensions of any given pore geometry. Three possible cases are considered below.

CASE I. *Uniform cylindrical pores.* From *equation 2*, the filtration rate per unit pressure difference is

$$\frac{\dot{Q}_f}{\Delta P} = \frac{A_f}{\Delta x} \cdot \frac{r^2}{8\eta} \qquad (2)$$

where A_f is the collective area of the pores available for filtration.

When referred to 100 gm. of muscle $\dot{Q}_f/\Delta P$ is the filtration coefficient K_f, experimental values for which are summarized in figure 1. Solving *equation 2* for r, the pore radius, we have

$$r = \sqrt{\frac{8\eta K_f}{A_f/\Delta x}} \qquad (6)$$

But the area per unit path length available to a molecule the size of water has already been evaluated by extrapolation of the results shown in figure 7 and was found to be

1.3×10^5 cm/100 gm. muscle.[6] Appropriate values for the numerical solution of *equation 6* are therefore:

$$K_f = 0.017 \text{ ml/minute/mm.Hg or } 2.1 \times 10^{-7} \text{ ml/second/dyne/cm.}^2$$
(average value for experiments shown in fig. 7)

$$A_f/\Delta x = 1.3 \times 10^5 \text{ cm.}$$

$$\eta = 0.007 \text{ dyne-second/cm}^2.$$

whence $$r = \sqrt{\frac{8 \times .007 \times 2.1 \times 10^{-7}}{1.3 \times 10^5}} = 30 \times 10^{-8} \text{ cm., or } 30\text{Å}$$

The number of such pores is defined by

$$N = \frac{A}{\Delta x} \cdot \frac{\Delta x}{\pi r^2} = \frac{1.3 \times 10^5 \times \Delta x}{\pi(30 \times 10^{-8})^2} = 0.45 \times 10^{18} \Delta x$$

For a path length, $\Delta x = 0.3\ \mu$ this becomes 1.3×10^{13} pores in 100 gm. muscle containing 7000 cm.^{2} of capillary surface or about 2×10^9 pores/cm.^{2}

CASE II. *Uniform rectangular slits.* In view of the small area in the capillary walls available for the interchange of the substances so far investigated it is of interest to consider the possibility of a fibrous matrix between endothelial cells. Structure of this type might offer long narrow slits rather than cylindrical pores as channels for filtration and diffusion. The laws of laminar flow through rectangular slits have been derived by Barr (61) and by Bjerrum and Mangold (18). For rectangular channels in which the width is small compared to the length

$$\frac{\dot{Q}_f}{\Delta P} = \frac{2L}{3\eta\Delta x}\left(\frac{w}{2}\right)^3$$

where $w/2$ is the slit half-width and L its length. This relation is analogous to Poiseuille's Law for flow through cylindrical tubes and leads to a solution for $w/2$ analogous to that for r in *equation 6*.

$$w/2 = \sqrt{\frac{3\eta K_f}{A_p/\Delta x}} = \sqrt{\frac{3 \times .007 \times 2.1 \times 10^{-7}}{1.3 \times 10^5}} = 18.5 \times 10^{-8} \text{ cm., or } 18.5\text{Å} \qquad (7)$$

which represents the slit half-width available to water molecules. The total length of such slits (assuming as before a path-length of 0.3 μ) is 2×10^7 cm. or about ten times the estimated total length of the capillaries in 100 gm. of muscle.

CASE III. *Distribution of pore sizes. Upper limit to pore size as defined by hemoglobin.* The effect of a distribution of pore sizes on the value for mean pore size, calculated as we have done from diffusion area and filtration constant, has been discussed in detail by Ferry (43). If there is a distribution of pore radii (or slit half-

[6] We are here assuming that the pore area for hydrodynamic flow of water is identical with the area available for diffusion of water molecules. It might be argued that the appropriate area to consider for flow would be that obtained by extrapolation of figure 7 to the area for a molecule which is infinitely small compared to the pore size (i.e. a molecule of zero molecular weight). This would lead to an effective pore area per unit path length of 1.62×10^5 cm. and an effective pore radius of 27 Å. Present knowledge of hydrodynamic flow through channels of molecular dimensions is insufficient to decide between these two alternative assumptions. The assumption which we have made, leading to a pore radius of 30 Å, predicts restriction to diffusion of large molecules (fig. 10) more accurately than does the alternative assumption.

widths) then the values calculated as above on the assumption of uniformity will always be greater than the true arithmetical mean of the radii (or half-widths). Thus the values of 30 Å for cylindrical pores or 18.5 Å for slits which were derived above for the case of uniform openings represent maximum values for the arithmetic mean of any distribution of pore sizes in the capillaries of the hindlimb.

An upper limit to such a distribution may be estimated from the behavior of hemoglobin which is, within the limits of error of our methods, completely retained by the capillary membranes of the perfused hindlimb. Figure 8 shows the osmotic effects of adding oxyhemoglobin in a typical experiment (*4-T*). A detailed account of figure 8 is given below.

After a control period during which the hindlimb was perfused with diluted cat blood, 130 ml. of 5.25 per cent hemoglobin solution were added to the reservoir, which contained 110 ml. plasma. The hemoglobin was prepared by shaking washed cat erythrocytes with distilled water, precipitating the stroma according to the method of Hamilton *et al.* (62), adjusting the pH (glass electrode) to 7.2 ± 0.2 with $NaHCO_3$, followed by dialysis against Ringer. Samples of perfusion fluid drawn from the reservoir were centrifuged, and the plasma analyzed spectrophotometrically for hemoglobin.

