

Passage of Molecules Through Capillary Walls

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FROM THE POINT OF VIEW of hemodynamics the blood is generally considered to circulate within a closed system of blood vessels. Even the smallest capillaries appear, under the microscope, as closed thin-walled vessels separating the blood from the extravascular fluid. Only occasionally are discontinuities in the capillary wall made evident by the diapedesis of one of the formed elements of the blood and even in such cases it is hard to be certain that a microscopically visible channel of egress is present. At high magnifications the blood appears to flow rapidly through individual capillaries thus forming a striking contrast to the relatively stagnant extravascular fluid and accentuating the role of the capillary membrane in providing a phase boundary separating the blood from the tissues. There are good reasons for supposing, however, that the capillary blood is in intimate contact with extravascular fluid and that the visible flow of blood through the capillaries is, in fact, very small in comparison with the *invisible* flow of water and dissolved materials back and forth through the capillary walls. Evidence to be reviewed below suggests that this invisible component of the circulation takes place at a rate which is many times greater than that of the entire cardiac output. Indeed, it is by means of this 'ultramicroscopic circulation' through the capillary wall that the circulatory system as a whole fulfills its ultimate function in the transport of materials to and from the cells of the body.

This review will deal with the physical properties of the ultramicroscopic circulation, its functional structure, the magnitude of flow through it and the physico-chemical mechanisms regulating the flow. Direct methods for the study of ultra-structure have not as yet been applied to the capillary wall and much of what can be said must be deduced from quantitative studies of capillary permeability. A wealth of evidence supports the view that the exchange of materials through the walls of living capillaries takes place by physical processes which involve no expenditure of energy on the part of the capillary endothelial cells themselves. This evidence has been reviewed previously (20, 30, 89, 162) and need not be considered here in detail. At least two types of capillary structure appear to be involved. On the one hand we have to consider the permeability characteristics of the plasma membranes which envelop the protoplasm of the capillary endothelial cells and which comprise the greater part of the visible capillary surface. On the basis of analogy with other known plasma membranes we may expect this type of structure to exhibit a relatively low order of permeability to ions and lipid insoluble molecules and a high order of permeability to oxygen, carbon dioxide and other lipid soluble substances. On the other hand, we have to consider specialized regions through or between endothelial cells which endow the capillary wall as a whole with a relatively high degree of permeability to water, ions and large lipid insoluble molecules. This type of permeability resembles that of artificial porous membranes and has given rise to the hypothesis that the blood communicates directly with the extravascular fluid via channels or

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pores which are in general too small to allow the passage of large protein molecules but are of sufficient size and number to account for the observed rates of passage of water and other lipid insoluble molecules. Chambers and Zweifach (20) have reviewed evidence, based on microscopic observations, that the structural basis for this type of permeability may be associated with the intercellular cement substance.

Although the capillary exchange may involve only 'passive' physical processes, we cannot say at this time that such processes are simple or well understood. The familiar physical laws, which govern the flow of materials in macroscopic and microscopic systems, do not necessarily apply to flow through channels of ultramicroscopic dimensions. At the present time, for example, we do not know precisely how the laws of laminar flow must be modified when the dimensions of the flow channel are of the same order of magnitude as the dimensions of the flowing molecules. No well-established theory exists to predict the restriction to free diffusion encountered by molecules diffusing through channels of molecular dimensions. Little is known concerning the effects of molecular shape on restricted diffusion, a problem which is of practical importance to the design of plasma volume expanders. No well-substantiated equations are available to predict the transient osmotic pressures developed across membranes which restrict, but do not prevent entirely, the free diffusion of solute molecules.

It is obvious that any attempt to analyze capillary permeability in terms of the ultrastructure of the capillary wall must involve a detailed consideration of these and related problems. PART I of this review is therefore devoted to a discussion of the basic physical processes involved in the flow of materials through membranes, especially porous membranes. The discussion will be limited to processes which are considered to be of importance in the passage of molecules through capillary walls, omitting such factors as electrostatic charge, electro-osmosis, or the migration of ions in potential fields which have no known representation in the capillaries although they may be important factors determining passive transfer of substances through cell membranes (148) or through certain artificial porous membranes (138, 146). In PART II some quantitative aspects of capillary permeability will be reviewed and the data analyzed in terms of the mechanisms discussed in PART I, leading to a description of molecular flow through capillary walls in terms of the properties of the flowing molecules on the one hand, and the structure of the capillary wall on the other.

PART I. PHYSICAL PROCESSES INVOLVED IN THE FLOW OF MATERIALS THROUGH MEMBRANES

1. Laminar Flow of Liquids Through Porous Membranes, Calculations of Effective Pore Size

Several lines of evidence suggest that laminar flow of liquids may occur through porous membranes. This evidence, which forms an important basis for interpreting measurements of fluid movement across capillary walls, may be summarized under the following headings. A. The rate of flow of liquids through porous membranes, like that through macroscopic laminar flow systems, is a) proportional to the gradient of hydrostatic pressure through the membrane, b) inversely proportional to the viscosity of the liquid, and c) independent of the temperature, except insofar as temperature alters the viscosity of the liquid. B. The effective pore size calculated on the assumption that flow through the membrane obeys Poiseuille's Law (laminar flow) agrees approximately with pore size calculated from a) the laws of capillarity

(surface tension), *b*) the dimensions of the largest molecule that will penetrate the membrane, and *c*) electron micrographs of membrane structure.

A. Rate of Flow of Liquids Through Porous Membranes. Darcy (31) was among the first to show that the rate of flow of liquids through porous media is proportional to the pressure gradient through the medium. Darcy's Law is stated by equation 1:

$$\dot{Q}_t = \frac{K_t A_m}{\eta} \times \frac{\Delta P}{\Delta x} \quad (1)$$

where \dot{Q}_t = volume of liquid filtering through the medium in unit time. K_t = the flow constant, sometimes called the 'Darcy coefficient' (68, 163) or the 'hydraulic conductivity' (14). In Darcy's original equation the viscosity was not considered. A_m = cross-sectional area of the medium. $\Delta P/\Delta x$ = gradient of pressure through the medium. η = viscosity of the filtrate. When expressed in c.g.s. units, the proportionality constant of flow, K_t , has the dimensions of square centimeters; it is frequently used as a coefficient of permeability in geophysical and industrial problems involving the permeation of liquids through soils, powders and fibrous materials (145, 163). Manegold (102) has advocated its use to characterize the 'Wasserdurchlässigkeit' or hydraulic conductivity of artificial porous membranes.

Darcy's Law appears to be applicable to membranes having porosities in the range of interest for comparison with capillaries. Bjerrum and Manegold (11, 12) and Manegold and Hofman (103) have shown that flow is proportional to pressure difference and inversely proportional to membrane thickness in partially dried collodion membranes with effective pore radii² ranging from 20 to 200 Å. (cf. also refs. 4, 10, 37, 42). This range of pore sizes probably includes the upper limit of pore dimensions to be expected in most capillaries. Thus egg albumin, which passes rapidly through glomerular capillaries (5, 13), is retained by collodion membranes having effective pore radii less than 30 Å (4, 43). Cellophane membranes, used clinically for *in vivo* dialysis, have effective pore radii in the range of 20 to 30 Å and severely restrict the passage of inulin (107) which is known to pass rather rapidly through the walls of peripheral capillaries (25, 79, 126, 129). Deviations from proportionality between pressure and flow have been noted by Lepeschkin (94) in connection with flow through fully dried collodion membranes. Membranes of this type are impermeable to molecules larger than glucose (157) and probably have pore radii which are less than 5 to 10 Å (the longest dimension of glucose is 9 Å as shown in table 2). This range of porosities is more of interest in connection with cellular membranes than with capillary membranes.

The effects of viscosity on the flow of liquids through collodion membranes were investigated by Bigelow (10) who found that the Darcy coefficient for water did not vary by more than 5 per cent when the viscosity was altered over a threefold range by changing the temperature from 0.9° to 56°C. The effective pore radius in this case was 55 Å³; membranes of this porosity retain more than 95 per cent of serum albumin during ultrafiltration (47) and are therefore comparable, in this respect, to living capillaries. More refined measurements by Duclaux and Errera (37) have shown that the Darcy coefficient for water flowing through cellulose acetate membranes varies

² See section B for definition of effective pore radius.

³ Bigelow did not characterize his membranes in terms of pore size. The value given above is calculated from the data of Hitchcock (64), Manegold and Hofman (103) and Elford and Ferry (42) which allow the pore radius to be estimated from the Darcy coefficient.

by less than 1 per cent in the temperature range 11° to 23°C. The dependence of flow on viscosity is strikingly demonstrated by the experiments of Duclaux and Errera (36-38) on the flow of various solvents and solutions through porous membranes. Concentrated solutions of saccharose or glycerine with viscosities up to 18-fold that of water, passed through the membranes at rates which were almost exactly in inverse proportion to their viscosities. Similar results were obtained with a series of organic solvents with relative viscosities ranging from 0.27 to 4.0. This result is of special importance to the theory of flow through porous membranes, for it eliminates the possible role of solubility or of surface tension as major factors determining the flow. Minor deviations from Darcy's Law were, however, noted. The viscosities of the organic solvents relative to water were slightly greater (about 10%) in the membranes than in a conventional capillary tube viscometer, whereas solutions of electrolytes tended to have a slightly lower relative viscosity in the membranes than in the viscometer. These deviations (confirmed by Manegold and Hofman) were interpreted by Duclaux and Errera in terms of electrokinetic potentials which are generated by aqueous solutions but not by organic solvents.

B. Calculation of Pore Dimensions in Artificial Membranes. The evidence discussed above provides experimental support for the basic assumption of viscous flow used in the calculation of pore dimensions from rate of flow measurements. This calculation, originally proposed by Guérout (58), has frequently been employed to characterize the porosities of artificial semipermeable membranes (4, 37, 40, 42, 57, 64, 103, 121) and has recently been applied also to estimate the porosity of capillary membranes (118). The calculation is simply illustrated for the case of a membrane containing n parallel cylindrical pores of uniform radius, r . In this case the total cross-sectional pore area, A_p , is $n\pi r^2$. If laminar flow occurs through these pores then we may apply Poiseuille's Law as follows

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

Combining equations 1 and 2 and solving for r , we have

$$r = \sqrt{\frac{8K_f}{A_p/A_m}} \quad (3)$$

which allows a numerical solution for the pore radius, r , in terms of the Darcy coefficient, K_f and the fractional pore area, A_p/A_m . The value of K_f is determined from rate of flow measurements and equation 1. The fractional pore area is usually estimated from the ratio of the dry to the wet weight of the membrane; with certain limitations it can also be estimated from the electrical conductivity through the membrane (64) or from diffusion measurements (101, 118).

Equation 3 applies only to membranes containing parallel uniform cylindrical pores. If a membrane contains a distribution of cylindrical pores, the flow rate through the large pores will be higher than through the small pores in proportion to the fourth power of the ratio of radii (Poiseuille's Law). Thus,

$$r = \sqrt[n]{F_1 r_1^4 + F_2 r_2^4 + \cdots F_n r_n^4} \quad (4)$$

where \bar{r} = effective pore radius and F_n = fraction of total number of pores having a radius of r_n . For this reason the 'effective pore radius' calculated from equation 3 will always be greater than the arithmetical mean of any distribution of pore sizes in the membrane.

Expressions similar to *equations 3* and *4* may theoretically be derived for pores or channels of any given geometrical configuration. For example, Bjerrum and Manegold (11) proposed a slit structure for the pores in collodion membranes and showed that in this case the slit width, *w* is given by

$$w = \sqrt{\frac{12K_f}{A_p/A_m}} \quad (5)$$

More recent evidence, reviewed by Ferry (48), suggests a uniform *ultragel* structure which would allow flow between rigid micelles of collodion oriented perpendicular to the plane of the membrane. The precise geometrical configuration of the pores is rarely, if ever, known, but *equation 3* is nevertheless valuable for characterizing any given membrane in terms of its 'effective' pore radius.

In ether-alcohol collodion membranes, the three variables—effective pore radius, hydraulic conductivity (Darcy coefficient) and fractional pore area—are not independently variable at all porosities. In membranes with pore radii less than 120 Å an empirical relation between porosity and fractional pore area has been found (42, 64, 103).

