Measurement of the Permeability
of Biological Membranes

Application to the glomerular wall

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ABSTRACT   The transport equation describing the flow of solute across a membrane has been modified on the basis of theoretical studies calculating the drag of a sphere moving in a viscous liquid undergoing Poiseuille flow inside a cylinder. It is shown that different frictional resistance terms should be introduced to calculate the contributions of diffusion and convection. New sieving equations are derived to calculate r and $A_p/\Delta x$ (respectively, the pore radius and the total area of the pores per unit of path length). These equations provide a better agreement than the older formulas between the calculated and the experimental glomerular sieving coefficients for [^{125}I]polyvinylpyrrolidone (PVP) fractions with a mean equivalent radius between 19 and 37 Å. From r and $A_p/\Delta x$, the mean effective glomerular filtration pressure has been calculated, applying Poiseuille's law. A value of 15.4 mm Hg has been derived from the mean sieving curve obtained from 23 experiments performed on normal anesthetized dogs.

In 1951, Pappenheimer et al. developed the so-called "pore theory" to account for the transcapillary transport of uncharged, lipid-insoluble solutes in mammalian muscles (24). According to this theory, convective flow and net diffusion contribute to solute flow across the membrane, in this case the capillary walls, both processes being impeded by the steric hindrance at the entrance of the "pores" (supposed to exist between the cells) and by frictional forces within the pores (20, 22, 23, 25).

The solute flow due to diffusion was calculated as $D(c_1 - c_2)A_w/\Delta x \times A_s/A_w$ where D is the free diffusion coefficient, c_1 and c_2, respectively, the solute concentrations in filtrand and filtrate and $A_w/\Delta x$ the pore area freely available to water per unit of length. The term A_s/A_w describes the restriction to the motion and can be calculated as $1/K_1 \times S_D$ where $S_D = [1 - (a_s/r)]^2$ is the steric hindrance term (a_s is the radius of the solute molecules

and r the radius of the pores) and $1/K_1$, called "wall correction factor" is the frictional resistance to diffusion in free solution relative to that in the pore. The wall correction factor takes into account the effect of the pore walls on the motion of the molecules; it is a function of a_s/r and was calculated, first by Ladenburg (14) (for small values of a_s/r), later by Faxen (5). These formulas will be given later.

The contribution of convective flow was calculated as $Q_f c_1 \times (A_s/A_w)$ where Q_f is the filtered volume per unit time. The same restriction factor A_s/A_w is used as for diffusion. Renkin later modified the restriction factor and replaced S_D by $S_F = 2[1 - (a_s/r)]^2 - [1 - (a_s/r)]^4$ which better describes the steric hindrance when Poiseuille flow takes place in the pores (25).

The transport equation for the solute is obtained by adding the contributions of diffusion and convection. Pappenheimer et al. (24) derived from the transport equation sieving equations which allow the calculation of two parameters characterizing the permeability properties of a membrane "equivalent" to the biological sieve: r the radius of cylindrical pores crossing the membrane and $A_p/\Delta x$ the total area of the pores per unit of path length. To derive these values, experimental values for Q_f and φ the sieving coefficient ($\varphi = c_2/c_1$) for at least two different solutes are needed (17). The validity of the pore theory to characterize biological membranes was justified by Solomon in 1968 (28).

Three objections concerning the solute transport equation may be raised. (a) The same steric hindrance term is used for diffusion and convection. Lambert et al. (18) recently pointed out that S_D should be used for the diffusion term and S_F for the convection term.

(b) The same wall correction factor is used in both terms. The validity of $1/K_1$ for diffusion is not questionable but its use in calculating the contribution of convective flow is incorrect. More recent work in the field of hydrodynamics (brought to our knowledge by B. M. Brenner [personal communication]) shows that the wall correction factor must be modified when the liquid inside the tube is not stationary (2, 8). All the authors who have applied the sieving equations to physiological problems, including ourselves, have neglected this fact (1, 18, 20, 21, 28).

(c) The concentration term c_1 used to calculate the contribution of convective flow is not correct if the concentrations on both sides of the membrane are not very similar. If they are different, the transport equation has to be integrated across the membrane. This results in introducing a mean concentration $\bar{c}_s$ instead of c_1 in the calculation of the contribution of bulk flow (12, 18).