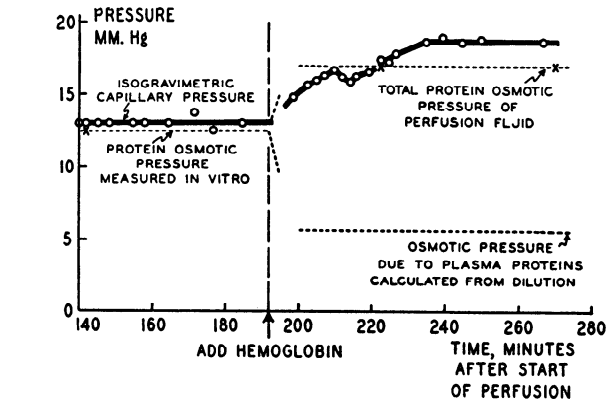
Mixing in the system was complete in less than 5 minutes, and after 8 minutes, the time required for washout of the limb vessels, there was no significant difference between the arterial and venous concentrations of hemoglobin, indicating that none was lost from the capillaries. The isogravimetric capillary pressure rose slowly from 13 to 18.5 mm. Hg, or slightly above the osmotic pressure of the hemoglobin plus plasma protein measured *in vitro*, and remained at this level for the remainder of the experiment: this is evidence that no protein leaked from the capillaries. In similar experiments there has been no detectable leakage of hemoglobin over a period of 2 hours.

The slow development of effective osmotic pressure following the addition of hemoglobin is a characteristic and puzzling feature of the osmotic behavior of all the proteins we have studied, including serum albumin (human, bovine and feline), globulin (human), hemoglobin (bovine and feline) and egg albumin. In an earlier paper on the effective osmotic pressure of proteins we reported that 2 samples of bovine albumin were not retained by the capillaries of the perfused hindlimb (2). We have since prepared samples of human, bovine and feline albumin which, after dialysis against Ringer's solution and titration to pH 7.4, have maintained stable isogravimetric capillary pressures for periods as long as 4 hours.

It is seen that the hemoglobin develops its full theoretical osmotic pressure and there is no detectable loss of osmotic pressure with time which might indicate diffusion through the capillaries. There was no detectable arteriovenous difference of hemoglobin concentration. We therefore conclude that the capillary area for diffusion of hemoglobin is not detectable by our methods (i.e. less than 5% of the diffusion area for water). The effective molecular radius of hemoglobin is approximately 31 Å (63, 64) and the end point porosity for penetration of hemoglobin through collodion membranes is 50 Å in radius. A Gaussian distribution of pore sizes about a mean pore radius of 24 Å and with a standard deviation of 12 Å is the broadest distribution which would account for the observed diffusion area and filtration coefficient and at the same time remain within the upper limit of pore size set by hemoglobin. However, the facts considered so far are equally well explained by uniform pores of radius 30 Å or slits of half-width 18.5 Å. The further consequences of a distribution of pore sizes will be discussed below in connection with steric hindrance to diffusion and molecular sieving during ultra-filtration.

B. Restriction to Diffusion of Large Molecules Through Pores of Molecular Dimensions. The derivation of pore dimensions given above for cylindrical pores

or rectangular slits is based on the area for free diffusion per unit path length for a molecule the size of water, which is very much smaller than the calculated pore size. The value for water was obtained from extrapolation of the data for molecules of graded sizes shown in figure 7. We have now to ask if the pore dimensions determined as above from a molecule which is small compared to the pore dimensions can satisfactorily explain the restricted diffusion areas for the larger molecules shown in figure 7 and table 7. On first thought one might suppose that the restricted diffusion area for sucrose, i.e. about one half the diffusion area for a molecule the size of water as shown in table 7 could be simply explained by assuming that half the pores were



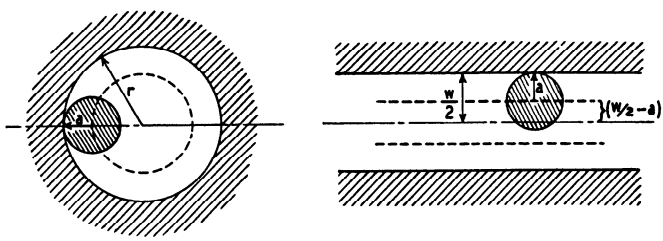


Fig. 8 (*upper*). OSMOTIC EFFECT OF HEMOGLOBIN, in the perfused cat's hindlimb preparation, *exper. 4-T.*

Fig. 9 (*lower*). SIMPLE THEORY FOR STERIC HINDRANCE to entrance of spherical molecules into cylindrical pores of radius r or rectangular slits of width w. It is assumed that penetration by a molecule of radius a will take place only if the molecule chances to fall within the area of a circle of radius $(r - a)$ or a slit of width $(w - 2a)$.

smaller than a sucrose molecule and half the pores were larger. This simple explanation must, however, be excluded, for the maximum radius of sucrose is probably less than 8 Å (table 9) and we have already shown that the broadest distribution of pore radii consistent with our data lies about a mean of 24 Å with a standard deviation of 12 Å (i.e. 67% of the pore radii must lie between 12–36 Å).

No well-established theory accounting for restricted diffusion through pores of molecular dimensions is at present available. However, the following treatment of the problem appears to explain the facts satisfactorily with regard to our data on diffusion through the capillary walls. A somewhat similar treatment may be found in the work of Friedman and Kraemer (65, 66) on the diffusion of nonelectrolytes into gels and

the reader's attention is called to the work of Ladenburg (67), of Westgren (68) and of Faxén (69) on corrections to Stoke's Law in systems where the particle diameter is comparable with the dimensions of the sedimentation chamber.

We consider two factors restricting the diffusion of spherical molecules through cylindrical pores or rectangular slits: *1*) A steric factor restricting the entrance of the molecule into the pore or slit; *2*) viscous drag at the wall once the molecule has entered the pore or slit.