It is obvious that if the effective pore radius of an isoporous membrane agrees with the radius calculated by independent methods, then this will provide indirect evidence in support of the use of Poiseuille's Law as a means of estimating pore size. Three such independent methods have been investigated. The first utilizes the law of capillarity which states that the critical pressure (*P_c*) required to cause a barely perceptible flow of fluid through a capillary tube which is already filled with a second fluid (immiscible with the first) is given by

$$P_c = 2\gamma/r \quad (6)$$

where γ is the surface tension at the interface between the two fluids and *r* is the radius of the tube. This method, originally devised by Bechhold (7), has been studied in detail by von Erbe (44) who utilized water-isobutyl alcohol and other solvent pairs. In a perfectly isoporous membrane no flow should occur until the critical pressure is exceeded. After the critical pressure is exceeded the pores become filled with pure solvent, there is no longer an interfacial tension and flow proceeds in accordance with Poiseuille's Law. If the membrane has a distribution of pore sizes, then the critical pressures due to the individual pores will summate to produce a sigmoid probability curve which gradually approaches Poiseuille flow only after the highest critical pressure (due to the smallest pore) has been exceeded. This sigmoid portion of the pressure-flow curve in membrane systems containing mixtures of immiscible liquids has been utilized by von Erbe (44), Pisa (121) and by Grabar and Nikitine (57) to estimate the distribution of pore sizes. The work of Grabar and Nikitine (57) is of special importance in this connection, for they compared pore size determined by the rate of flow method with pore size determined by the surface tension method in a series of membranes of graded porosities prepared by the method of Elford (41). Elford membranes are characterized by a fairly high degree of isoporosity as determined both by the surface tension method and by their properties as molecular sieves. In the membranes studied by Grabar and Nikitine (57) the distribution of pore radii determined by the surface tension method showed a high peak at values slightly less than the effective pore radii determined by the rate of flow method, a result which would be expected of Poiseuille flow through a narrow distribution of pore sizes (*eq. 4*). Thus in a typical membrane with an effective pore radius of 75 Å

as determined by rate of flow, the mean pore radius estimated from surface tension was 64 Å and 75 per cent of the pore radii were within the range of 45 to 110 Å.

A second method of estimating pore size for comparison with the rate of flow is based on restriction to diffusion and filtration of molecules of graded sizes through isoporous membranes. The practical application of this method involves a consideration of many complicating factors, including the influence of molecular shape, the exact degree of isoporosity, interactions between the test molecules and the pores, the distribution of flow velocity within the pores (47) and the relations between filtration velocity and diffusion velocity. For the present argument, however, it is only necessary to note the general correspondence between pore size as estimated from rate of flow measurements and the radius of particles which just fail to penetrate the membrane. Thus Bauer and Hughes (4) found an endpoint porosity of about 70 to 80 Å diameter in ultrafiltration experiments with oxyhemoglobin. X-ray diffraction studies (73) indicate that hemoglobin is cylindrical in shape with a diameter of 57 Å and length of 34 Å; its diffusion coefficient (122) is equivalent to that of a sphere of diameter 62 Å (Einstein-Stokes diameter). Similarly egg albumin is withheld by membranes with effective pore diameters in the range 55 to 65 Å (4, 43); the Einstein-Stokes diffusion diameter of egg albumin is 56 Å and its dimensions calculated from its diffusion coefficient and intrinsic viscosity correspond to an ellipse with axes 22 x 96 Å. Similar figures for several well-characterized proteins have been summarized by Ferry (48). In general the data indicate that the effective pore radius calculated on the assumption of Poiseuille flow is from 25 to 50 per cent greater than the Einstein-Stokes diffusion radius of the molecule which just fails to penetrate the membrane. Some of the factors which may enter into this discrepancy have been mentioned above; the correspondence is nevertheless sufficiently close to provide evidence justifying the use of Poiseuille's Law in the estimation of pore size.

A third method of estimating pore size has recently been described by Bugher (18). Replicas of free surfaces and microscopic sections of calibrated collodion membranes were made by evaporating silicon monoxide normal to the specimen surface. Electron micrographs of such replicas revealed a structure consisting of "... somewhat irregular, anastomosing passages giving a spongy character to the whole." The orifices were irregular and the passages variable in bore but they were of the same magnitude as the effective pore size calculated on the basis of Poiseuille's Law.

The various lines of evidence reviewed above support the hypothesis that the energy required for flow through porous membranes is utilized in overcoming viscous resistance between fluid laminae as in the usual type of laminar flow system considered in hydrodynamics. The evidence applies to porosities extending down to about 20 Å (about 20 water molecules in diameter) which is well below the range of porosity required to explain capillary permeability to molecules such as inulin, myoglobin or egg albumin. Laminar flow through a cylindrical tube (Poiseuille flow) implies a parabolic distribution of flow velocity within the tube. The velocity is zero at the walls and increases parabolically to a maximum at the axis of the tube. In macroscopic systems, where the tube diameter may be several million times larger than the diameter of the flowing molecules, it is relatively easy to conceive of this smooth parabolic velocity profile demanded by Poiseuille's Law. In this case the random kinetic movements of the individual molecules would have little effect on the average velocity vector in the direction of flow. In membranes having pore dimensions of the order of 20 to 100 water molecules in diameter, it is more difficult to imagine a smooth velocity profile within an individual pore. Possibly we should con-

sider the picture from a statistical point of view; within any given pore the velocity distribution may be discontinuous, owing to the random movements of individual molecules, but the average of the velocity gradients in many millions of pores might well lead to a smooth average distribution.

As the pore radius is reduced below about 20 Å we may expect substantial corrections to Poiseuille's Law. Barrer (3) has discussed physical aspects of flow through certain crystals, but on the whole very little experimental work has been carried out in systems with known pore dimensions in this range. In the next section reasons will be given for supposing that net diffusion, as contrasted with hydrodynamic flow, becomes an increasingly important mechanism as the pore radius is reduced below 10 Å.

2. Role of Diffusion and Osmotic Pressure in the Net Flow of Solvent Molecules Through Porous Membranes

Net flow of solvent molecules through porous membranes may result from osmotic, as well as from hydrostatic, forces operating across the membrane. It seems reasonable to assume that the energy required to produce a given net flow rate through a given membrane would be the same whether hydrostatic or osmotic forces are involved. Indeed, this assumption is implicit in the 'Starling Hypothesis' (140), which supposes that the rate of net fluid movement through capillary walls is proportional to the difference between hydrostatic and osmotic forces acting across the capillary walls. Thermodynamical reasons justifying this assumption have been advanced by Laidler and Shuler (83), Staverman (143) and by Chinard (21, 22). Experimental evidence that net rate of flow is proportional to the difference between hydrostatic and osmotic pressures has been obtained using both artificial membranes (111, 113) and the walls of living capillaries (85, 117) (cf. also fig. 3A).

The thermodynamic formulation (21, 83, 143) is applicable to the flow of both solvent and solute; for the case of water flowing through porous membranes it may be written in the form,

$$\dot{Q} = k \frac{A_p}{\Delta x} (\Delta P - \Delta \pi) \quad (7)$$

where \dot{Q} = flow of water; k = constant of proportionality; A_p = cross-sectional pore area; Δx = path length through the membrane; ΔP = difference in hydrostatic pressure across the membrane and $\Delta \pi$ = difference in osmotic pressure across the membrane. The factors entering into the proportionality constant, k , depend upon the mechanism of flow. For the case of laminar flow through cylindrical pores, equation 7 becomes

$$\dot{Q}_f = \frac{K_f A_m}{\eta \Delta x} (\Delta P - \Delta \pi) = \frac{A_p r^2}{8 \eta \Delta x} (\Delta P - \Delta \pi) \quad (8)$$

where K_f is the Darcy coefficient. Equation 8 is identical with equations 1 and 2 when $\Delta \pi$ is negligible compared with ΔP .

At osmotic equilibrium, when hydrostatic pressure is equal and opposite to osmotic pressure, there is no net flow of solvent and therefore no energy is required to overcome viscous resistance to shear between fluid laminae. In this case, the rate of diffusion caused by the higher concentration (activity) of water on the side of the membrane containing no osmotically active solute molecules is exactly balanced by the increased thermodynamic activity of the water molecules subjected to hydrostatic pressure on the other side of the membrane. Chinard (21, 22) has recently

pointed out that during net flow caused by hydrostatic pressure the increased thermodynamic activity of the solvent molecules due to the applied pressure will cause net diffusion of the solvent molecules and that this mechanism must be considered in addition to the mechanism of hydrodynamic flow. In the case of free diffusion of water through pores, Chinard (21) has shown that *equation 7* becomes,

$$\dot{q} = \frac{D_{H_2O} \bar{V}_{H_2O}}{RT} \times \frac{A_p}{\Delta x} (\Delta P - \Delta \pi) \quad (9)$$

where A_p , Δx , ΔP and $\Delta \pi$ are the same as in *equation 7* and \dot{q} = net flow rate of water diffusing across the membrane; R = gas constant; D_{H_2O} = free diffusion coefficient of water in water; T = absolute temperature; and \bar{V}_{H_2O} = partial molal volume of water.

From *equations 8* and *9* we can assess the relative importance of diffusion as compared with hydrodynamic flow. Of the total net flow of water ($\dot{Q} + \dot{q}$), the fraction which may be expected to take place by diffusion is:

$$\frac{\dot{q}}{\dot{Q} + \dot{q}} = \frac{\frac{r^2 RT}{1 + \frac{8\eta D_{H_2O} \bar{V}_{H_2O}}{r^2 RT}}} \quad (10)$$

It is evident from inspection of *equation 10* that the relative importance of net diffusion will increase greatly with decrease in pore size. At 37°C. the various constants in *equation 10* have the following values: $RT = 25 \times 10^9$ dyne-cm/mol; $D_{H_2O}^{18} = 4.05 \times 10^{-5}$ cm.²/sec. (154); $\eta = 0.007$ (dyne-sec/cm.²); and $\bar{V}_{H_2O} = 18$ cm.³/mol.

Figure 1, based on these values and *equation 10*, shows the fraction of net flow of water which may be expected to take place by diffusion in membranes of varying porosities. It is evident from figure 1 that for membranes with pore radii greater than about 20 Å, the net flow of water which may be expected to result from net diffusion is negligible compared to that from hydrodynamic flow, a conclusion which is consistent with the evidence for hydrodynamic flow reviewed in *section 1* above. It therefore appears unlikely that diffusion plays an important role in the net flow of water through capillary membranes which allow the rapid passage through them of molecules the size of inulin (Einstein-Stokes diffusion radius $R_E = 15$ Å). The recent work of Koefoed-Johnsen and Ussing (75) suggests that even in cellular membranes the role of net diffusion of water may be subordinate to that of hydrodynamic flow. They point out that the permeability to water of frog skin (61) or intestine (152) as measured by the diffusion of D_2O is always considerably less than 'permeability' determined from rate of flow per unit osmotic pressure difference. A similar result has been found in the case of various ova (165). Under the influence of pituitary hormones, the permeability of frog's skin, as determined by rate of flow, could be increased two- or threefold without a corresponding change in permeability as measured by diffusion of D_2O . They have derived an equation relating hydrodynamic flow to diffusion which is more general than *equation 10* above in that it assumes no specific pore geometry. The conclusion, however, is the same, "For pore membranes the permeability as measured by osmotic flow is always larger than the permeability as calculated from the diffusion of isotopic water."

Failure to distinguish between hydrodynamic flow and net diffusion of solvent through porous membranes has given rise to some confusion in the literature. For example, the experiments of Landis (85) showed that a net volume of 56×10^{-8} ml. would flow each second through 1 cm.² of capillary wall (frog mesentery) under the

influence of a pressure difference of 1 cm. H₂O across the capillary wall. Davson (32) has converted the units of pressure into 'equivalent' concentration units ($\Delta C = \Delta P/RT$) and volume flow to mols per second (mols = volume \times density/mol. weight). With this transformation the original constant becomes 7.9×10^{-1} cm/sec.), these dimensions being considered comparable with those obtained in diffusion studies utilizing Fick's Law. Similar transformations were made for data on net rate of flow into cells. The implicit assumption is that the pressure difference provides the force required to overcome resistance to diffusion rather than to hydrodynamic flow. Chinard (21, 22) has recently made similar calculations in connection with glomerular filtration.