A supplementary remark has to be made on which Pappenheimer himself had already drawn the attention (20). If the molecular radius (a_s) is greater than $0.5r$, Faxen's approximate solution for K_1 is no longer accurate enough.

Recently exact values for K_1 have been computed by Haberman and Sayre up to $a_s/r = 0.8$ (8).

The purpose of this paper is to show that sieving equations can be derived in a consistent way from the Kedem and Katchalsky equation describing the transport of solute across a membrane in terms of irreversible thermodynamics (13). Different frictional coefficients will be proposed for bulk flow and diffusion, starting from Haberman and Sayre studies.

The modified equations will be used to extract from the mean sieving curve for [^{125}I]polyvinylpyrrolidone (PVP) of 23 normal dogs the values of the membrane parameters (r and $A_p/\Delta x$). The effective glomerular filtration pressure (GFP$_e$) will then be calculated by means of Poiseuille's law. These results will be compared with those published recently (18).

THEORY

The following mathematical model is based on these assumptions: (*a*) The biological sieve is comparable to an artificial membrane crossed by cylindrical pores with a uniform radius r; Δx, their length, is much greater than r. (*b*) Solute molecules are simulated by rigid spheres of radius a_s moving slowly inside the pores. (*c*) The solvent flows according to Poiseuille's law. (*d*) The filtration rate is constant during the experiment and steady state is assumed. (*e*) The concentrations of solute are so small that there is no interaction between solute molecules inside the pores. (*f*) Finally it is assumed that all gradients are along the x coordinate. Thus the forces, flows, and velocities are along the x axis.

Let us call c_1 the solute concentration in the filtrand, c_2 that in the filtrate ($c_1 > c_2$), and c_s the concentration at any point in the pore. It must be kept in mind that c_s will change along the pore.

A molecule of solute at point x in a particular pore will be acted upon by thermodynamic forces as well as by frictional forces. It is assumed that in a steady flow, the thermodynamic force, f_s, acting on the solute is counterbalanced by the frictional forces and that these latter forces are additive (13). Thus:

$$f_s = -\phi_{sw} - \phi_{sm}, \qquad (1)$$

where ϕ_{sw} and ϕ_{sm} are the frictional forces between one molecule of solute and water or membrane.

According to hydrodynamic convention, the frictional forces between two components are proportional to their relative velocity (13). Choosing the membrane as reference:

$$\phi_{sw} = -f_{sw}(v_s - v_w),$$
$$\phi_{sm} = -f_{sm}v_s, \qquad (2)$$

where v_s and v_w are the velocities of solute and water relative to the membrane, f_{sw} and f_{sm} the frictional coefficients for one molecule of solute. Thus:

$$f_s = f_{sw}(v_s - v_w) + f_{sm}v_s = v_s(f_{sw} + f_{sm}) - f_{sw}v_w . \qquad (3)$$

The two terms on the right-hand side of Eq. 3 are, respectively, (a) the drag force on a molecule of solute moving with speed v_s in a pore containing stationary water and (b) the drag force exerted on a stationary molecule of solute by a flow of water moving with speed v_w .

Haberman and Sayre (8) have calculated the drag force of a sphere of radius a_s moving slowly on the axis of a cylinder of radius r containing a viscous liquid. The drag force is enhanced by the presence of the walls of the cylinder. Thus the drag felt by a sphere moving in a stationary liquid is K_1 times greater than the drag in an infinite medium: $v_s f^o_{sw}$. Likewise, the drag on a stationary sphere in a moving liquid (according to Poiseuille's law) is K_2 times greater than $f^o_{sw} v_w$. K_1 and K_2 are both functions of a_s/r. The values to be given to K_1 and K_2 will be discussed later.