The first of these factors is illustrated diagrammatically in figure 9. For the case of cylindrical pores, the virtual area, A, for penetration is

$$A = \pi(r - a)^2 \text{ or } A/A_o = (1 - a/r)^2 \qquad (8)$$

where a is the radius of the diffusing molecule, r is the radius of the pore, and A_o is the actual area of the opening. The corresponding expression for the case of rectangular slits is

$$A/A_o = \left(1 - \frac{a}{w/2}\right) \qquad (9)$$

The second restricting factor, namely viscous drag at the walls, has been determined for the case of cylindrical tubes by Ladenburg (67) and is given by

$$f/f_o = 1 + 2.4\frac{a}{r}$$

where f_o is the viscous drag when the particle radius, a, is negligible compared to the tube radius, r. The complete equation predicting the restricted diffusion of spherical molecules through cylindrical pores is then

$$\frac{D_{\text{restricted}}}{D_{\text{free}}} = \frac{(1 - a/r^2)}{1 + 2.4\ a/r} \qquad (10)$$

The effects of viscous drag on sedimentation (and diffusion) between parallel plates have been studied over a wide range of particle sizes by Westgren (68) who showed empirically that

$$f/f_o = 1 + 3.4\left(\frac{a}{w/2}\right)^2$$

A theoretical derivation of this factor in the form of an infinite series has been shown by Faxén (69) to apply with slightly greater accuracy, but the Faxén correction is cumbersome to use and is unnecessarily precise for our purposes. The complete equation predicting the restriction to diffusion of spherical molecules through slits is then

$$\frac{D_{\text{restricted}}}{D_{\text{free}}} = \frac{\left(1 - \frac{a}{w/2}\right)}{1 + 3.4\left(\frac{a}{w/2}\right)^2} \qquad (11)$$

Table 8 shows the restriction to diffusion to be expected on this theory (i.e. from *equations 10* and *11*) for spherical molecules diffusing through uniform cylindrical pores of 30 Å (*column 2*) or uniform rectangular slits of 18.5 Å (*column 4*). It is

obviously possible also to extend the prediction to any given distribution of pore sizes and in *column 3* this has been done for the case of the broadest Gaussian distribution of pore radii consistent with our experimental data for filtration and diffusion of water and hemoglobin (cf. the first section of *Case III*).

In comparing the theoretical values of table 8 with our experimental values for relative diffusion areas there is some doubt as to the proper choice of values to be used for molecular dimensions.

Table 9 shows the molecular dimensions of the substances so far studied as calculated from diffusion, viscosity and x-ray diffraction data. It is evident from inspection of table 9 that none of the molecules are actually spherical; their molecular 'radii' calculated from the Stokes-Einstein Equation (as in *column 1*) represent the radii of spherical molecules of equivalent diffusion coefficient and may not be appropriate for applications involving steric hindrance. An alternative assumption is to consider that the effective molecular volume presented to the pore is that derived

TABLE 8. THEORETICAL RESTRICTION TO DIFFUSION AS A FUNCTION OF MOLECULAR RADIUS

(1) MOLECULAR RADIUS, Å	(2) RELATIVE VIRTUAL AREA FOR FREE DIFFUSION THROUGH UNIFORM PORES OF RADIUS 30 Å (*eq. 10*)	(3) SAME AS *col. 2* BUT FOR EQUIVALENT GAUSSIAN DISTRIBUTION, $r_m = 24 \pm 12$ Å	(4) RELATIVE VIRTUAL AREA FOR FREE DIFFUSION THROUGH UNIFORM SLITS OF HALF-WIDTH 18.5 Å (*eq. 11*)
0	1.00	1.00	1.00
2	0.75	0.75	0.82
5	0.49	0.49	0.59
10	0.25	0.25	0.23
15	0.11	0.12	0.06
20	0.04	0.07	0
25	0.01	0.025	0
32	0	0.003	0

from the longest molecular dimension. This assumption would be expected to be particularly untrustworthy in the case of a long-chain molecule such as inulin. Also, in the case of inulin, we may be dealing with a polydisperse system. It must be remembered, however, that the kinetic movements of the test molecules involve some 10^{12} random collisions per second (70), and the time during which a molecule could present an oriented position to a pore may be small. In the absence of a well-established theory relating molecular shape to diffusion through membranes of known pore size, we shall consider both of the two extremes of molecular dimensions discussed above.

In figure 10 the experimental values for relative diffusion areas are plotted as a function of molecular dimensions and compared with the theoretical predictions of table 9. In this graph the radius of a sphere of equivalent free diffusion coefficient (*column 1*, table 9) is denoted by a cross (X) and the maximum molecular dimension is represented by a horizontal line of appropriate length. The agreement between experiment and theory is reasonably close. It is clear that the pore dimensions as calculated from filtration coefficient and diffusion area for water (*equation 6*) will

account remarkably well for the permeability of the capillaries to lipoid-insoluble molecules ranging in size from NaCl to hemoglobin. In general the results favor cylindrical pores rather than rectangular slits as the more likely pore geometry but one of the disappointing features of our theory for restricted diffusion is that it predicts such small differences between these two types of pore structure. Further experiments, using myohemoglobin (effective diffusion radius 19 Å) may provide a more clear cut distinction between round as contrasted with rectangular openings. Figure 10 suggests also that for a long chain molecule such as inulin the significant dimension is that of a sphere of equivalent diffusion coefficient rather than the longest molecular dimension.