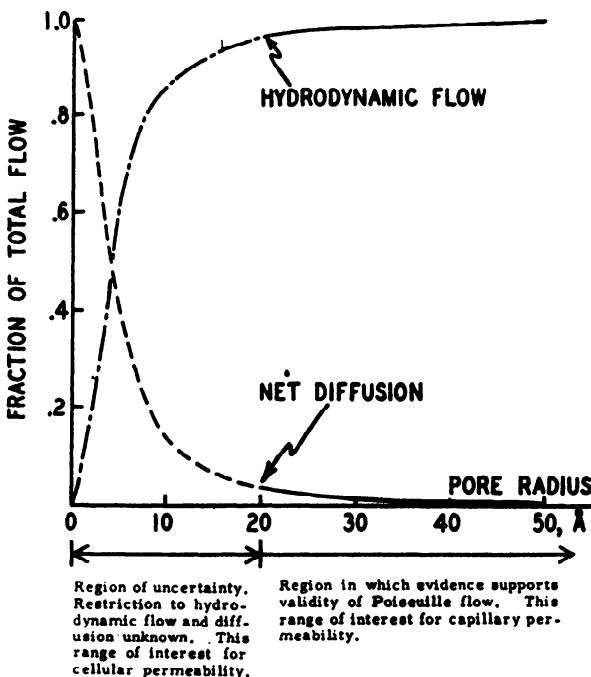


FIG. 1. Net diffusion and hydrodynamic flow of water as a function of pore size during flow induced by hydrostatic or osmotic pressure at 37°C. Calculation from equation 10, to illustrate relative importance of hydrodynamic flow and diffusion in the net transport of water through porous membranes under the influence of hydrostatic or osmotic forces (or both) operating across the membrane. For membranes with effective pore radii greater than about 20 Å, the net flow of water by diffusion is negligible compared to the hydrodynamic flow.

We cannot emphasize too strongly that data obtained from rate of flow measurements are not directly comparable with diffusion data. The comparison is valid only in connection with flow through nonporous (phase) membranes in which flow takes place by solution in the membrane followed by diffusion through the membrane. It is indeed unfortunate that the term 'permeability' should have become attached to measurements based on both flow and diffusion through porous membranes. For a given cross-sectional pore area and activity gradient, diffusion is practically independent of pore size unless the pore dimensions are comparable with those of the diffusing molecules as discussed in section 3, whereas hydrodynamic flow varies with the square of the effective pore radius as shown in equation 8. The distinction between the two mechanisms is so important that it is perhaps worth while to give one more numerical example in addition to figure 1. Consider two membranes, each of the same thickness. Let the total cross-sectional pore area in each membrane be 1 cm.², but in one membrane let there be a single pore of radius, $r = \sqrt{\pi^{-1}}$ cm., whereas in the second membrane let there be 10^4 pores each of radius, $r = \sqrt{\pi^{-1}} \times 10^{-2}$ cm.

Rates of diffusion per unit activity gradient will be the same in the two cases. However, the membrane with the single pore will allow fluid to pass 10^4 times more rapidly per unit pressure difference than the membrane with 10,000 pores having the same cross-sectional area for diffusion. In the case of both membranes hydrodynamic flow caused by unit pressure difference will be over a million times faster than flow by net diffusion (eq. 10).

The above discussion refers to *net* volume flow of water by diffusion as contrasted with hydrodynamic flow. It should not be confused with rates of diffusion *in both directions* through the membrane, nor with the *net* diffusion rates of small solute molecules under the influence of large concentration gradients. In PART II, sections 5 and 6, evidence will be reviewed which indicates that in these latter processes, diffusion rates may be overwhelmingly more important than hydrodynamic flow through the membrane.

3. Diffusion of Solutes Through Membranes

The physical factors which determine the rate of diffusion of molecules through nonporous membranes have been discussed in detail in numerous reviews and monographs (33, 65, 114, 115, 161) and will not be considered here except in connection with capillary permeability to lipid-soluble molecules in PART II below. Relatively little is known, however, of the basic physical factors which determine the rate of diffusion of molecules through membrane pores, especially when the porous channels are of dimensions comparable with those of the diffusing molecules. It is the purpose of this section to define various terms which are useful in describing diffusion through porous membranes and to call attention to some unsolved physical problems, the solutions of which are important to the understanding of the capillary exchange and of cellular permeability in general. The definitions may be made most simply with the aid of the familiar model consisting of a vessel which is separated into two compartments by means of a porous membrane. It will be assumed that the membrane is uncharged and that no electrokinetic, electrostatic or chemical interactions take place between the pores and the molecules which diffuse through them.

A. Free Diffusion, Calculation of Pore Area From Diffusion Data. If the pores are sufficiently large, the process of free diffusion can occur in both directions through the pores. The only effect of the membrane is to reduce the total area available for free diffusion. The rates of free diffusion in either direction may be computed from Fick's Law, utilizing the free diffusion coefficient and the total cross-sectional pore area in the membrane. Net free diffusion will occur if the thermodynamic activity of molecules in one compartment exceeds that in the other. Steady-state net diffusion will occur if the activities in the two compartments are maintained at a constant difference. The equations governing diffusion under these circumstances are well known and are frequently utilized for the experimental determination of free diffusion coefficients (97, 112, 130). Conversely, if the free diffusion coefficient of a molecular species is known, it should be possible to compute the cross-sectional area of the membrane pores, A_p , from the remaining variables in Fick's Law which can be measured. When the path length, Δx , for diffusion through the membrane is unknown, as in most biological membranes, it should be possible to solve the equations for $A_p/\Delta x$. This principle affords the basis for recent estimates of the pore area per unit path length through the walls of living capillaries (118); it is somewhat surprising that the method has not been applied to artificial porous membranes in which the results can be compared with those obtained by independent methods for determining pore area. How-

ever, all the data required for this calculation are available from Manegold's detailed studies on the rate of diffusion of HCl, urea and sucrose through calibrated collodion membranes with effective pore radii⁴ ranging from 23 to 520 Å (101). When the pore radii were greater than 150 Å (i.e. more than 30 times larger than the Einstein-Stokes diffusion radius of sucrose), the relative diffusion rates of the test molecules were proportional to their free diffusion coefficients, and independent of pore size, thus indicating that the pores were sufficiently large to permit free diffusion. Under these conditions the fractional pore areas available for free diffusion through the membranes (as calculated by the present author from Fick's Law and Manegold's data) were 75 to 85 per cent of the fractional pore areas estimated from the ratios of wet to dry weights of the membranes. This discrepancy is not large; in the membranes used by Manegold the ratio of membrane thickness to effective pore diameter was never less than 10,000 and it would be surprising, under these circumstances, if all the channels which took up water were direct thoroughfares through the membrane. The correspondence between pore area, computed from free diffusion on the one hand and from the ratio of wet to dry weight on the other, is sufficiently close to encourage the use of similar calculations in connection with diffusion through the walls of living capillaries as described in PART II below.

B. Restricted Diffusion Through Pores. The diffusion rate of any given molecular species through a membrane of given pore area and thickness may be greatly slowed if the dimensions of the pores are comparable with those of the diffusing molecules. Under these circumstances the diffusion coefficient, calculated from Fick's Law, is less than the free diffusion coefficient and may be termed the *restricted* diffusion coefficient. The well-known work of Collander (24) and of Michaelis and his associates (55, 157) has shown clearly that the rates of diffusion of organic non-electrolytes through membranes with very small pores decrease, as a function of molecular size, far more rapidly than can be accounted for on the basis of the free diffusion coefficients of the test molecules. The membranes utilized for these studies were probably heteroporous (157) and they were not calibrated in terms of the Darcy coefficient or by the method of surface tension; the most permeable membranes employed prevented the passage of sucrose, the longest dimension of which is 12 Å (table 2).

The only quantitative data, known to this reviewer, which relate molecular diffusion to pore dimensions in the range of interest for capillary permeability are those of Manegold (101). These data indicate that the diffusion of sucrose or urea is significantly restricted in membranes with effective pore radii of 150 Å or less (cf. footnote 4). When the porosity was reduced to 68 Å, the diffusion rate of sucrose was less than 50 per cent of that expected on the basis of free diffusion and the diffusion of urea was similarly restricted in membranes with effective pore radii of 42 Å. These results are indeed surprising, for the Einstein-Stokes diffusion radii of sucrose and urea are only 4.4 and 1.7 Å, respectively, and it would therefore appear that diffusion is severely restricted even when the pores are of the order of 20 times larger than the diffusing molecules. Evidence has already been cited (*section 1, B*) which indicates that ether-alcohol collodion membranes of the type used by Manegold are relatively isoporous; it therefore seems unlikely that his results are attributable to a distribution of pore sizes.

A theoretical approach to restricted diffusion has been suggested by Friedman

⁴ In Manegold's paper the pore dimensions are given in terms of an equivalent 'slit half-width,' β , rather than in terms of effective pore radius, r . The two are related as follows, $r = 1.34 \beta$.

and Kraemer (54) and Friedman (52, 53) in connection with their experiments on the diffusion of nonelectrolytes through gelatin gels. These authors point out the applicability to restricted diffusion of the Ladenburg-Faxén correction (45, 81, 82) of Stokes Law in systems where the particle size is comparable with the dimensions of the sedimentation chamber. For the case of spherical particles of radius (a), sedimenting (diffusing) in cylindrical vessels of radius r , this correction takes the form:

$$f/f_0 = 1 + 2.4(a/r) \quad (11)$$

where f is the frictional force opposing sedimentation and f_0 is the frictional force opposing sedimentation when a is negligible compared to r (i.e. free sedimentation or free diffusion). The Ladenburg equation is derived on purely hydrodynamical grounds to take account of viscous drag between the sedimenting particles and the walls of the cylinder. An analogous equation has been shown by Westgren (158) to apply to ultramicroscopic particles sedimenting in a wedge-shaped container; Westgren utilized the Tyndall effect to follow the rate of sedimentation of the suspended particles. A summary of this work may be found in Barr's monograph (2).

A further restriction to diffusion may result from steric effects at the entrance to the pore. This factor has been considered by Ferry (47) and by Pappenheimer *et al.* (118) who make the simplifying assumption that the diffusing molecules can enter a pore only if they do not strike the edges of the pore. For the case of spherical particles entering circular openings the effective target area for entrance relative to the true area of the pore is $(1 - a/r)^2$. Steric hindrance and viscous drag at the walls would thus account for a restriction to free diffusion given by

$$\frac{D'}{D} = \frac{\left(1 - \frac{a}{r}\right)^2}{1 + 2.4\frac{a}{r}} \quad (12)$$

Equation 12 predicts a 50 per cent restriction to diffusion when the radius of the pore is six times the radius of the diffusing molecule; it therefore accounts for only a part of the restriction to diffusion actually found in the experiments of Manegold cited above. A closer correspondence between theory and experiment has been found in the case of diffusion through capillary walls (118), but some of the assumptions made for this comparison must be considered doubtful as will be discussed in PART II below.

It is clear, however, from the few quantitative data available to us, that diffusion may be greatly restricted through pores, even when the pores are many times larger than the diffusing molecules. Further experimental work using systems of known pore dimensions and molecules of known shape would be required to elucidate this problem which appears to be a major one in accounting for passive transfer processes through cell membranes as well as through capillary walls. The experiments of Manegold, which have been neglected in previous reviews of permeability, offer one approach to the problem and deserve careful study. The use of D_2O to determine the effective pore area per unit path length might greatly improve the accuracy of such experiments. Molecular diffusion into or through certain crystals, the pore dimensions of which can be determined by x-ray diffraction, offers another promising approach (3, 100, 125).