The drag forces on our molecule are thus:

$$v_s(f_{sw} + f_{sm}) = f^o_{sw}K_1v_s , \qquad (4)$$

and

$$v_w f_{sw} = f^o_{sw}K_2v_w .$$

The values for K_1 and K_2 have been calculated only for spheres moving on the axis of a cylinder. But we shall assume that K_1 and K_2 are constant throughout the pore. This will be justified in the discussion. By substitution in Eq. 3, Eq. 5 is obtained:

$$\frac{f_s}{f^o_{sw}} = K_1v_s - K_2v_w . \qquad (5)$$

Let us write this equation for 1 mol of solute acted upon by a thermodynamic force $X_s = N_o f_s$ and let $F^o_{sw} = N_o f^o_{sw}$ be the frictional coefficient of 1 mol of solute in an infinite medium (N_o is the Avogadro number).

$$\frac{X_s}{F^o_{sw}} = K_1v_s - K_2v_w . \qquad (6)$$

The thermodynamic force is equal to the gradient of chemical potential (μ_s). As all gradients are along the x coordinate:

$$X_s = -\frac{d\mu_s}{dx} . \qquad (7)$$

Following Kedem and Katchalsky (12) we shall make the assumption that the chemical potentials for ideal solutions may be used:

$$\frac{d\mu_s}{dx} = \bar{V}_s \frac{dP}{dx} + \frac{RT}{c_s} \frac{dc_s}{dx},\qquad(8)$$

where $\bar{V}_s$ is the partial molar volume of solute, P the effective filtration pressure, R is the ideal gas constant, T is the absolute temperature. Introducing Eqs. 7 and 8 in Eq. 6 we find:

$$-\bar{V}_s \frac{dP}{dx} - \frac{RT}{c_s} \frac{dc_s}{dx} = F^\circ_{sw}(K_1 v_s - K_2 v_w),\qquad(9)$$

and multiplying both sides by c_s

$$-\bar{V}_s c_s \frac{dP}{dx} - RT \frac{dc_s}{dx} = F^\circ_{sw}(K_1 v_s c_s - K_2 v_w c_s).\qquad(10)$$

$v_s c_s$ is the solute flow per unit area and unit time, j_s. Thus:

$$-\frac{\bar{V}_s c_s}{F^\circ_{sw}} \frac{dP}{dx} - \frac{RT}{F^\circ_{sw}} \frac{dc_s}{dx} = K_1 j_s - K_2 v_w c_s,\qquad(11)$$

or

$$j_s = -\frac{D}{K_1} \frac{dc_s}{dx} + \frac{K_2}{K_1} v_w c_s - \frac{1}{K_1} \bar{V}_s \frac{c_s}{F^\circ_{sw}} \frac{dP}{dx},\qquad(12)$$

where $D = RT/F^\circ_{sw}$ is the free diffusion coefficient of the solute in water.

Let us now calculate the total solute flow for one pore, (j_s) pore. We shall assume that the flow of water in the pore is laminar. Then, $v_w = V(1 - (\rho^2/r^2))$ where V is the axial velocity of the water and ρ the distance from the axis. Besides it will be assumed that the molecules are evenly distributed over the cross section of the pore, their centers being located inside a circle of radius $r - a_s$. Then, c_s is constant for $\rho \le r - a_s$ and is equal to 0 for $\rho > r - a_s$.

$$(j_s)\,\text{pore} = \frac{1}{K_1} \int_0^{r-a_s} 2\Pi\rho \, d\rho \left\{ -D \frac{dc_s}{dx} + K_2 c_s V\left(1 - \frac{\rho^2}{r^2}\right) - \bar{V}_s \frac{c_s}{F^\circ_{sw}} \frac{dP}{dx} \right\}.\quad(13)$$

As

$$\frac{1}{K_1} \int_0^{r-a_s} 2\Pi\rho \, d\rho D \frac{dc_s}{dx} = \frac{D}{K_1} \frac{dc_s}{dx} \Pi(r - a_s)^2,$$

$$\frac{1}{K_1} \int_0^{r-a_s} 2\Pi\rho \, d\rho K_2 c_s V = \frac{K_2}{K_1} c_s V \Pi(r - a_s)^2,$$

$$\frac{1}{K_1} \int_0^{r-a_s} \frac{2\Pi\rho^3}{r^2} \, \mathrm{d}\rho K_2 c_s V = \frac{K_2}{K_1} c_s V \frac{\Pi}{2r^2} (r - a_s)^4,$$

$$\frac{1}{K_1} \int_0^{r-a_s} 2\Pi\rho \, \mathrm{d}\rho \bar{V}_s \frac{c_s}{F^0{}_{sw}} \frac{\mathrm{d}P}{\mathrm{d}x} = \frac{\bar{V}_s}{K_1} \frac{c_s \Pi}{F^0{}_{sw}} \frac{\mathrm{d}P}{\mathrm{d}x} (r - a_s)^2,$$