It should be noted from table 8 that the maximum Gaussian distribution of pore sizes consistent with our data (i.e. 24 ± 12 Å as explained in the first section of *Case III*) leads to practically the same restriction to diffusion of large molecules

TABLE 9. CALCULATION OF MOLECULAR DIMENSIONS

MOLECULE	CALCULATIONS BASED ON MEASUREMENTS OF FREE DIFFUSION (71, 72)			CALCULATIONS BASED ON VISCOSITY OF DILUTE SOLUTIONS (72, 73)			DIMENSIONS FROM X-RAY CRYSTALLOGRAPHY (HALF-AXES)
	As a sphere of equivalent diffusion coefficient, Effective radius, $r = \frac{RT}{6\pi\eta DN}$ (1)	As an ellipsoid of revolution about axis, a — Half-axes		As a sphere of equivalent viscosity. Effective radius	As an ellipsoid of revolution about axis, a — Half-axes		
		a (2)	b (3)	(4)	a (5)	b (6)	(7)
	Å	Å	Å	Å	Å	Å	Å
Na^+Cl^-	1.4						Cl^- = 1.81 Na^+ = 0.95
Glucose	3.6	3.6	3.6	3.8	5.0	3.2	4.5 × 3.5 × 2.5
Sucrose	4.4	4.4	4.4	5.0	8.3	3.4	5.8 × 5.3 × 4.0
Raffinose	5.6	7.5	4.7	5.7	8.2	4.4	
Inulin[1]	15.2	40.0	6.3	15.3	35.2	6.7	
Hemoglobin	30.8	12.0	41.0	34.8	8.3	49.5	16 × 27 × 27

[1] Mean values; inulin may be polydisperse.

as an isoporous structure of 30 Å and these two possibilities cannot easily be distinguished experimentally by our methods. However, a change of ±4 Å in the uniform pore size, or its Gaussian equivalent, would be readily detectable by its effects on both filtration coefficient and diffusion areas.

C. Diffusion Velocity as Compared to Filtration Velocity and its Significance for Molecular Sieving During Ultrafiltration. The experimental data presented in PARTS I and II may be utilized to estimate the velocity of transfer by diffusion as compared to that by filtration (hydrodynamic flow) in the passage of substances across the capillary wall. Let the crossectional pore area available for filtration and diffusion of water molecules be A_w, and the effective crossectional area for any test molecule be A as shown in table 7. Then the rate of transfer of the test substance by filtration through any section along the length of the capillary wall is given by,

$$\dot{T}_f = \frac{A}{A_w} \times \dot{Q}_f \times c_o \qquad (12)$$

where $\dot{T}_f$ is the rate of transfer (e.g. mol/sec.), $\dot{Q}_f$ is the filtration rate through the section and c_o is the plasma concentration of the test substance at this section.

The corresponding rate of transfer by diffusion, $\dot{T}_d$, through the same section is given by Fick's Law,

$$\dot{T}_d = DA \frac{\Delta c}{\Delta x} = DA \frac{(c_o - c_e)}{\Delta x} \qquad (13)$$

where c_e is the concentration of the test substance at the distal side of the capillary wall. Dividing *equation 13* by *equation 12* we have,

$$\frac{\dot{T}_d}{\dot{T}_f} = \frac{D \frac{A_w}{\Delta x}}{\dot{Q}_f} \left(1 - \frac{c_e}{c_o}\right) \qquad (14)$$

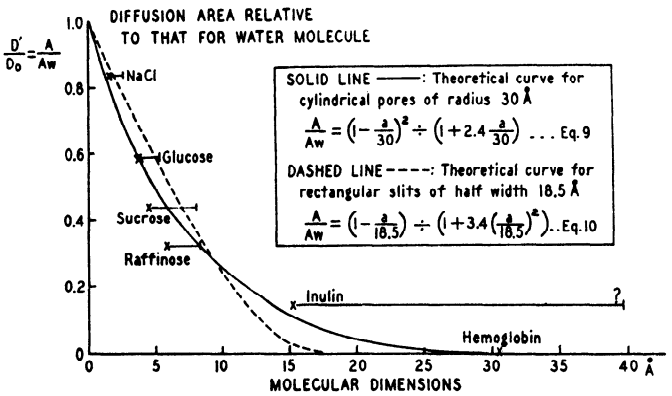


Fig. 10. COMPARISON OF THEORETICAL WITH EXPERIMENTAL VALUES for restriction to diffusion of lipoid insoluble molecules through the capillaries of perfused hindlimb. Experimental values for restriction to diffusion of NaCl, glucose, sucrose, raffinose, inulin and hemoglobin through the capillary walls are here compared with the values predicted theoretically in table 8 for a pore radius of 30 Å or a slit width of 18.5 Å, these dimensions being determined from the hydrodynamical resistance to flow of fluid through the capillary walls and the diffusion area per unit path length for a molecule the size of water. The data favor cylindrical pores rather than slits as the more likely pore geometry, but more exact data with nearly spherical molecules would be required to establish this conclusion. The behavior of inulin is of particular interest because it indicates that effective diffusion radius rather than maximum molecular length is the important dimension to consider in the diffusion of molecules through pores of molecular dimensions. However, the behavior of inulin may be complicated by some degree of polydispersion. Further experiments utilizing these theories for the study of molecular shape in relation to diffusion through capillaries and artificial membranes are in progress.

In choosing numerical values for substitution in *equation 14* we shall consider first the relations between filtration and diffusion during extremely rapid filtration. In a typical hindlimb preparation a very large filtration rate would be 0.6 ml/min. or 0.01 ml/sec/100 gm. muscle. In order to produce such a filtration rate the mean capillary pressure would have to exceed the protein pressure by 35 mm. Hg (cf. fig. 1) and filtration would be expected to occur over the entire capillary length. The preparation would become edematous at the rate of 36 per cent of its weight per hour. In such a typical hindlimb the area for passage of water per unit path-length ($A_w/\Delta x$) is 1.3×10^5 cm. as shown in figure 7. Substituting these values in *equation 14* we have,

$$\frac{\dot{T}_d}{\dot{T}_f} = \frac{1.3 \times 10^5}{.01} D \left(1 - \frac{c_e}{c_o}\right) \qquad (15)$$

Table 10 shows the relations between the rates of transfer by diffusion as compared to filtration to be expected from *equation 15* for various molecular species. *Line I* shows the ratio of transfer by diffusion to that by filtration at the onset of diffusion when $c_e/c_o = 0$. *Line II* shows the fraction of diffusion equilibrium which must be attained before transfer by diffusion is reduced sufficiently to equal transfer by filtration (i.e. when $\dot{T}_d = \dot{T}_f$).