C. Restricted Diffusion in Relation to Filtration and its Significance for Molecular Sieving. We have now to discuss factors which determine the degree of molecular sieving during ultrafiltration through porous membranes. The degree of molecular sieving of a given solute may be defined as the ratio of its concentration in the filtrate (c_2) to its concentration in the filtrand (c_1). It is often supposed that during ultrafiltration of a monodisperse solute through an isoporous membrane, the value of c_2/c_1 will be either 0 or 1. According to this view c_2/c_1 will be zero when the pores are smaller than the solute molecules and unity when the pores are larger than the solute molecules. If intermediate values are actually observed, they are said to be evidence for heteroporosity. This concept is frequently applied to the process of ultrafiltration through living membranes. If, for example, the concentration of a given solute in the ultrafiltrate is 50 per cent of its free concentration in the plasma, it is concluded that half the pores in the membranes are larger than the solute molecules and half are smaller (13, 90, 108).

The following considerations suggest that the degree of molecular sieving of a monodisperse solute through an isoporous membrane depends greatly upon the restricted diffusion coefficient of the solute in the membrane and upon the rate of filtration. Consider the filtration of a solution through an isoporous membrane which restricts the passage of solute molecules but does not offer appreciable restriction to the passage of solvent. Let the pore area for passage of solvent be A_p , and the restricted pore area for the passage of a particular solute be A'_p . The restricted diffusion coefficient of the solute through the membrane is then $D' = (A'_p/A_p) D$. Let the concentration of the particular solute in the filtrand be c_1 and that in the filtrate be c_2 . At equilibrium $c_1 = c_2$. Hydrostatic pressure applied to one side of the membrane will cause hydrodynamic flow through the pores. If the passage of solute molecules is restricted in relation to the solvent molecules, then the filtrate will be diluted, thereby causing a concentration gradient and net diffusion of solute molecules. Let the transport rate of solute by bulk flow (filtration) be \dot{T}_f (mols/sec.). Then

$$\dot{T}_f = (A'_p/A_p) Q_f c_1 \quad (13)$$

If there is no restriction to passage of solute then $A'_p = A_p$, and in this case the transfer rate by filtration is merely the product of the filtration rate and the concentration as in the filtration of glucose solution through ordinary filter paper. If there is restriction, however, the filtrate will be diluted to C_2 , thus causing net transfer by diffusion, \dot{T}_D

$$\dot{T}_D = D' A_p \frac{(c_1 - c_2)}{\Delta x} \quad (14)$$

The concentration in the filtrate during steady ultrafiltration is

$$c_2 = (\dot{T}_f + \dot{T}_D) \div \dot{Q}_f \quad (15)$$

Solution of equations 13, 14 and 15 for the ratio of transport of solute by diffusion as compared to that by filtration (\dot{T}_D/\dot{T}_f) and for the degree of molecular sieving (c_2/c_1) during steady ultrafiltration yields

$$\frac{\dot{T}_D}{\dot{T}_f} = \frac{D A_p}{\dot{Q}_f \Delta x} \left(1 - \frac{c_2}{c_1} \right) \quad (16)$$

and

$$\frac{c_2}{c_1} = \frac{\frac{D'}{D} + \frac{D'A_p}{\dot{Q}_t \Delta x}}{1 + \frac{D'A_p}{\dot{Q}_t \Delta x}} \quad (17)$$

In the transient case, when solute is suddenly added to the filtrand during bulk flow, the initial concentration in the filtrate (c_2) is zero and the initial rate of diffusion relative to filtration becomes

$$\dot{T}_D / \dot{T}_f = (DA_p) / (\dot{Q}_t \Delta x) \quad (18)$$

Inspection of *equation 16* shows that high rates of filtration and thick membranes will tend to diminish the importance of transport by diffusion relative to that by filtration. This consideration led Ferry (47) to assume that diffusion plays a negligible role in the sieving of protein molecules during ultrafiltration at high pressures through artificial membranes. At low filtration rates, however, diffusion may be an important factor determining the passage of solutes, even in relatively thick artificial membranes.

Figure 2 shows the degree of molecular sieving to be expected on the basis of *equations 16* and *17* during the ultrafiltration of sucrose through one of the membranes characterized by Manegold (101). It is seen that the (calculated) concentration of sucrose in the filtrate decreases with increasing rates of filtration (volume flow) through the membrane. Experimental studies of the type suggested by figure 2 have not yet been carried out. However, several observations suggest that the theory is at least qualitatively correct. Thus Trautman (147) has investigated the sieving of NaCl through cellophane membranes; in this case c_2/c_1 decreased from unity at zero filtration to 0.66 at the highest rate of filtration. Similar observations for the case of protein molecules have been reviewed by Ferry (48, p. 416). In PART II, *section 5* below, evidence will be reviewed that restricted diffusion plays an important role in determining the degree of molecular sieving of large molecules during ultrafiltration of plasma through capillary membranes.

PART II. QUANTITATIVE ASPECTS OF CAPILLARY PERMEABILITY

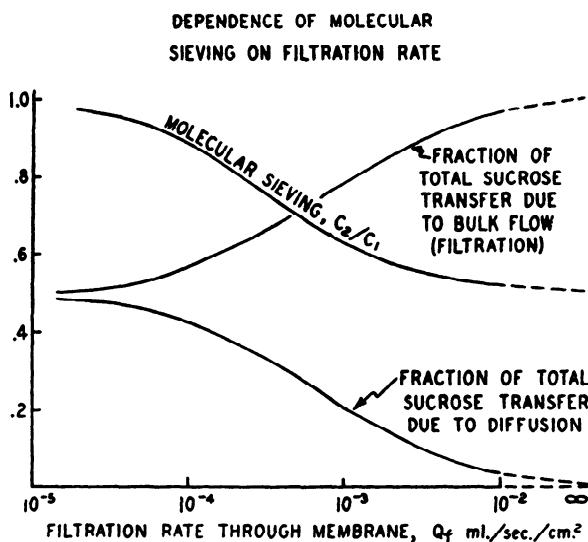
In the preceding sections we have seen how measurements of flow through artificial porous membranes can be utilized, in conjunction with auxiliary data, to deduce the dimensions of the pores and the mechanism of flow of solvents through them. We have now to consider the results of similar measurements and similar reasoning applied to the walls of living capillaries.

1. Fluid Movement in Relation to Hydrostatic and Osmotic Pressures

Measurements of net flow through capillary walls as a function of hydrostatic and osmotic pressures have been made in the capillaries of the frog's mesentery (15, 85, 86), the capillaries in perfused extremities of frogs (27), rats (66), cats and dogs (117, 118) and in the capillaries of the human forearm (49, 78, 91). In only two of these tissues, however, has it been possible to make a direct comparison between the rate of net fluid movement and the hydrostatic and osmotic forces operating across the capillary membranes.

In the individual capillaries of the frog's mesentery, Landis (85, 86) found that the net rate of flow through the wall was, on the average, proportional to the difference between the hydrostatic pressure within the capillary and the protein osmotic pressure of the plasma as measured *in vitro*. When capillary pressure exceeded protein osmotic pressure, fluid passed from within the capillary to without (filtration); when capillary pressure fell below protein osmotic pressure, fluid was withdrawn from the extravascular space into the capillary (absorption). This work is unique in that the primary quantities necessary to test the validity of the Starling Hypothesis (140) were measured by direct methods in single capillaries. Similar results, using very different methods, have been obtained in studies of fluid movement through the walls

FIG. 2. Effects of restriction to diffusion on the passage of a solute through a porous membrane. Calculated from the theory of molecular sieving summarized by equations 16 and 17. The actual values employed refer to the passage of sucrose through collodion membrane no. 506 characterized by Manegold (101). The effective pore radius in this membrane was 68 Å as compared to a molecular radius of 4.4 Å for the sucrose molecule. The ratio of the diffusion coefficient of sucrose through the membrane (restricted diffusion coefficient) to the free diffusion coefficient was 0.5 and this value sets a limit to the degree of molecular sieving (c_2/c_1) at high filtration rates (eq. 17). At low filtration rates the rate of (restricted) diffusion becomes relatively important (eq. 16) and the concentration ratio approaches unity at zero flow (dialysis).



of capillaries in the perfused hindlegs of cats and dogs (117). In this preparation the mean hydrostatic capillary pressure was determined from the blood flow, the post-capillary resistance to blood flow and the venous pressure; net fluid movement between blood and tissues was measured from changes of weight. When normal plasma proteins were used for perfusion the mean capillary pressure at which no net transfer of fluid took place (isogravimetric capillary pressure) was, on the average, 93 per cent of the protein osmotic pressure as measured *in vitro* (fig. 3A). During net filtration or absorption, the rate of gain or loss of weight was accurately proportional to the difference between the mean capillary pressure and the isogravimetric capillary pressure (fig. 3B). The proportionality constant was independent of the absolute value of the isogravimetric capillary pressure when this was varied by diluting or concentrating the plasma proteins. The results shown in figure 3 leave little room for doubt that the net rate of flow through the walls of capillaries in muscle, like that through frog's mesenteric capillaries or through collodion membranes, is proportional to the difference between hydrostatic and osmotic forces acting across the membrane as originally proposed by Starling (140, 141).

2. Capillary Filtration Coefficients; Comparison With Cell Membranes and With Artificial Membranes

The proportionality constant relating flow of fluid to the pressure differences operative across the capillary wall is a measure of capillary 'permeability' to fluid. In view of the fundamental differences between permeability as measured from diffusion rates and permeability as measured from flow data (cf. PART I, section 2) it is perhaps more appropriate to consider this proportionality constant as a unit of hydraulic conductivity, reserving the term *permeability* to describe rates of diffusion under conditions which entail no changes of volume.

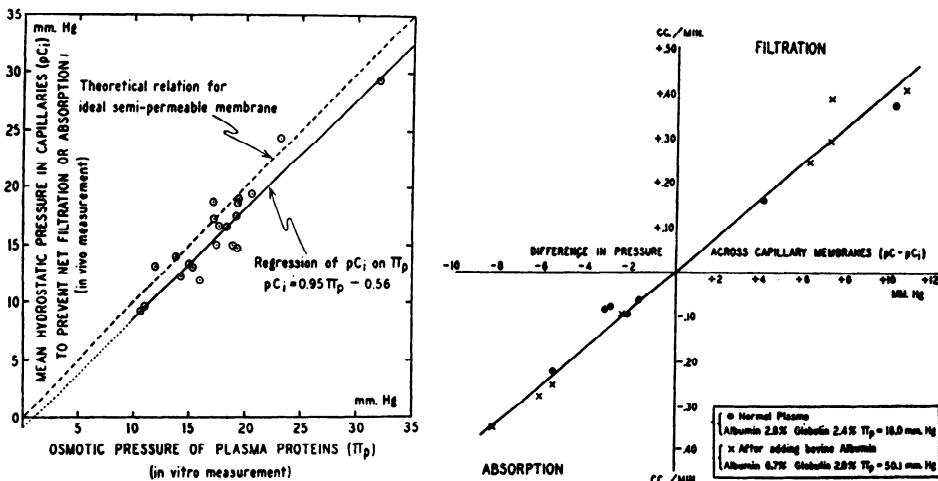


FIG. 3A (left). Effective osmotic pressure of plasma proteins in the hindlimb capillaries of cats and dogs. The mean hydrostatic pressure in the capillaries required to prevent net transfer of fluid is, on the average, slightly less than the *in vitro* osmotic pressure of the plasma proteins. B (right). Fluid exchange in hindleg of a cat. Experiment C-25, limb weight 302 gm. The rate of net fluid movement is proportional to the difference between the mean hydrostatic pressure in the capillaries, p_C , and the sum of all pressures opposing filtration (isogravimetric capillary pressure, pC_i). The filtration coefficient is defined by the slope of the line shown. When expressed per 100 gm. tissue its value is 0.014 ml/min/mm. Hg. In 81 similar experiments the mean value was 0.011 ml/min/mm. Hg (for statistical analysis see ref. 118). The results illustrated in figure 3 show that the Starling Hypothesis (140) is applicable to the capillaries of the perfused hindleg. Reprinted by permission from PAPPENHEIMER, J. R. AND A. SOTO-RIVERA. *Am. J. Physiol.* 152: 471, 1948.