$$(j_s) \text{ pore} = -\frac{D}{K_1} \frac{\mathrm{d}c_s}{\mathrm{d}x} \Pi r^2 \left(1 - \frac{a_s}{r}\right)^2 + \frac{K_2}{K_1} c_s V \Pi r^2 \left\{\left(1 - \frac{a_s}{r}\right)^2\right.$$
$$\left. - \frac{1}{2}\left(1 - \frac{a_s}{r}\right)^4\right\} - \frac{\bar{V}_s}{K_1} \frac{c_s}{F^0{}_{sw}} \frac{\mathrm{d}P}{\mathrm{d}x} \Pi r^2 \left(1 - \frac{a_s}{r}\right)^2, \tag{14}$$

The mean solute flow per unit area and unit time $J_s = (j_s) \text{ pore}/\Pi r^2$ is:

$$J_s = -\frac{D}{K_1} \frac{\mathrm{d}c_s}{\mathrm{d}x} \left(1 - \frac{a_s}{r}\right)^2 + \frac{K_2}{K_1} c_s J_v \left\{2\left(1 - \frac{a_s}{r}\right)^2 - \left(1 - \frac{a_s}{r}\right)^4\right\}$$
$$- \frac{\bar{V}_s}{K_1} \frac{c_s}{F^0{}_{sw}} \frac{\mathrm{d}P}{\mathrm{d}x} \left(1 - \frac{a_s}{r}\right)^2. \tag{15}$$

In this equation J_v is the water volume flow; its value is $\bar{c}_w \bar{V}_w$ times the mean velocity of water ($\bar{c}_w$ and $\bar{V}_w$ being, respectively, the mean concentration and partial molar volume of water). As the mean velocity in Poiseuille flow is half the axial velocity and as $\bar{c}_w \bar{V}_w$ is very close to unity, $J_v = V/2$.

Eq. 15 is a local equation. To become useful for the interpretation of experimental data, this equation must be integrated across the membrane (13). As $-\mathrm{d}P/\mathrm{d}x$ is constant, it may be replaced by $\Delta P/\Delta x$ the gradient of effective filtration pressure. Let us perform the integration:

$$\int_0^{\Delta x} J_s \, \mathrm{d}x = \int_0^{\Delta x} \left(-\frac{D}{K_1} S_D \, \mathrm{d}c_s + \frac{K_2}{K_1} c_s J_v S_F \, \mathrm{d}x + \frac{1}{K_1} \frac{\bar{V}_s c_s}{F^0{}_{sw}} \frac{\Delta P}{\Delta x} S_D \, \mathrm{d}x\right). \tag{16}$$

As J_s is constant along the pore in a steady flow, we find:

$$J_s = \frac{D}{K_1} S_D \frac{c_1 - c_2}{\Delta x} + \frac{K_2}{K_1} J_v S_F \bar{c}_s + \frac{1}{K_1} \frac{\bar{V}_s \bar{c}_s}{F^0{}_{sw}} \frac{\Delta P}{\Delta x} S_D, \tag{17}$$

where

$$\bar{c}_s = \frac{1}{\Delta x} \int_0^{\Delta x} c_s \, \mathrm{d}x,$$

$$S_D = \left(1 - \frac{a_s}{r}\right)^2,$$

and

$$S_F = 2\left(1 - \frac{a_s}{r}\right)^2 - \left(1 - \frac{a_s}{r}\right)^4.$$

Eq. 17 has a simple physical interpretation. The first term on the right-hand side describes the transport of solute by diffusion, the second term, the transport by convection (molecules being dragged along by the fluid) whereas the third term represents the flow of solute due to a difference in effective pressure across the membrane. The steric hindrance factors S_F and S_D describe the "reflection" of molecules hitting the rim of the pore (these molecules are unable to enter the pore and hence do not contribute to the flow) (25). As the laminar flow drives proportionally more molecules through the central area of the pore than diffusion, the steric hindrance factor for convection S_F is greater than that for diffusion, S_D .