It is evident from table 10 that even during rapid filtration the initial rate of transfer by diffusion greatly exceeds that by filtration. Only in the case of molecules as large or larger than inulin does filtration become an appreciable factor determining the rate of transfer. Under normal circumstances when there is no net fluid exchange, the filtration and absorption rates at the arterial and venous ends of the capillary are of the order of one tenth the rapid rate assumed for calculating the values of table 10 and under these conditions the initial rate of transfer by diffusion must be over-

TABLE 10. RATE OF TRANSFER BY DIFFUSION AS COMPARED TO THAT BY FILTRATION DURING EXTREMELY RAPID FILTRATION (0.01 ML/SEC/100 GM. MUSCLE)

	NaCl	GLUCOSE	RAFFINOSE	INULIN
I. $\dot{T}_d/\dot{T}_f$ at onset of diffusion when $c_e/c_o = 0$	260	116	83	27
II. Concentration ratio across capillary when $\dot{T}_d = \dot{T}_f$	0.996	0.991	0.988	0.963

whelmingly more rapid than transfer by filtration, even in the case of slowly diffusing molecules such as inulin.

Various other methods and assumptions have been considered in estimating the relations between diffusion and filtration. These include: *a*) The use of Ferry's probability theory to calculate the pore area available for filtration (ref. 11, p. 98, *equation 4*). This method eliminates the assumption we have made in *equation 12* that the area for filtration of the test molecules is equal to the restricted area for diffusion. *b*) The velocity of diffusion may be considered in terms of the velocity of a wave-front of concentration $c = c_o/2$. This velocity may be evaluated from Fick's first law for the case of diffusion into a semi-infinite layer.

Both these methods of calculation yield slightly smaller ratios of diffusion to filtration than the method leading to *equation 14*. The added complexities involved do not appear to justify their presentation in detail.

These relations between diffusion velocity and filtration velocity lead to important predictions concerning molecular sieving during ultrafiltration. We have seen that the area in the capillary walls which is available for the transfer of inulin is only 14 per cent of that for water (table 7). This does not mean, however, that during ultrafiltration the concentration of inulin in the ultrafiltrate will be 14 per cent of that in plasma. Thus if water molecules pass through areas which restrict the passage of inulin molecules then the filtrate will be diluted and this will set up a concentration gradient leading to the rapid diffusion of inulin through the restricted area available to it. The concentration of inulin in capillary filtrate will therefore be greater than that expected if diffusion were slow compared with filtration. In order to test this prediction we devised the following methods for estimating the concentration of substances in the capillary filtrate of the perfused hindlimb.

A measured quantity of the test molecular species is added to the perfusing blood

plasma, the volume of which is known. The test molecules are allowed to reach osmotic equilibrium with the tissue fluid and their concentration in plasma is determined by chemical analysis. A substantial fraction of the plasma volume is then filtered into the interstitial fluid by raising the venous pressure for a considerable period; the volume filtered is measured from the gain in weight of the tissue. The concentration of the test molecules in plasma is again determined. The concentration in the filtrate (c_f) can then be computed from, $c_f = m/v_f$, where m = molecules lost from reservoir and v_f = volume filtered.

Table 11 shows the details of such an experiment with inulin as the test molecular species. It is seen that the concentration of inulin in the ultrafiltrate was 71 per cent of that in plasma (*item* 7, table 11) and not 14 per cent as would be predicted if transfer by filtration were large compared with transfer by diffusion.

TABLE 11. ULTRAFILTRATION OF INULIN AND PLASMA PROTEIN IN PERFUSED HINDLIMB

QUANTITY	BEFORE FILT.	AFTER FILT.
	A. Measured Quantities	
1. Limb weight	499 gm.	540 gm.
2. Plasma volume	125 ml.	83 ml.
3. (Inulin) plasma	6.32 mg/ml.	7.18 mg/ml.
4. (Protein) plasma	.039±.004 gm/ml.	0.061±.003 gm/ml.
	B. Calculated Quantities	
5. Total inulin in filtrate (2.,3.) = 125 × 6.32 − 83 × 7.18 = 197 mg.		
6. (Inulin) filtrate (5.,1.) = 197/41 = 4.8 mg/ml.		
7. (Inulin) filtrate/(Inulin) plasma (6.,3.) = 4.8/6.75 = 0.71		
8. Total protein in filtrate (2.,4.) = 125 × .039 − 83 × .061 = −0.15 ± .4 gm.		
9. (Protein) filtrate (8.,1.) = −0.15 ± .4/41 = −0.4±1, or less than 0.6%		

Exper. 37R., 41 gm. filtered into limb at average rate of 0.72 ml/minute by raising venous pressure. Inulin analyzed by method of Harrison (60). Protein concentrations determined from osmotic pressure (74).

An alternative method of demonstrating molecular sieving of inulin during ultrafiltration through the capillaries is shown in figure 11. Inulin was added to the perfusion reservoir and allowed to diffuse into the interstitial fluid while the limb was maintained in the isogravimetric state. The period from 180 to 218 minutes shows the end of the osmotic transient so produced. Rapid filtration was then produced for 45 minutes by raising the venous pressure at constant blood flow. The isogravimetric capillary pressure rose from 15 to 30 mm. Hg during the course of this filtration and when the limb was again made isogravimetric the increment of osmotic pressure declined exponentially with a time constant typical of inulin. Presumably the diffusion of inulin through the capillary wall could not keep pace with the rapid rate of filtration so that some molecular sieving occurred. Molecular sieving is not detectable by this method in the case of smaller molecules such as glucose or NaCl whose diffusion velocities greatly exceed filtration velocity as shown in table 10.