A. Mesenteric Capillaries, Frog. In Landis' experiments with single capillaries, the capillary surface area could be measured directly and the proportionality between flow and pressure difference expressed as flow rate per unit pressure difference per unit area of capillary surface (filtration constant). Thus at room temperatures the filtration constant of the frog's mesenteric capillary was found to average 56×10^{-8} ml/second/1 cm. H_2O pressure difference/cm.² of capillary wall (85, 86) as shown in table 1. When converted to c.g.s. units and multiplied by the viscosity, the filtration constant defined by Landis is equal to the Darcy coefficient of hydraulic conductivity divided by the path length through the capillary wall (eq. 8).

B. Capillaries in Perfused Extremities. Data similar to those shown in figure 3B have been obtained from about 100 cat hindleg preparations in this laboratory. The proportionality constant relating flow to pressure difference across the capillary

walls at 37°C. averaged 1.3×10^{-4} ml/sec/cm. H₂O/100 gm. tissue or 1.8×10^{-4} ml/sec/cm. H₂O/100 gm. muscle (for a statistical evaluation cf. ref. 118). These values are of the same magnitude as those estimated from less direct measurements in mammalian extremities. In the human forearm a rise of 1 cm. H₂O in venous pressure produces, on the average, a volume increase of 0.55×10^{-4} ml/sec/100 ml. tissue (78, 91). Assuming that 80 per cent of the rise in venous pressure is transmitted to the capillaries, this value becomes 0.7×10^{-4} ml/sec/cm. H₂O/100 ml. tissue or

TABLE I. FLOW THROUGH VARIOUS MEMBRANES

	Species, Tissue, or Membrane	Temp. °C.	Filt. Con- stant, * $\times 10^4$	Darcy Coefficient, † $\text{cm.}^2 \times 10^{10}$	References	Notes
<i>Cell membranes</i>	Arbacia egg (un-fertilized)	20	0.016		95	
	Fibroblasts (mouse, rat, chick)	20-2	.06-.16		17	
	Leucocytes (rabbit, man)	20-23	.05-.2		133	
	Erythrocytes (ox, man)	20	0.4-0.5		70	Value at 37°C. by extrapolation from data in range 0-30°C.
37	1.0-1.3					
<i>Capillary membranes</i>	Muscle (cat, dog)	37	2.5	6	117, 118	Assume path length Δx , = 0.3μ through capillary wall for calc. of Darcy coeff.
	Mesenteric (frog)	22-26	56	150	85, 86	
	Glomerular (frog)	25	220	570	118	
	Glomerular (mammal)	37	300-600	600-1200	118, 137, 151	
<i>Collodion membranes</i>	$r_e = 30\text{\AA}$ with-holds egg albumin			5,700	4, 43	Fractional pore area
	$r_e = 40\text{\AA}$ with-holds hemoglobin			11,000	4, 41	$A_p/A_m = .52$
	$r_e = 50\text{\AA}$ with-holds serum albumin			19,000	41, 48	$A_p/A_m = .58$

* ml/sec/cm. H₂O/cm.²† $K_f = (\dot{Q}\eta\Delta x)/[A_m(\Delta P - \Delta\pi)]$, eq. 8.

about one-half that of the perfused leg. Rates of change of net filtration per unit change in protein osmotic pressure are also comparable with this value, both in the human forearm (78) and in the perfused hindquarters of rats (66).

In order to compare these values with similar values obtained from other membranes (table I), it is necessary to express the results per unit capillary surface area. Precise figures for capillary surface area are hard to obtain. Usual histological techniques, involving perfusion with India Ink, followed by fixation, paraffin embedding and capillary counts have led to estimates ranging from 18,000 to 80,000 cm.²/100 gm. muscle (77, 105, 116, 119, 136, 144). These figures are almost certainly too high,

for they imply capillary volumes of 3 to 14 per cent of the muscle volume and the entire vascular volume in mammalian muscle is probably less than 4 per cent (99). Our own estimates of capillary surface area in the perfused hindleg are considerably lower ($7000 \text{ cm.}^2/100 \text{ gm.}$), possibly because we have used frozen sections which avoid shrinkage and thus result in fewer capillary counts per square millimeter. The average total vascular volume was 2.5 per cent (hemoglobin method, ref. 28) and the volume of the large vessels, as determined from the increase in weight following arterial and venous injections of mercury at appropriate pressures, averaged 0.9 per cent leading to a maximum capillary volume of 1.6 per cent of the limb weight. These estimates lead to a reasonable value for capillary radius, thus $r = 2 V/S = 2 \times 1.6 \div 7000 = 4.5 \mu$. When our estimate of $7000 \text{ cm.}^2/100 \text{ gm.}$ muscle is applied to the filtration data from the hindleg we arrive at a filtration constant of $2.5 \times 10^{-8} \text{ ml/sec/cm. H}_2\text{O/cm.}^2$ capillary wall, as shown in table 1.

C. Glomerular Capillaries. In the case of glomerular capillaries, all of the factors required to compute the filtration constant have been measured except for the hydrostatic pressure difference across the capillary walls. Reasonable assumptions as to the value of this pressure difference have led to estimates of filtration constant in the range 300 to $600 \times 10^{-8} \text{ ml/sec/cm. H}_2\text{O/cm.}^2$ in mammalian kidneys (118, 137, 151) and $220 \times 10^{-8} \text{ ml/sec/cm. H}_2\text{O/cm.}^2$ in frog's kidneys (118) as shown in table 1. These values suggest that flow through glomerular capillary membranes occurs 100 times more readily than through the capillary walls in muscle and serve to emphasize the fact that the capillary walls in different tissues may offer widely varying resistances to the flow of fluid through them.

D. Comparison With Cell Membranes. The rate of flow of fluid through the cell membranes of marine ova, erythrocytes, leucocytes and other single cells is proportional to the difference in osmotic pressure across the cell membranes (96). When expressed per unit surface area, the proportionality constant has the same dimensions as the filtration constant defined above in connection with the capillaries. Landis (89) has noted that the filtration constant of the frog's mesenteric capillary is more than 100-fold that of the mammalian erythrocyte and more than 3000-fold that of the unfertilized Arbacia egg as shown in table 1. The filtration constant of muscle capillaries is not, however, so very much greater than that of the mammalian erythrocyte, although it is from 10- to 100-fold greater than the filtration constants of the nucleated cells listed in table 1. In section 4A evidence will be cited showing that urea diffuses into mammalian red cells very nearly as rapidly as through the walls of muscle capillaries (per unit surface area and concentration difference). From the point of view of mechanics, however, such comparisons have little quantitative significance. Neither the filtration nor the diffusion data take into account the thickness of the membranes, nor the fractional surface area which may be involved in the flow or diffusion process. It is possible also that in small-pored cell membranes the flow occurs in part by net diffusion and is therefore not directly comparable with flow through capillary membranes as shown in figure 1. However, all of these factors would operate in a direction tending to emphasize the important qualitative conclusion that cell membranes offer more resistance to flow of fluid than do capillary membranes.

E. Comparison With Collodion Membranes. The characteristics of flow through collodion membranes have already been discussed in detail in section 1, PART I. In table 1 the Darcy coefficients of selected collodion membranes are listed for comparison with capillary membranes. A nominal value of 0.3μ has been assumed for the path length through the capillary membranes, this value being approximately

that of the thickness of capillary endothelium in regions which do not include nuclei. It is possible, indeed likely, that flow through capillary membranes occurs in regions between cells which could provide a shorter path length than that estimated from microscopic observations. If this is the case, however, the Darcy coefficients of capillary membranes will be even smaller in relation to collodion membranes than indicated in table 1. It is clear from the table that collodion membranes which withhold proteins to about the same degree as capillary membranes offer far less resistance to flow than do capillary membranes. On the pore theory of capillary permeability, this behavior could be explained if the fractional pore area available for flow through capillary membranes were smaller than in collodion membranes in inverse proportion to their respective Darcy coefficients. For example, in comparing muscle capillaries, which retain hemoglobin (118), with a collodion membrane of comparable permeability to hemoglobin ($r_s = 40 \text{ \AA}$, $A_p/A_m = 0.58$), we note that the ratio of Darcy coefficients is $6 \div 11,000 = 0.54 \times 10^{-3}$, leading to a fractional pore area in muscle capillaries of only $0.54 \times 0.58 \times 10^{-3}$ or 0.0003. In making this comparison we should note that the values of Darcy coefficient for the collodion membranes listed in table 1 were actually obtained using membranes in which Δx was 50μ or more. We assume that the Darcy coefficient will be at least as great in a membrane 0.3μ thick.

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. Further evidence in support of this conclusion comes from measurements of capillary permeability to small lipid-insoluble molecules as described in section 4 below.

3. Fluid Movement in Relation to Temperature

Flow through artificial porous membranes changes with temperature in inverse proportion to viscosity as discussed in section 1, PART I. We have now to consider the effects of temperature on flow through capillary membranes.

Brown and Landis (15) have studied flow through the walls of single capillaries in the frog's mesentery at $24^\circ \pm 2^\circ \text{C}$. and during local cooling of the tissue to $0 \pm 2^\circ \text{C}$. In the control series the filtration constant (milliliters per second per square centimeter capillary wall per 1 cm. H_2O change of capillary pressure) averaged 70×10^{-8} . The standard error of the slope of the regression line was 10.5×10^{-8} . This mean value is slightly greater than the mean value of 56×10^{-8} found in the earlier and more extensive measurements by Landis (85, 86). At the low temperatures the filtration constant was decreased to 19×10^{-8} (S.E. ± 7.3). The ratios of the control values to the low temperature value are 3.7 and 2.9, depending upon which series of measurements is taken for the control. These ratios appear to be considerably higher than the ratio of viscosities ($\eta_{24^\circ}/\eta_{24^\circ} = 2.0$) but this conclusion is not justified on a statistical basis, owing chiefly to the large scatter among the experimental data obtained at the low temperatures.

The effects of temperature on fluid exchange in the human forearm have been studied by Landis and Gibbon (91) and by Brown *et al.* (16). In these studies the forearm was immersed in water at various temperatures and the filtration coefficient estimated from the rate of increase of arm volume as a function of venous pressure. The complicating effects of changes in vascular volume were excluded by means of the 'pressure' plethysmograph. The results showed that the filtration coefficient decreases considerably with decrease in temperature but the quantitative interpretation of the results in relation to viscosity is complicated by several factors. A con-

siderable temperature gradient may exist between the water bath and the deep tissues. An approximate estimate of this gradient may be made from the data of Barcroft and Edholm (1) and of Kreyberg (76). When the filtration coefficient is plotted as a function of deep tissue temperature there appears to be a reasonably close correspondence between the rate of change of filtration and the rate of change of reciprocal viscosity over the range 36° to 20°C. However, at tissue temperatures above 36°C. the filtration coefficient increases more than expected on the basis of the viscosity change and below about 20°C. it decreases less than expected from viscosity and may actually increase when the temperature of the water bath is changed from 15° to 4° or 5°C. Several possible explanations of these results may be mentioned. The effects of unit rise of venous pressure on mean capillary pressure (and hence filtration rate) depend upon the ratio of arteriolar to venular tone (6, 117) and there are no reasons to suppose that this ratio remains constant during the large alterations of over-all vascular tone which are known to result from changes of temperature (1). It is possible also that such changes may be accompanied by changes in total capillary area. Moreover, at the lowest temperatures the capillaries may suffer damage, particularly in the cutaneous and subcutaneous region where the temperature is lowest. In view of these possibilities it seems unjustified to attach undue significance to the relations between filtration coefficient and viscosity in the human forearm, even in the range of temperatures where these appear to be related.

In the perfused hindleg preparation the relation between the temperature coefficients of filtration and viscosity are more clear-cut. In this preparation the temperature of the deep tissues can be varied over a wide range by controlling the temperature of the arterial blood. The capillary membranes retain their normal impermeability to plasma proteins over a temperature range of 8° to 44°C. and, within this range, measurements similar to those shown in figure 3 can be made reversibly and without evidence of capillary damage. In 15 hindleg preparations the ratio of the filtration coefficient measured at 36° ± 2°C. to that measured at 10° ± 2°C. averaged 1.68 (S.E. = ±0.08). This value is within 10 per cent of the ratio of viscosities of water at the two temperatures ($\eta_{10}/\eta_{36} = 1.85$) and the difference between the two ratios is not significant ($P > .05$). These results⁶ are consistent with the view that filtration and absorption of fluid take place predominantly by viscous flow through aqueous channels penetrating the capillary walls.