To calculate the value of $\bar{c}_s$, we must know how the solute concentration c_s behaves along the pore. $\bar{c}_s$ is calculated as follows: J_s in a steady flow, being constant along the pore, $(d/dx)J_s = 0$. Derivation of Eq. 15 with respect to x is:

$$- \frac{D}{K_1} S_D \frac{d^2 c_s}{dx^2} + \left\{ \frac{K_2}{K_1} J_v S_F + \frac{1}{K_1} \frac{\bar{V}_s}{F^\circ_{sw}} \frac{\Delta P}{\Delta x} S_D \right\} \frac{dc_s}{dx} = 0.$$

Resolving this linear differential equation:

$$c_s = k_1 e^{\frac{K' x}{\Delta x}} + k_2 \tag{18}$$

where

$$K' = \frac{K_2}{D} J_v \frac{S_F}{S_D} \Delta x + \frac{\bar{V}_s}{F^\circ_{sw}} \frac{\Delta P}{D} .$$

k_1 and k_2 are constants which are determined by the limit conditions $c_s = c_1$ at $x = 0$ and $c_s = c_2$ at $x = \Delta x$.

$$k_1 = \frac{c_1 - c_2}{1 - e^{K'}}$$

$$k_2 = \frac{c_2 - c_1 e^{K'}}{1 - e^{K'}} .$$

The mean value of the concentration in the pores, $\bar{c}_s$, is

$$\bar{c}_s = \frac{1}{\Delta x} \int_0^{\Delta x} c_s \, dx = \frac{c_2 - c_1 e^{K'}}{1 - e^{K'}} - \frac{c_1 - c_2}{K'} \tag{19}$$

Let us replace in Eq. 17 $\bar{c}_s$ by its value. We find the transport equation for the solute:

$$J_s = \left(\frac{K_2}{K_1} J_r S_F + \frac{1}{K_1} \frac{\bar{V}_s}{F^\circ_{sw}} \frac{\Delta P}{\Delta x} S_D \right) \left(\frac{c_2 - c_1 e^K}{1 - e^{K'}} \right) . \tag{20}$$

This equation differs from the equation we used in a previous work (18) on two points. First, the formula giving the mean concentration $\bar{c}_s$ is more accurate. Then, we have shown that different wall correction factors should be used: $1/K_1$ for diffusion and K_2/K_1 for convection.[1]

Several authors have calculated the drag force on a sphere (of radius a) moving axially in a cylinder containing a viscous liquid. When the liquid is stationary, the drag force is

$$\text{drag} = 6\Pi\eta a v_s K_1$$

when η is the dynamic viscosity (poises) and v_s the sphere velocity.

The first formula given for the coefficient $1/K_1$ is due to Ladenburg (14):

$$\frac{1}{K_1} = \frac{1}{1 + 2.4\frac{a}{r}},\tag{21}$$

valid only within narrow limits ($0 < a/r < 0.1$).

Faxen has proposed the following equation (5):

$$\frac{1}{K_1} = 1 - 2.104\frac{a}{r} + 2.09\left(\frac{a}{r}\right)^3 - 0.95\left(\frac{a}{r}\right)^5,\tag{22}$$

which is valid in the range $0 < a/r < 0.5$. Since Eq. 22 neglects the terms of power higher than 5, Bohlin (2) has introduced higher order terms, extending the validity of his formula up to $a/r < 0.6$. Bohlin's equation, however, is of little help in solving our problem. For $a/r > 0.6$ his formula leads to negative values. Haberman and Sayre have given a more accurate formula, although still approximate (8):

$$\frac{1}{K_1} = \frac{1 - 2.105\frac{a}{r} + 2.0865\left(\frac{a}{r}\right)^3 - 1.7068\left(\frac{a}{r}\right)^5 + 0.72603\left(\frac{a}{r}\right)^6}{1 - 0.75857\left(\frac{a}{r}\right)^5}\tag{23}$$

When the liquid is moving inside the cylinder (with axial speed V far from the sphere) Haberman and Sayre have shown that the drag is

$$\text{drag} = 6\Pi\eta a(v_s K_1 - V K_2),$$

and they give for K_2 the formula

$$K_2 = \frac{1 - \frac{2}{3}\left(\frac{a}{r}\right)^2 - 0.20217\left(\frac{a}{r}\right)^5}{1 - 2.1050\frac{a}{r} + 2.0865\left(\frac{a}{r}\right)^3 - 1.7068\left(\frac{a}{r}\right)^5 + 0.72603\left(\frac{a}{r}\right)^6}.\tag{24}$$

[1] See Addendum.