The relations between diffusion and filtration velocities in the capillaries of the hindlimb are in contrast to those which ordinarily obtain in ultrafiltration experi-

ments with artificial membranes. As pointed out by Ferry (ref. 43, p. 375), "Under actual conditions of ultrafiltration the hydrodynamical flow through the (artificial) membrane due to the applied pressure is usually so much greater than any diffusion effects, that the latter may be considered negligible." An additional factor of importance is that artificial membranes are ordinarily thicker than the capillary walls and diffusion rate varies inversely with the square of the path length whereas filtration varies inversely with the first power of the path length. It follows that molecular sieving during the ultrafiltration through the capillary walls will be less than that through an artificial membrane of the same porosity unless experimental

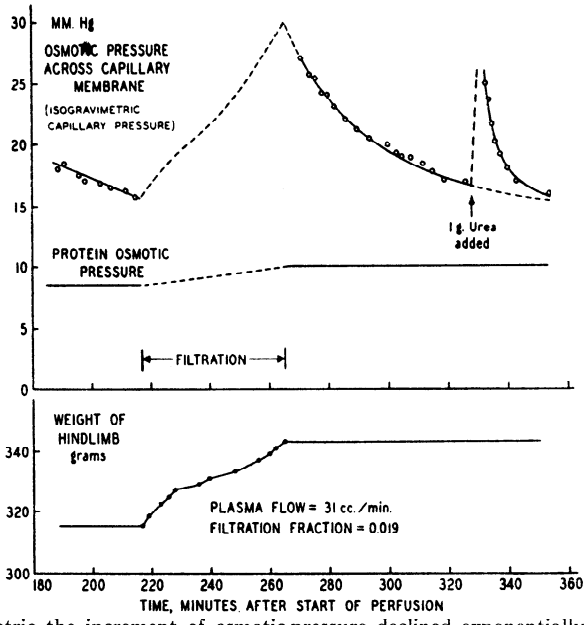


Fig. 11. Separation of inulin by ultrafiltration through peripheral capillaries. *Exper. 30-R.* T_A = 38° C. During rapid net filtration the filtration velocity becomes an appreciable fraction of the diffusion velocity for inulin (table 10). Under these circumstances inulin may be sieved, thus giving rise to an osmotic pressure across the capillary membranes. In this experiment inulin was added to the perfusion reservoir and allowed to diffuse into the interstitial fluid while the limb was maintained in the isogravimetric state. The period from 180 to 218 minutes shows the end of the osmotic transient so produced. Rapid filtration was then produced by raising the venous pressure at constant blood flow. The isogravimetric capillary pressure rose from 15 to about 30 mm. Hg during the course of this filtration and when the limb was again made isogravimetric the increment of osmotic pressure declined exponentially with a time constant typical of inulin. A 'urea transient' was induced for comparison. The average concentration of inulin in capillary filtrate during rapid ultrafiltration is about 70 per cent of that in plasma as determined by the chemical method summarized in table 11. Molecular sieving is not detectable by these methods in the case of smaller molecules such as glucose or NaCl whose diffusion velocities greatly exceed filtration velocity as shown in table 10.

conditions are such that the ratio of diffusion to filtration velocities is the same in the two cases.

Discussion

The theoretical methods developed in this paper to calculate capillary pore dimensions from osmotic transients, Fick's Laws and hydrodynamical resistance to filtration are, no doubt, greatly over-simplified. Likewise, the theory developed to account for restricted diffusion through pores of molecular dimensions is probably incomplete and will undergo modification as more experimental data accumulate. Nevertheless, when applied to the capillaries of the hindlimb, the present theories ac-

count remarkably well for the observed hydrodynamical resistance to flow of fluid across the capillary membranes and for the observed restrictions to diffusion of a wide variety of lipoid insoluble molecules. The results are quantitatively consistent with a description of capillary permeability to these substances in terms of a porous structure having an average pore radius of 30 Å (or an equivalent, but limited, distribution of radii) and a total area per unit path length of 1.3×10^5 cm. in the capillary walls of 100 gm. of muscle.

The discovery that only a minute fraction of the capillary wall is involved in the capillary exchange of water and lipoid-insoluble molecules provides a simple explanation of the fact that glomerular capillaries may be many times more permeable to water than peripheral capillaries without losing their impermeability to plasma protein. Molecular sieving of inulin during ultrafiltration and retention of hemoglobin indicate that the average pore size of the peripheral capillaries is less than that of glomerular capillaries. An increase in effective pore radius from 30 to 50 Å and an increase of collective pore area from less than 0.2 per cent to 1 or 2 per cent could easily account for a 100 fold increase of filtration coefficient without appreciable leakage of plasma protein.

The theoretical developments described in this paper appear to be new to the field of membrane physical chemistry. It would be interesting to apply them to the further study of factors affecting the diffusion of variously shaped molecules through artificial membranes. For such a study it would be desirable to employ extremely thin membranes so that the ratio of effective pore diameter to pore length would be comparable to that of the capillaries.

In a recent series of papers on the kinetics of membrane processes, Laidler *et al.* (75, 76) have demonstrated the feasibility of utilizing osmotic transients for the study of diffusion of various sugars through collodion membranes.

Much of the detailed discussion relevant to our present picture of capillary permeability has been included with the development of the theoretical and experimental sections in PARTS I, II and III above. Two principal topics remain for discussion.

1. Permeability to Gases and to Other Lipoid Soluble Molecules. A simple calculation suffices to show that the small area in the capillary walls available for the filtration of water and diffusion of lipoid insoluble molecules could not account for the observed rate of transport of oxygen by simple diffusion.

For the diffusion of oxygen,

$$\dot{Q}_{O_2} = D_{O_2} \cdot \alpha_{O_2} \cdot \frac{A}{\Delta x} \overline{\Delta P} \qquad (16)$$

where $\dot{Q}_{O_2}$ = oxygen consumption, ml/second, STPD.
D_{O_2} = free diffusion constant of O_2 at 37° C. = 3.0×10^{-5} cm.²/second
α_{O_2} = solubility of oxygen at 37° C. = 2.5×10^{-5} ml/ml/mm. Hg.
ΔP = mean pressure difference of oxygen across the capillary walls.