4. Diffusion of Lipid-Insoluble Molecules Through Capillary Walls, Calculations of Pore Dimensions

Rates of molecular diffusion through cell membranes have been studied extensively, using a wide variety of single cells and molecular species. The diffusion rates are generally measured in terms of the number of mols of the test molecules which penetrate 1 cm.² of membrane in 1 second under the influence of a concentration difference across the membrane of 1 mol/l. This unit of permeability has the dimensions of centimeters × seconds⁻¹ and represents the restricted diffusion coefficient through the membrane surface divided by the membrane thickness. Many of our concepts of the morphology and physiology of cell membranes are derived from quantitative studies of this kind.

Until very recently no comparable measurements of capillary permeability to small molecules were available. From a qualitative point of view it was known that

Not previously published. The experiments were carried out in 1947-49 by S. L. Eversole, E. M. Renkin and the author.

capillary walls in the body as a whole could offer a measurable restriction to the diffusion of small lipid-insoluble molecules. Starling (139, 141) showed that the degree of hemodilution which follows the intravenous injection of hypertonic salt solutions is related to the size of the molecules injected; he attributed this result to the osmotic withdrawal of fluid from the extravascular compartments into the circulation during the (restricted) diffusion of the injected molecules through the capillary walls. On the basis of similar evidence, Keys (74) noted that urea, glucose and sucrose pen-

TABLE 2. PERMEABILITY OF MUSCLE CAPILLARIES TO LIPID-INSOLUBLE MOLECULES OF GRADED SIZES*

Molecular species and no. of determinations	Molecular Dimensions† (cm. $\times 10^6$)			Capillary Permeability (av. values, $\times 10^6$)	
	Radius of equivalent sphere		Dimension estimated from x-ray diffraction or from frictional ratios	Mols/sec/mm. Hg osmotic pressure developed during diffusion process in 100 gm. muscle	Mols/sec/cm. ² capillary wall/mol/l. conc. diff.
	From free diffusion	From intrinsic viscosity		Observed	Calculated‡
NaCl (8).....	1.4		Cl ⁻ , radius 1.8 Na, radius 0.95	11.4	31
Urea (4).....	1.6			8.7	23
Glucose (3).....	3.6	3.8	5 \times 7 \times 9	3.3	9
Sucrose (2).....	4.4	4.4	8 \times 11 \times 12	1.8	5
Raffinose (1).....	5.6	5.7	Axes of equiv. ellips., a = 16, b = 10	1.4	4
Inulin (1).....	15.2	15.3	Axes of equiv. ellips., a = 75, b = 13	0.2	0.5
Myoglobin§ (2).....	19		Cylinder, d = 54, h = 8	0.02	0.05
Hemoglobin (4).....	31	35	Cylinder, d = 54, h = 32	$\rightarrow 0$	$\rightarrow 0$

Some of the permeability data recorded in this table have been published previously in slightly different form (118). I am indebted to Dr. E. M. Renkin, of the Brookhaven National Laboratories, for further data relating to urea and sucrose and for help in performing experiments with myoglobin. Most of the NaCl measurements were made in this laboratory in collaboration with W. B. Kinter.

† Details concerning the calculation of these dimensions may be found in refs. 8, 9, 23.

‡ Calculated on the basis of 7000 cm.² of capillary surface per 100 gm. muscle (PART II, section 2, B) and on the assumption that $\Delta C = \Delta P \times RT$. For a discussion of this assumption see PART II, section 4, B.

§ Prof. H. Teorell generously supplied the myoglobin for these measurements.

trated capillary walls at rates which were inversely related to their molecular size. Kruhoffer (79) showed clearly that inulin leaves the circulation more slowly than sucrose, the respective rates being approximately proportional to the aqueous diffusion coefficients of the test molecules.

Quantitative methods for studying capillary permeability to lipid-insoluble molecules in perfused hindlegs have recently been developed in this laboratory (118). The transcapillary diffusion rates of NaCl, urea, glucose, sucrose, raffinose, inulin, myoglobin and hemoglobin have been measured in terms of the number of mols of each substance which penetrated the capillary walls in 100 gm. of tissue in 1 second under the influence of unit pressure difference for diffusion through the capillary walls. The results are summarized in table 2 together with recent estimates of the

dimensions of the test molecules. The following resumé of the methods employed to obtain the permeability data shown in table 2 is included as an aid to their interpretation by readers who are unfamiliar with the original paper.

If an inert lipid-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, through the capillary membranes, to the extravascular 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 walls approaches zero as a result of loss of molecules from blood to extravascular fluid and dilution of the blood by the absorbed interstitial fluid. In the isolated perfused hindleg preparation the osmotic withdrawal of fluid throughout such an osmotic transient may be prevented by continually adjusting the mean hydrostatic pressure in the capillaries by known amounts just sufficient to maintain the leg at constant weight. Under these conditions the increment of mean capillary pressure, over and above the pre-existing isogravimetric pressure, is a measure of the partial osmotic pressure (or diffusion pressure) exerted by the added molecules across the capillary membranes. The diffusion pressure measured in this way reaches a maximum shortly after addition of the test molecules to arterial blood; it then declines exponentially with time, the isogravimetric capillary pressure eventually returning to a value close to the stable osmotic pressure of the plasma proteins. All other factors being constant, the magnitude of the osmotic transient depends upon the size and concentration of the added molecules; the time constant of the osmotic transient is inversely related to the diffusion coefficient of the test molecular species. The net rate of diffusion of the test molecules through the capillary walls can be determined at any time during the osmotic transient from the product of the blood flow and arteriovenous concentration difference. In practice the flow is maintained constant and arteriovenous differences are determined from analyses of samples taken at intervals throughout the transient.

It is therefore possible to obtain a measure of the two variable factors in Fick's Law, namely the net rate of diffusion and the mean pressure difference for diffusion through the membranes. The remaining factors, which are constant, include the (restricted) diffusion coefficient of the test molecular species and the cross-sectional pore area divided by the path length for diffusion through the membranes ($A_p/\Delta x$). Experimentally, it was found that the ratio of net diffusion rate to diffusion pressure remained constant throughout the diffusion process. Factors such as the rate of blood flow or final distribution volume, which may affect the time course of the transient, do not appear to affect this ratio which is presumably determined by the restricted diffusion coefficient of the test molecular species and the geometry of the pore structure in the capillary wall.

A. Magnitude of Permeability. The permeability values shown in table 2 are in general large compared to similar values found in cell membranes, but they are small in comparison with collodion membranes of comparable thickness and pore size. Thus the permeability of most cells to urea is less than one-hundredth of the value shown in table 2 and permeability to molecules the size of sucrose or larger is negligible by comparison (33). The single recorded exception is the mammalian red cell which is almost as permeable to urea (69) as the muscle capillary (18×10^{-8} as compared with 23×10^{-8} mol/sec/cm.²/mol.¹ l. at 37°C.). It will be recalled that

the resistance to flow of water offered by the mammalian red cell is also comparable with that of the muscle capillary. However, direct comparisons between cell membranes and capillary membranes are difficult to interpret from the point of view of membrane structure for the reasons given above in section 2, *D*.

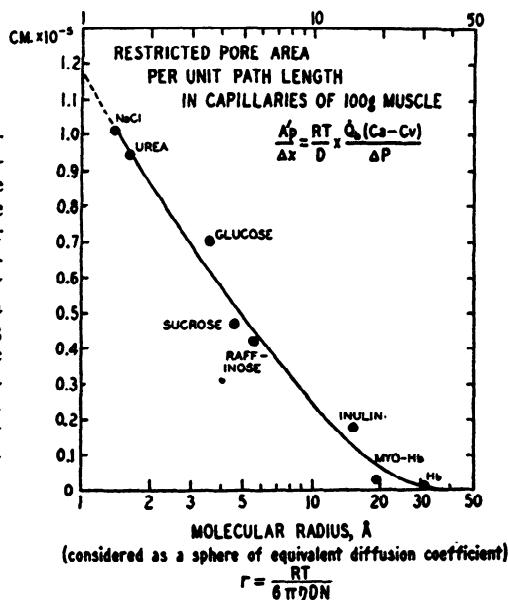
B. Area Available for Diffusion of Lipid-Insoluble Molecules Through the Capillary Wall. In our original paper (118) we assumed that the partial osmotic pressure exerted by the test molecules across the capillary membranes was directly related to the mean concentration difference by Van't Hoff's Law. On this assumption it was possible to solve the Fick diffusion equation for the restricted pore area per unit path length ($A'_p/\Delta x$), available for the free diffusion of the test molecules

through the capillary membranes. Thus the net rate of diffusion was determined experimentally from the product of blood flow and arteriovenous concentration difference, $\dot{Q}_B(c_A - c_V)$, and the mean concentration difference was calculated from the Van't Hoff relation, $\Delta c = RT/\Delta P$, where ΔP was the observed partial osmotic pressure difference across the membranes. Substitution in Fick's Law yielded

$$\frac{A'_p}{\Delta x} = \frac{RT}{D} \times \frac{\dot{Q}_B(c_A - c_V)}{\Delta P} \quad (19)$$

If the free diffusion coefficient of the test molecular species is used in equation 19, then the value of $A'_p/\Delta x$ is a virtual one representing the area in the capillary walls which would be needed to allow the observed diffusion rates if diffusion were un-

FIG. 4. Restricted diffusion as a function of molecular size in the capillary circulation of perfused hindlegs. The restricted pore areas are calculated from the data of table 2 and equation 19. The limiting molecular radius of 30 to 40 Å agrees well with the effective capillary pore radius calculated by equation 21 from the data of figure 3B and a pore area per unit path length of $0.9 \pm 0.3 \times 10^{-5}$ cm. The precise relations between the restricted pore area and the true pore area are unknown; further work relating restricted diffusion to osmotic pressure may be necessary to elucidate this relation as discussed in PART II, section 4, B.



restricted. We define this virtual area as the restricted pore area; if equation 19 is correct, then $A'_p = (D'/D) A_p$, where D'/D is the ratio of the restricted to the free diffusion coefficient, and A_p is the true pore area. If there is no restriction to diffusion, then $A'_p = A_p$, as found by Manegold (101) for collodion membranes in which the pore radii were very much larger than the radii of the diffusing molecules (see PART I, section 3, A). Values of restricted pore area per unit path length for various molecular species diffusing through the capillary walls in perfused hindlegs are shown in figure 4. In 8 hindlegs the mean value for NaCl was 1.03×10^{-5} cm. (S.D. $\pm 0.3 \times 10^{-5}$) and the values for the other test molecules decreased progressively with molecular size as would be expected of restricted diffusion. Since the path length through the capillary wall is not likely to be more than 1μ , the cross-sectional pore areas required to explain the observed rates of diffusion are extremely small. Thus for NaCl the area is less than $1.03 \times 10^{-5} \times 10^{-4}$ or about 10 cm^2 . Extrapolation of the results to the molecular radius of water yields a value of 12 cm^2 . These values may be compared with the value of 7000 cm^2 estimated for the histological surface area of the capillaries in 100 gm . of muscle (PART II, section 2, B). In terms of the fractional area of

the capillary wall available for the diffusion of a molecule the size of water, we have $13 \div 7000$ or less than 0.2 per cent. 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 water and lipid-insoluble molecules, a conclusion which was already indicated on the basis of the flow data reviewed in PART II, section 2, E.