In addition they have calculated "exact" values for K_1 and K_2 for several values of a/r up to 0.8. Fig. 1 shows $1/K_1$, K_2/K_1, as well as S_D/K_1 and $S_F K_2/K_1$ in function of a_s/r.

APPLICATION TO GLOMERULAR SIEVING

We shall now express the transport equation in terms of renal physiology. J_s and J_v will be replaced by quantities readily derived from sieving measurements.

Let Q_f be the filtration rate, A_p the total area of the pores. The capillary and urinary compartments are assumed to be well stirred so that the solute
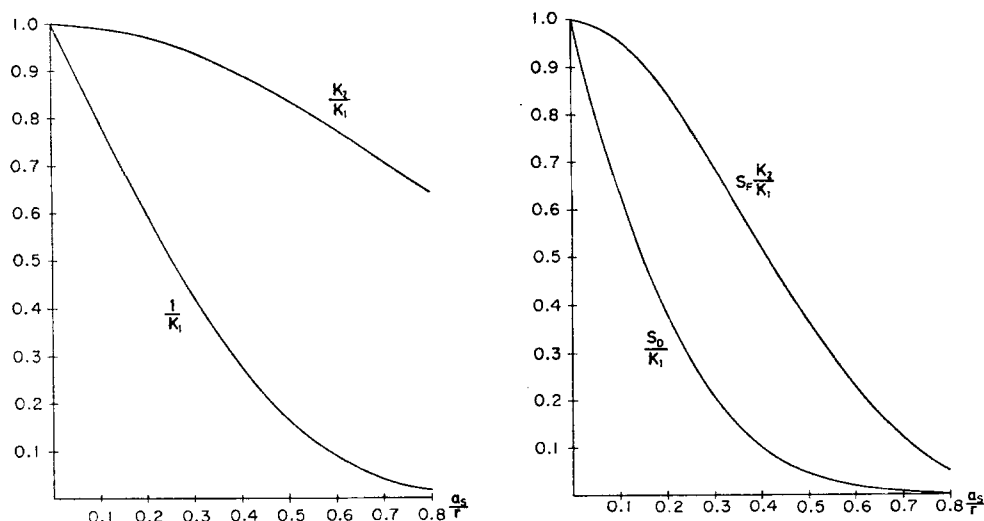

FIGURE 1. Relationship between $1/K_1$, K_2/K_1, S_D/K_1, $(K_2/K_1) \times S_F$, and a_s/r.

concentration in each is homogeneous. The sieving coefficient φ is the ratio of filtrate to filtrand concentration $\varphi = c_2/c_1$. Then $J_v = Q_f/A_p$, and $J_s = c_2 J_v = \varphi c_1 Q_f/A_p$.

The third term in Eq. 17 is negligible as long as $\bar{c}_w \bar{V}_w$ is close to unity; $\bar{c}_s \bar{V}_s$ is then very small (dilute solutions). We find a simplified formula for $\bar{c}_s$ by replacing K' by

$$K = \frac{K_2}{D} \frac{Q_f}{A_p} \frac{S_F}{S_D} \Delta x. \tag{25}$$

Replacing J_s and J_v in Eq. 20 by their values and dividing both members by Q_f/A_p, we find

$$\varphi c_1 = \frac{K_2}{K_1} S_F \left(\frac{c_2 - c_1 e^K}{1 - e^K} \right),$$

and, by using the relation $\varphi = c_2/c_1$, one obtains

$$\varphi = \frac{\dfrac{K_2}{K_1} S_F}{1 - e^{-K}\left(1 - \dfrac{K_2}{K_1} S_F\right)}. \tag{26}$$

An explicit expression for $A_p/\Delta x$ may be obtained starting from Eq. 26,

$$e^{-K} = \frac{1 - \dfrac{K_2}{K_1}\dfrac{S_F}{\varphi}}{1 - \dfrac{K_2}{K_1} S_F}.$$