In a typical perfused cat hindlimb the oxygen consumption at 37°C. is 5×10^{-3} ml. O_2/second/100 gm. muscle (77). The value of $A/\Delta x$ for water is 1.3×10^5 cm. as described above. Solving *equation 16* for $\overline{\Delta P}$ we have

$$\overline{\Delta P} = \dot{Q}_{O_2} \div D_{O_2} \alpha_{O_2} \frac{A}{\Delta x} = \frac{5 \times 10^{-3}}{3 \times 10^{-5} \times 2.5 \times 10^{-5} \times 1.3 \times 10^5} = 50 \text{ mm. Hg.}$$

The true difference of oxygen pressure across the capillary walls must be very much less than this, for the oxygen pressure in arterial blood may be reduced to less than 50 mm. Hg without interfering appreciably with the transfer of oxygen across the capillary membranes. Moreover, a considerable additional pressure drop must be allowed for the diffusion of oxygen into the muscle cells (78). We are therefore led to suppose that the area in the capillary walls available for the diffusion of oxygen is greater than that available to water even though the oxygen molecule is larger than the water molecule.

The answer to this discrepancy appears to be concerned with lipoid solubility. The partition coefficient of oxygen between cottonseed oil and water is 5.0 in contrast to oil-water partition coefficients of less than 0.001 for all the molecules so far considered in this paper. It seemed possible that oxygen by virtue of its lipoid solubility, could dissolve in the plasma membranes of the capillary endothelial cells and in this way utilize the entire capillary surface for diffusion. With this hypothesis in mind, one of us (*E.M.R.*) has examined capillary permeability to various lipoid-soluble molecules including urethane, paraldehyde, triacetin and antipyrine. All of these molecules penetrate the capillary membranes so rapidly that no osmotic transient is detectable, even in concentrations calculated to give many times the osmotic activity of the lipoid-insoluble molecules used in the present investigation.

In view of these facts, it appears likely that capillary permeability to gases and other lipoid-soluble molecules involves the whole surface of the endothelial cell and is, in this respect, similar to cellular permeability in general. Whereas, capillary permeability to water and lipoid-insoluble solutes involves only a minute fraction of the capillary wall. The total area is so small that it might be localized to intercellular regions, although our experiments give no direct evidence on this point.

2. Interpretation of 'Arterial Disappearance Curves' of Tracer Substances Injected into the Circulation. The experiments described in this paper are of major importance to the interpretation of 'arterial disappearance curves' of tracer molecules injected into the circulation. In recent years a considerable literature has accumulated in this field; the 'transcapillary exchange rates' of various labelled ions have been calculated from analysis of their disappearance rates from arterial plasma (50–56). For these calculations it has been assumed implicitly that the mean capillary concentration of the tracer molecule is equal to its concentration in arterial blood throughout the distribution time.

In the present investigation we have found that this assumption is not even approximately correct. There is a large arteriovenous difference in concentration of injected molecules (figs. 3*a*, *b*, *c*, *d*, 4 and 5) and the initial partial osmotic pressure in the capillaries is only a small fraction of that in arterial blood (fig. 2*b*). Previous estimates of transcapillary exchange rates, based on the assumption that the mean capillary concentration is equal to that in arterial blood, are therefore in error by a large but unknown amount. An estimate of the order of magnitude of this error may be made as follows.

The basic equation from which these estimates of transcapillary exchange rate were made has been stated by Flexner, Cowie and Vosburgh (54) in the following form:

$$C_t - C_{eq} = (C_o - C_{eq})e^{-(R/Q)t} \qquad (17)$$

where C_t, C_o and C_{eq} are the concentrations of the tracer molecules in *arterial plasma* at time t, at time $t = 0$ and at equilibrium, respectively. R is the proportion of the plasma tracer which exchanges per unit time with the extravascular fluid and q is the proportion of the total distribution volume occupied by the extravascular compartment. This equation is similar to previous formulations of diffusion processes in uniformly stirred two compartment systems (49, 79). When the two compartments of volumes V_1 and V_2 are separated by a membrane of diffusion area A and thickness Δx, then the concentration (c_t) in *compartment I* at any time after addition of the test molecule is given by,

$$C_t - C_{eq} = (C_o - C_{eq})e^{-DA/\Delta x[(V_1+V_2)/V_1V_2]t} \qquad (18)$$

Equation 18 is identical with *equation 17* where

$$R = \frac{D}{V_1} \times \frac{A}{\Delta x} \qquad \text{and} \qquad q = V_2/(V_1 + V_2)$$

The area per unit path length available for diffusion of a test molecule added to V_1 is therefore $(A/\Delta x) = (RV_1/D)$.

Application of *equation 17* or *18* to the disappearance of sodium or chloride from the arterial plasma of the guinea pig leads to a value for R of 0.6/minute or 0.01/second (55). The plasma volumes V_1 of the guinea pig is about 4 ml/100 gm. tissue and the diffusion constant of NaCl is about 2×10^{-5} cm.^{2}/second. The value of R as calculated from *equation 17* therefore implies that the area per unit path length available for the exchange of Na or Cl in the capillaries of 100 gm. of tissue is

$$\frac{A}{\Delta x} = \frac{R \cdot V_1}{D} = \frac{.01 \times 4}{2 \times 10^{-5}} = 0.005 \times 10^5 \text{ cm.}$$

This value for $A/\Delta x$ is 200 times smaller than the area per unit path length available to NaCl in the capillaries of 100 gm. of muscle in the cat hindlimb (fig. 7). It would imply that the total capillary area for diffusion of NaCl is less than 0.05 cm.^{2}/100 gm. of guinea pig (assuming a maximum path length of 1 μ) and would lead to improbably low values for permeability to water or improbably high values for capillary pore size.