The above values for diffusion areas are derived on the assumption that the mean concentration difference for diffusion may be calculated from osmotic pressure data by Van't Hoff's Law. Staverman (143) and Ussing (149, 150) have recently pointed out that the full Van't Hoff pressure may not be attained under conditions where the restriction to diffusion of solute molecules is not complete. Under these conditions it is more likely that the observed osmotic pressure is a function of the restricted diffusion coefficient of the solute relative to that of the solvent. For aqueous solutions Van't Hoff's Law would then be modified to the form

$$\Delta P = \Delta c RT \left(1 - \frac{D'}{D'_w} \right) \quad (20)$$

where D' is the restricted diffusion coefficient of the test solute molecules and D'_w is the restricted diffusion coefficient of the solvent (water). *Equation 20* suggests that the osmotic transient will become zero if the solute molecules are comparable in size and diffusion coefficient with the solvent molecules. Indeed, if the membrane is more permeable to the solute than to the solvent the osmotic transient should be negative. Shuler *et al.* (135) have observed a negative osmotic transient of this type for the case of urea diffusing through collodion membranes with glycerol as the solvent. The full Van't Hoff pressure would be expected only when $D' = 0$ as in a truly semi-permeable membrane.

It will be evident from these considerations, that the mean concentration difference for diffusion will be greater than that calculated (on the basis of Van't Hoff's Law) for insertion into *equation 19*. The discrepancy will be greatest for small molecules and will tend to make our original estimates of diffusion areas (shown in fig. 4) too high. It seems unlikely, however, that the corrections will be large; on the basis of *equation 20* our original estimates should be multiplied by the factor $(1 - D'/D'_w)$ and this factor lies between 0.5 and 1.0 for the case of the molecular species shown in figure 4. A more accurate appraisal of the magnitude of the correction must await further study of the basic physical laws relating restricted diffusion to the osmotic pressures developed across porous membranes.

C. Estimation of Effective Pore Radius. The effective pore radius of artificial membranes was defined in terms of the hydraulic conductivity (Darcy coefficient, K_t) and the fractional pore area, A_p/A_m , (*eq. 3*). Evidence was reviewed that the radius so calculated is in approximate agreement with the radius determined by the surface tension method, from the dimensions of molecules which just fail to penetrate the membrane and from electron micrographs of membrane surfaces. Similar reasoning may be employed to estimate the effective pore radii in capillary walls from data of the type illustrated in figures 3B and 4. Thus *equation 2* may be rearranged as follows:

$$r = \sqrt{\frac{8\eta}{A_p/\Delta x} \times \frac{\dot{Q}_t}{\Delta P}} \quad (21)$$

The value of $\dot{Q}_t/\Delta P$ is determined directly from the slope of the line shown in figure 3B. The average value in 81 hindleg preparations was 0.011/ml/min/mm. Hg/100

gm. tissue or 2.0×10^{-7} ml/sec/dyne/cm.²/100 gm. muscle. The value of $A_p/\Delta x$ is determined from the data of figure 4; for a molecule the size of water ($r = 1 \text{ \AA}$) its value is estimated to be 1.2×10^6 cm. in 100 gm. of muscle, although this value must be regarded as a high estimate for the reasons suggested by Ussing (section 4, B). Substitution of these values in equation 21 yields an effective pore radius of 31 \AA or an equivalent slit width of 37 \AA (eq. 5). If our estimate of $A'_p/\Delta x$ is too large by a factor of 2 as suggested above, then the effective pore radius will be 44 \AA . Until more is known of the relations between restricted diffusion and osmotic pressure we must consider that effective pore radii in the range of 30 to 45 \AA would adequately explain the data. The number of such pores per square centimeter of membrane surface (A_m) is defined by $n = A_p/A_m\pi r^2 = (A_p/\Delta x)(\Delta x/A_m\pi r^2)$. Assuming a path length, Δx , of 0.3μ and a total membrane surface of $7000 \text{ cm.}^2/100 \text{ gm. muscle}$, this value becomes $1-2 \times 10^9$ pores/cm.² Perhaps the most significant aspect of this estimate of pore size lies in its relation to the observed permeabilities to large molecules. Hemoglobin and serum albumin, which have Einstein-Stokes diffusion radii (R_{ES}) of 31 \AA and 32 \AA , respectively, are almost completely retained by the capillaries in perfused muscle,⁶ whereas inulin ($R_{ES} = 15 \text{ \AA}$) and myoglobin ($R_{ES} = 20 \text{ \AA}$) penetrate the capillary walls at readily detectable rates (fig. 4). It is therefore evident that pore dimensions calculated from hydrodynamic flow and aqueous diffusion of small lipid-insoluble molecules correspond closely with pore dimensions estimated from the size of molecules which just fail to penetrate the membranes. This correspondence provides further evidence in support of the hypothesis that hydrodynamic flow and diffusion take place through aqueous channels penetrating the capillary walls.

D. Possible Distribution of Pore Sizes. It might be supposed that the restricted diffusion areas shown in figure 4 could be explained in terms of a distribution of pore sizes rather than in terms of restricted diffusion through pores of uniform size. Thus the restricted pore area for the diffusion of sucrose is only half that for NaCl and this might be explained by supposing that half the total cross-sectional pore area was made up of pores with effective pore radii less than that of the sucrose molecule (i.e. less than 4.4 \AA). If this were the case, however, all of the remaining pores would have to be greater than 40 \AA in radius in order to explain the observed filtration coefficient and this would not be consistent with the observed permeability to raffinose, inulin or myoglobin. On the other hand, the experimental observations of Manegold (101), Friedman and Kraemer (54) and Westgren (158), and the theoretical papers of Ladenburg (81) and Faxén (45) suggest that diffusion through uniform channels becomes progressively restricted as the dimensions of the diffusing particles approach the dimensions of the channel. The theoretical considerations summarized by equation 12 and the few data supplied by Manegold (101) indicate that the restriction is sufficiently large to explain the results of figure 4 in terms of an isoporous structure.

5. Relative Rates of Transport of Solutes by Net Diffusion and by Filtration or Absorption Through Capillary Walls. Significance for Molecular Sieving

Two different hypotheses have been advanced to explain net transport of solutes through porous channels in the capillary wall. One hypothesis supposes that the

⁶ In terms of the mechanisms of permeability considered here the small amount of protein in capillary filtrate from extremities is negligible, although it may be extremely important in explaining the high protein content of lymph and the slow extravascular circulation of the plasma protein (34, 155, 156).

noncolloidal constituents of the plasma are brought to the interstitial fluid by a process of 'bulk' flow during filtration through the capillary walls; the rate of transport by this mechanism would then be determined by the rate of flow of filtrate and not by the individual diffusion coefficients of the various solutes (*eq. 13*). This hypothesis has been supported by Danielli and Davson (29) and by Danielli and Stock (30) on the basis of their observation that maltose and galactose enter interstitial fluid at about the same rate in spite of a twofold difference in molecular size. A similar explanation is generally offered in connection with glomerular filtration to account for the fact that the concentrations of small molecules in glomerular filtrate are approximately the same as in plasma.

More recent work by Kruhoffer (78, 79) and others (25, 50, 118) has shown clearly that the rates at which lipid-insoluble molecules are brought to the interstitial fluid may depend very greatly on molecular size. Kruhoffer concluded that diffusion is the dominant mechanism concerned and pointed out the interesting example of *p*-aminohippuric acid and related substances which pass rapidly out of the peritubular capillaries of the kidney, in the direction *opposite* to a high rate of 'bulk' flow (peritubular capillary absorption). A similar argument in favor of the diffusion hypothesis has recently been advanced by Hyman *et al.* (67) who have shown that the rates of clearance of Na²⁴ and I¹³¹ from human skin are not appreciably affected by concurrent edema formation.

In the perfused hindleg it is easy to demonstrate that the rates of transcapillary transport of small molecules added to arterial blood are not significantly affected by large changes in the rate of net filtration or absorption induced by changes of arterial or venous pressures. Substitution of the appropriate values of $A_p'/\Delta x$ (fig. 4) into *equation 18* suggests that during extremely rapid filtration (0.007 ml/sec./100 gm. tissue caused by a net pressure difference of 50 cm. H₂O across the capillary wall) the initial rates of passage of NaCl, glucose, raffinose, inulin and myoglobin would be, respectively, 400, 180, 120, 40 and 30 times their rates of passage by bulk flow. In steady state conditions of net filtration at this rate, the concentration ratios calculated from *equation 17* are, respectively, 0.99⁺, 0.99, 0.98, 0.87 and 0.34. At lower, more normal rate of filtration, the relative importance of diffusion would be even greater and the concentration ratios closer to 1.0. On the basis of the theory of restricted diffusion, we would therefore predict that molecular sieving of lipid-insoluble molecules the size of raffinose or smaller would not be detectable ($C_1/C_2 > 0.98$) even during rapid net filtration. However, molecular sieving of molecules the size of inulin or larger should be readily demonstrable in the capillaries of the perfused hindleg ($c_1/c_2 = 0.87$). This prediction has been verified in the case of inulin which has been shown to be present in the capillary filtrate in only 70 to 80 per cent of its concentration in plasma during rapid net filtration through the capillaries of the perfused hindleg (118). In the case of still larger molecules, including the plasma proteins, the restriction to diffusion becomes sufficiently great to allow a high degree of molecular sieving even at normal filtration rates. Wasserman and Mayerson (156) have shown that serum albumin leaves the circulation about 1.6 times more rapidly than serum globulins. This ratio may be compared with the ratio of the diffusion coefficients of the two molecular species (D albumin $\div D$ globulin = 1.5, ref. 23). At abnormally low filtration rates, however, even protein molecules may be expected to approach diffusion equilibrium with the ultrafiltrate. It has been observed that lymph (160) or capillary filtrate (92) obtained during rapid net filtration produced by venous congestion contains only small concentrations of protein

($0.3 \pm 0.1\%$), whereas lymph obtained during low rates of net filtration contains comparatively high concentrations (2–4%) of protein (34, 60). These observations have previously been explained on a spatial basis, it being supposed that in regions of the capillary where net filtration occurs, the concentration of protein is relatively small, but this filtrate is subsequently concentrated with respect to protein in regions of the interstitial fluid where absorption of fluid into the capillary occurs (35, 89). Both mechanisms may, of course, be operative.

Although data comparable to those shown in figure 4 have not yet been obtained for glomerular capillaries, it seems possible that in this case also diffusion as well as filtration may be an important mechanism involved in the passage of solutes. The large filtration coefficient of the glomerular membranes (table 1) and their relative permeability to myoglobin (164), egg albumin (5, 13) and hemoglobin (84, 98, 108) indicate that the effective pore radii and the (restricted) pore areas per unit path length are larger than in peripheral capillaries. The permeability of the glomerular membranes to molecules of graded sizes is usually interpreted in terms of a distribution of pore sizes. Thus Bott and Richards (13) in summarizing their work on glomerular permeability to proteins suggested that "In the light of present knowledge of the size of protein molecules it appears that most of the mesh surface of the glomerular membranes is coarse enough to permit the passage of particles about 20 \AA in diameter and that only one-half of it allows particles of about 50 \AA to go through." The evidence relating to restricted diffusion would make it more probable that the effective pore size of the glomerular membranes is closer to 100 \AA in diameter and that the observed permeability to molecules of graded sizes is a result of restricted diffusion during ultrafiltration through these relatively large channels. If glomerular filtration is momentarily reduced, as a result of transient vasoconstriction or other interference with the blood supply, large concentrations of protein may subsequently appear in the urine (71, 142, 159). This observation is usually explained in terms of an increase in capillary permeability brought about by anoxia (137, 159), but it could equally well be accounted for by the theory of molecular sieving summarized by equation 17 and figure 2. Lambert *et al.* (84) have recently shown that the renal glomerular clearance of hemoglobin relative to that of creatinine (in the dog) increases greatly when the filtration rate is reduced. The results suggest that the concentration of hemoglobin in glomerular filtrate relative to that in plasma increases from about 0.03 at normal filtration rates to about 0.1 when the filtration rate is reduced to 50 per cent of its control value during the infusion of adrenaline. This observation would be difficult to interpret on the basis of a distribution of pore sizes but is readily explicable by the theory of molecular sieving shown in figure 2.