Taking the natural logarithm of both members,

$$-K = \ln\left\{\frac{1 - \dfrac{K_2}{K_1}\dfrac{S_F}{\varphi}}{1 - \dfrac{K_2}{K_1} S_F}\right\},$$

and, by using Eq. 25

$$\frac{A_p}{\Delta x} = \frac{K_2 Q_f}{D}\frac{S_F}{S_D}\frac{1}{\ln\left\{\dfrac{1 - \dfrac{K_2}{K_1} S_F}{1 - \dfrac{K_2}{K_1}\dfrac{S_F}{\varphi}}\right\}}. \tag{27}$$

The effective glomerular filtration pressure, ΔP (dyn cm^{-2}) is calculated by using Poiseuille's law:

$$\Delta P = \frac{8\eta Q_f}{r^2}\frac{1}{\dfrac{A_{wf}}{\Delta x}}, \tag{28}$$

where η is the dynamic viscosity (poises), A_{wf} the pore area for water flow equal to

$$A_p\left\{2\left(1 - \frac{a_w}{r}\right)^2 - \left(1 - \frac{a_w}{r}\right)^4\right\}.$$

a_w is the radius of the water molecules (1.5 Å); since A_{wf} and A_p are very close together, ΔP will be calculated introducing A_p in Eq. 28.

RESULTS

Fig. 2 shows the mean sieving curve for [125I]PVP derived from 23 experiments performed on normal anesthetized dogs with a mean arterial pressure between 120 and 150 mm Hg. The sieving coefficients (ordinates) were calculated as:

$$\frac{\text{urinary clearances of } [^{125}\text{I}]\text{PVP fractions}}{\text{GFR}}$$

The glomerular filtration rate (GFR) was measured as the urinary clearance of inulin. Separation of PVP equimolecular fractions from the urine and the plasma was performed by Sephadex G-200 gel filtration (Pharmacia Fine Chemicals Inc., Uppsala, Sweden) (10, 16). Since PVP is not significantly reabsorbed by the renal tubules (15), the ratio

$$\frac{\text{urinary clearance of } [^{125}\text{I}]\text{PVP fractions}}{\text{GFR}} = \frac{c_2}{c_1}$$

Molecular sizes (abscissas) were calculated as radii of equivalent spheres from the chromatographic data according to Hardwicke et al. (9). Since the values for K_2, K_1, S_F, and S_D in Eq. 26 and Eq. 27 depend on the pore radius,
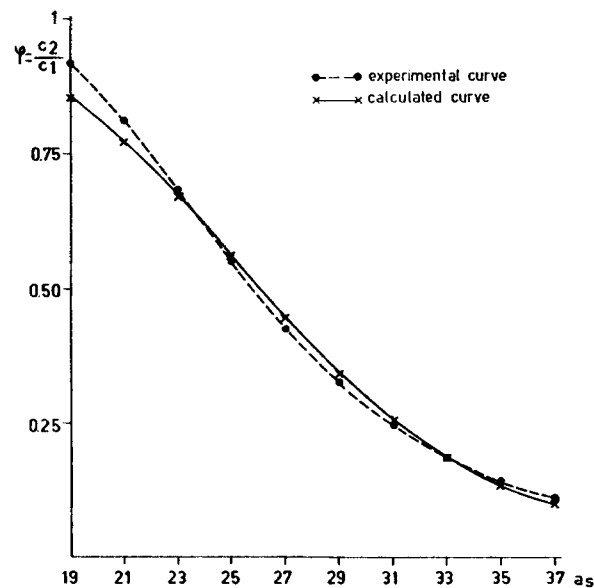

Fɪɢᴜʀᴇ 2. Glomerular sieving coefficients φ for [125I]PVP fractions with an equivalent molecular radius a_s between 19 and 37 Å.

r, all being functions of a_s/r and since the value for K in Eq. 25 depends on the value given to $A_p/\Delta x$, those values for r and $A_p/\Delta x$ giving the best fit to the experimental data, within the limits $a_s = 19$ to 37 Å were calculated by computer using a step by step approach. The solution minimizes a sum of weighted quadratic errors, ΣE which is calculated as follows:

$$\sum_{a_s=19}^{37} \left(\frac{\varphi_{calc} - \varphi_{exp}}{\sqrt{\varphi_{exp}}} \right)^2 .$$

K_2 and K_1 were obtained by interpolation on a logarithmic scale from the "exact" values given in Haberman and Sayre's tables (8). The calculated curve is also represented in Fig. 2.