We therefore conclude that the erroneous assumptions involved in the use of *equation 17* to calculate transcapillary exchange rates lead to a value which is approximately 200-fold too low in the case of NaCl. For larger molecules the magnitude of this error will be less, and for smaller molecules, such as D_2O, it will be more.

Summary

Part I. Filtration. The capillaries in mammalian muscle were estimated to be 100-fold less permeable to water than glomerular capillaries and approximately 4000-fold less permeable to water than a collodion membrane of comparable thickness and permeability to protein (table 4). On the pore theory of capillary permeability this implies that the fractional area of the capillary walls available for filtration of water must be exceedingly small.

Part II. Diffusion. *1*) A new method is described for measuring the rates at which substances diffuse across the capillary walls in the perfused hindlimbs of cats. Addition of test molecules to the perfusing blood causes a rise of effective capillary osmotic pressure (fig. *2a*) above the preexisting protein pressure, due to the partial osmotic pressure exerted by the test molecules during their passage across the capillary membranes. This partial osmotic pressure is measured by the increment of mean capillary hydrostatic pressure required to prevent osmotic withdrawal of interstitial fluid into the capillaries during the diffusion process. The increment of partial osmotic pressure diminishes exponentially with time as diffusion equilibrium between blood and interstitial fluid is approached (fig. *2b*).

2) The half-times of osmotic transients produced by various substances at tissue temperatures of 37 ± 2°C. and 10 ± 2°C. are summarized in table 5. The products of half-time and free diffusion coefficient are approximately constant for inulin, raffinose, glucose, urea and NaCl in spite of a 20:1 spectrum of diffusion coefficients. Sucrose diffuses slightly more rapidly than predicted from its free diffusion coefficient.

3) The rates of net molecular transfer across the capillary walls were also measured from the blood flow and the arteriovenous difference of concentration. Combination of these results with the osmotic pressure data theoretically allows calculation of the area per unit path length in the capillary membranes which is available for free diffusion of the various test molecules (*equation 5*). This quantity remains constant throughout the diffusion process (figs. *3a*, *b*, *c*, *d* and 4) and is independent of the blood flow in the range of flows so far investigated (fig. 5, table 6).

4) The calculated area available for free diffusion per unit path length in the capillary walls decreases progressively with increasing molecular size (fig. 7). For a molecule the size of water the average value is 1.3×10^5 cm/100 gm. of muscle; for inulin the diffusion area is reduced to less than 15 per cent of that for water (table 7). The total area available for the passage of water is estimated to be less than 0.2 per cent of the histological surface of the capillary walls. This supports the conclusion reached in PART I that the fractional area in the capillary membranes available for the filtration of water is exceedingly small.

Part III. *1*) *Derivation of pore dimensions.* A method is presented for calculating the dimensions of cylindrical pores or rectangular slits which would account for *a*) the diffusion area per unit path length in the capillaries for a molecule the size of water and *b*) the filtration coefficient of the capillaries. Uniform pores of radius 30 Å or uniform rectangular slits of half-width 18.5 Å would successfully account for the data. The values calculated for uniform openings represent maximum values for the mean pore size in any distribution of pore sizes. The upper limit to a distribution of pore sizes is set by hemoglobin which, within the limits of error of our methods, is completely retained by the hindlimb capillaries (fig. 8). The broadest Gaussian distribution compatible with the data predicts a mean pore radius of 24 Å with a standard deviation of 12 Å.

2) *Restricted diffusion as a function of molecular size.* A theory is developed, (*equations 10* and *11* and table 8) to account for restricted diffusion, as a function of molecular size, through pores of dimensions calculated as above from the filtration

coefficient and the area per unit path length for diffusion of a molecule the size of water. The theory accounts remarkably well for the permeability of the hindlimb capillaries to lipoid-insoluble molecules ranging in size from NaCl to hemoglobin (fig. 10).

3) *Relation between filtration and diffusion velocities and its significance for molecular sieving during ultrafiltration.* The data indicate that under normal circumstances diffusion velocity is overwhelmingly greater than filtration velocity. This tends to diminish molecular sieving during ultrafiltration; it is shown that during extremely rapid filtration the concentration of inulin in capillary filtrate is 70 per cent of that in plasma (table 11) although the effective area in the capillary walls available for penetration of inulin is only 14 per cent of that available to water (table 7). It is pointed out that in ultrafiltration experiments with artificial membranes filtration rather than diffusion is usually the dominant process and this favors molecular sieving.

Discussion. 1) *Permeability to oxygen and other lipoid-soluble substances.* The small capillary area available for the passage of water and lipoid-insoluble molecules is insufficient to account for the observed rate of transfer of oxygen by diffusion. Preliminary experiments are cited which show that paraldehyde, urethane, triacetin etc. (which, like oxygen, are soluble in oils as well as in water) penetrate the capillary membranes so rapidly that no osmotic transient is detectable, even in concentrations calculated to give many times the osmotic activity of the lipoid-insoluble molecules used in the present investigation. The hypothesis is advanced that oxygen and other lipoid-soluble molecules can penetrate the plasma membranes of the capillary endothelial cells and are not restricted to aqueous intercellular spaces.

2) *'Transcapillary exchange rates' as calculated from arterial disappearance curves of tracer molecules.* Our experiments have revealed a major error in the equations commonly employed to calculate transcapillary exchange rates from the disappearance rates of tagged molecules injected into the circulation. These equations assume that the effective capillary concentration of the test molecules is equal to that in arterial plasma. The large arteriovenous differences (figs. 3*a*, *b*, *c*, *d*, 4 and 5) and the fact that the initial effective osmotic pressure is only a small fraction of that in arterial plasma (fig. 2*b*) show that this assumption is not correct, even to a first approximation. The error is such that the value of transcapillary exchange rate of Na^+ or Cl^- as previously calculated from tracer studies is about 200-fold too low.

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