6. Exchange Rates Through Capillary Membranes

A. Filtration and Absorption. Under normal conditions the volume of a given tissue remains relatively constant; filtration of fluid in regions where hydrostatic forces within the capillary exceed osmotic forces operating across the membrane is almost counterbalanced by absorption in regions where osmotic forces exceed hydrostatic forces. A slight tendency for filtration to exceed absorption is ordinarily accounted for by drainage through the lymphatics. The direct measurements of capillary pressure made by Landis (87, 88) allow an estimate to be made of the magnitude of filtration and absorption under normal conditions involving little or no net flow. In the mammalian circulation the pressure at the arteriolar end of the capillary is ordinarily less than $60 \text{ cm. H}_2\text{O}$ and that at the venular end must be greater

than zero. Since the effective osmotic pressure of the proteins is approximately 30 cm. H₂O the average pressure gradient across the capillaries available for filtration or for absorption is something less than 15 cm. H₂O. This pressure difference, operating over one-half the total capillary area, would produce a flow of 0.001 ml/sec./100 gm. tissue in the perfused cat hindleg and only 0.0005 ml/sec. (or 40 ml/24 hr.) in 100 ml. of the human forearm (see PART II, section 2, A, for filtration coefficient). It will be evident, from these estimates, that the transport of substances to and from these tissues would be an extremely slow process if the mechanism of transport were limited to bulk flow through the filtering and absorbing regions of the capillaries.

B. Exchange by Diffusion. Diffusion of water and small molecules in both directions through the capillary wall may be expected to occur at rates which are prodigious in comparison to their rates of transport by filtration and absorption. The pore area per unit path length available for the diffusion of water through the capillary walls in 100 gm. of muscle is about 1.2×10^5 cm. (fig. 4). The concentration (activity) of water available for diffusion in either direction is about 55 molar (0.99 gm/ml.) and the diffusion coefficient of H₂O¹⁸ in water at 37°C. is 4.0×10^{-6} cm.²/sec. (154). Substitution of these values in Fick's diffusion equation leads to a calculated diffusion rate of 5 gm/sec. Since the total volume of plasma in the capillaries of 100 gm. of tissue is only about 1 ml. (PART II, section 2, B), this suggests that plasma water exchanges five times per second or 300 times per minute with the interstitial fluid water immediately surrounding the capillaries. Similar calculations for NaCl, urea and glucose yield exchange rates of 120, 100 and 40 times the plasma content of these substances per minute, respectively. An alternative method of expressing the results is in terms of the ratio of exchange rate to plasma flow. The latter is generally in the range 2 to 4 ml/min./100 gm. tissue. Taking 3 ml/min. as an average, we would estimate that the diffusion of water, NaCl, urea and glucose back and forth through the capillary wall occurs at rates which are, respectively, 80, 40, 30 and 10 times the rate at which these substances are brought to the capillary by the incoming blood plasma. These high rates of exchange by diffusion occur despite an extremely small pore area for the diffusion process; the structural feature of the capillary wall which makes this exchange possible is the short path length for the diffusion process. The net rates of diffusion of these solutes will, of course, depend upon mean concentration differences operative across the membrane; under physiological conditions such differences may be maintained by the metabolic activities of the surrounding tissues.

The above estimates of capillary exchange rates are very much higher than previous estimates based on 'arterial disappearance rates' of isotopic trace substances injected into the circulation (19, 26, 50, 51, 56, 106, 109, 134, 153). Thus the transcapillary exchange rates of sodium or chloride in the guinea pig (26, 106), the rabbit (109) and in man (19) have previously been estimated to be of the order of 0.6 times the plasma water per minute instead of 120 times per minute as estimated above. A reason for this discrepancy may be found in the assumptions used to calculate exchange rates from arterial disappearance curves. For these calculations it has been assumed that the concentration of tracer substances in arterial plasma is representative of its mean concentration within the capillary. It is now known, however, that the concentration of the test molecules at the venous end of the capillary circulation may be very much less than that at the arterial end, the difference being large during the initial phase of the diffusion process and decreasing with time as

diffusion equilibrium is approached (39, 46, 72, 118, 131). The true concentration gradients for diffusion are therefore less than those estimated on the basis of arterial concentration, thus leading to estimates of exchange rates which are too low. In the case of small, rapidly diffusing molecules such as D₂O, NaCl or urea, this error leads to estimates of transcapillary exchange rates which are of the order of 100-fold too low. Indeed, the rates of disappearance of these molecules from the blood may be limited by the rate of blood flow rather than by restriction to diffusion through the capillary walls. Johnson *et al.* (72) have shown that the rate of distribution of D₂O in cardiac and skeletal muscle is blood flow limited over a wide range of flows. In this laboratory we have noted that the distribution rates of urea and NaCl in skeletal muscle become limited by flow (rather than by capillary permeability) when the plasma flow is reduced below about 6 ml/min./100 gm. muscle.

The mathematical relationships between arterial or venous concentrations and capillary exchange rates appear to be extremely complex. Among the variables which need be considered are the blood flow, the geometrical structure of the distribution volume in relation to the capillary and the apparent diffusion coefficient(s) of the diffusing molecules in their distribution volume(s). For lipid-soluble molecules such as the respiratory or anesthetic gases, the problem has often been treated as one of cylindrical diffusion (62, 63, 77, 110, 128, 132). In this case the diffusion path through the surrounding tissues is long compared to the radius of the capillary and a single apparent diffusion coefficient is employed. The mathematical treatment of cylindrical diffusion along a capillary with variable concentration gradient along its length is difficult but by no means insoluble. This treatment would not apply, however, to the case of lipid-insoluble molecules such as NaCl, urea, glucose etc., which penetrate the surrounding cells slowly if at all. In the case of muscle, which comprises almost half the body weight, the radial path length for diffusion of these molecules in the interstitial fluid is extremely small and the whole diffusion problem may be complicated by reflection from the surface of the muscle cells and by linear diffusion in the plane of the muscle fibers. Some of the complexities of the capillary exchange considered in terms of tissue structure are illustrated in recent analyses by Harris and Burn (59) and by Schmidt (132).

7. Passage of Lipid-Soluble Molecules Through Capillary Walls

Calculations based on Fick's Law have indicated that the small area in the capillary wall available for the passage of water and lipid-insoluble molecules would be insufficient to account for the observed rates of diffusion of oxygen and carbon dioxide through the capillary walls of the perfused hindleg (118). Similar calculations, based on the diffusing capacity of the lungs, indicate that at least one-half of the histological area of the lung capillaries would be required to explain the observed respiratory exchange rate on the basis of aqueous diffusion (127). The respiratory gases have relatively high partition coefficients between oil and water (93) and with this fact in mind, Renkin (123, 124) investigated the relations between lipid solubility and capillary permeability in the perfused hindleg. Lipid-soluble substances such as urethane (M.W. 89), paraaldehyde (M.W. 132) and triacetin (M.W. 218) traversed the capillary walls so rapidly that no osmotic transients were detectable. Glycerol and glycerol derivatives were shown to pass through the capillary walls at high rates which varied in order of their lipid:water partition coefficients but in the order opposite to that expected on the basis of their aqueous diffusion coefficients. The temperature coefficients of capillary permeability to antipyrine and

antipyrine derivatives were found to be related to the temperature coefficients of their lipid solubilities rather than to their aqueous diffusion coefficients.

These results indicate that lipid-soluble molecules can diffuse through regions in the capillary wall which are relatively impermeable to lipid-insoluble molecules. The permeability characteristics of this additional pathway are similar to those of cell membranes in general. It seems reasonable, therefore, to identify the diffusion pathway for lipid-soluble molecules with the plasma membranes of the capillary endothelial cells themselves, as opposed to the system of water-filled pores penetrating through or between these cells, which is capable of accounting for the passage of water and lipid-insoluble molecules.

CONCLUSION

"We have no right to deny the entrance of air into the blood because, on account of the bluntness of our senses we cannot actually see the vessels by which it makes its entrance. . . . For in order that the aereal particles should mix with the mass of blood in a state of fine division and in a most intimate manner, it is necessary that they should enter the blood through channels or rather orifices, almost infinite in number, distributed here and there over the whole mass of the lungs." John Mayow, *De Respiratione*, 1668.

Our scientific senses have sharpened considerably since these words were written. Yet, we must admit, in this year 1953, that no means are available to visualize the channels ('infinite in number') which subserve the ultimate function of the circulatory system in the transport of materials to and from the cells.

In this review I have attempted to combine the facts of capillary permeability with theory and fact from physical chemistry, to form some crude estimate of the number and dimensions of these pathways, the magnitude of flow through them and the physical mechanisms which regulate the flow. The picture which has emerged may be summarized as follows, choosing the capillary bed in mammalian extremities as a quantitative example.

The passage of water and lipid-insoluble molecules takes place through aqueous channels or pores penetrating the capillary wall. The total cross-sectional area of the pores comprises less than 0.2 per cent of the histological surface of the capillaries and may well be limited to areas between endothelial cells as suggested by Chambers and Zweifach (20).

Uniform cylindrical pores of radius 30 to 45 Å and a population density of $1-2 \times 10^9$ per cm.² of capillary wall would account for the observed rates of passage of water and lipid-insoluble molecules of various sizes. However, there are no reasons for supposing that the channels are actually cylindrical and we may regard this value of 'effective' pore radius as analogous to the Einstein-Stokes molecular radius which, by itself, tells nothing of the actual shape of the molecule. Net volume flow of fluid through these channels takes place by hydrodynamic processes. Under normal circumstances the transport of materials by hydrodynamic flow (filtration and absorption) is extremely slow, the rate of fluid movement being something less than 2 per cent of the plasma flow. Diffusion, rather than hydrodynamic flow, constitutes the chief process whereby small molecules can exchange rapidly between blood plasma and interstitial fluid. The diffusion of water, NaCl, urea and glucose in both directions through the capillary wall occurs at rates estimated to be, respectively, 80, 40, 30 and 10 times the rates at which these substances are brought to the tissues by the incoming blood. These high rates of exchange occur in spite of the small pore area, the chief structural factor concerned being the short path length for diffusion

through the capillary wall. Although the diffusion rates of these small molecules may be rapid, they are nevertheless considerably less rapid than can be accounted for on the basis of free diffusion. The concept of restricted diffusion is developed to take account of this fact, to explain the progressive decrease in apparent pore area as a function of molecular size (fig. 4) and to explain molecular sieving during ultrafiltration. According to this concept the effective pore size in the capillary wall is sufficiently great to allow even large plasma protein molecules to penetrate through the pores. The degree of molecular sieving of any given solute depends upon the ratio of its restricted diffusion coefficient to the rate of volume flow through the capillary wall (filtration rate). For small molecules the restriction to diffusion is small, relative to physiological rates of filtration, and no appreciable concentration gradients are maintained across the capillary membranes. Theoretically, however, molecules as small as urea or NaCl would be sieved if the filtration rates were made sufficiently large (fig. 2). For large lipid-insoluble molecules the restriction to diffusion becomes so great that the degree of molecular sieving is determined largely by the rate of filtration. Thus molecular sieving of inulin through the capillary walls in muscle is readily demonstrable at high rates of filtration produced by venous congestion (118). In the case of still larger molecules, including the plasma proteins, the restriction to diffusion is sufficient to allow a high degree of molecular sieving at normal rates of filtration. However, at abnormally low rates of filtration even protein molecules may be expected to approach diffusion equilibrium with the ultrafiltrate.

Finally, we conceive that most or all of the capillary endothelial surface is available for the passage of oxygen, carbon dioxide and other molecules which are soluble in lipids as well as in water. The rates of transcapillary diffusion of these molecules are correlated with their oil-water partition coefficients and are many times greater per unit concentration difference than the transcapillary diffusion rates of lipid-insoluble molecules of comparable size. This type of permeability is characteristic of cell membranes in general and leads to the hypothesis that lipid-soluble molecules can penetrate the plasma membranes of the capillary endothelial cells.

The information which forms the basis of this picture of the capillary exchange is indeed limited. Measurements of capillary permeability, in terms of volume flow or diffusion rates per unit driving force operating across known capillary areas, have been made in only three types of capillaries. The large quantitative differences which characterize each of these three types may be considered as a warning not to generalize from our present data. Further information from the field of membrane physical chemistry is also needed to clarify our present concepts, particularly in relation to restricted diffusion. The theoretical considerations and experimental evidence reviewed above suggest that restricted diffusion plays a major role in determining the rates of transcapillary exchange and the degree of molecular sieving during ultrafiltration.

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