Table I shows the values derived for r, $A_p/\Delta x$, GFP_s (equal to ΔP) and ΣE according to Eqs. 26 and 27. For comparison the same parameters have been calculated within the same limits according to the equations previously proposed by Lambert et al. (18). The latter do not take into account Poi-

TABLE I

VALUES DERIVED FOR r, $A_p/\Delta x$, GFP_s (EQUAL TO ΔP) AND ΣE
ACCORDING TO EQS. 26 AND 27

	r	$A_p/\Delta x$	GFP_s	ΣE
	Å	cm	mm Hg	
Eqs. 26 and 27	50.66	7,510,000	15.4	0.0108
Lambert et al. (18)	49.47	15,340,000	9.5	0.0183

seuille flow in calculating the "wall factor" restricting convective flow. These authors also use the approximate values derived for K_1 by Faxen (5) instead of Haberman's exact values.

The new equations do not modify the mean value for r but increase GFP_s by approximately 60%. It is noteworthy that ΣE is much lower using the new sieving equations. The improvement in the alignment of the sieving curves results from a better fit between the sieving coefficients (calculated and experimental) for the smallest molecules here considered (φ_{19} and φ_{21}).

A more analytical method has been applied to calculate r and $A_p/\Delta x$. Paired values for φ and D are introduced in Eq. 27; $A_p/\Delta x$ is then eliminated from this system of equations and r is determined. The same procedure is used for the pairs: $a_s = 19$ and 23 Å and so on until $a_s = 35$ and 39 Å. The mean value for $r(\bar{r})$ is thereafter introduced in Eq. 27 to calculate the $A_p/\Delta x$ value corresponding to each experimental value for φ. The same procedure is used to calculate other values for $\bar{r}$ and $\bar{A}_p/\Delta x$ by utilizing the sieving data within more narrow limits. Those paired values for $\bar{r}$ and $\bar{A}_p/\Delta x$ minimizing ΣE are definitively accepted.

Fig. 3 illustrates how r varies according to the molecular sizes used in the calculations; r is almost constant when the new sieving equations are utilized (Fig. 3 B) but increased progressively with molecular size when calculated according to the older equations (Fig. 3 A). Although the introduction of the φ_{19} still provides a lower value for r, the new equations make the isoporous model much more reliable, at least in the range of molecular sizes 21–37 Å.

Whatever the mathematical model used, the value derived for $A_p/\Delta x$ is too high when the φ_{19} is introduced together with $\bar{r}$. The same observation was
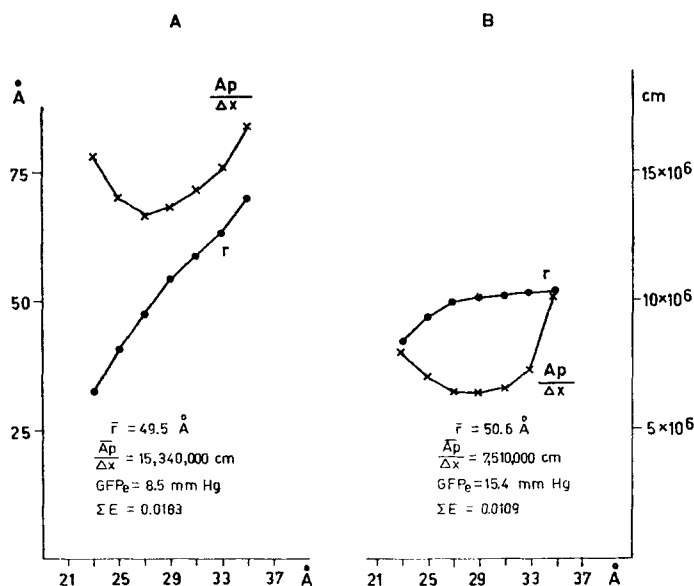

FIGURE 3. Values for r and $A_p/\Delta x$ calculated according to the "analytical method" (see text). At left, using the sieving equations previously proposed (18). At right, using the new sieving equations developed in the present study.

made with the older model. The difference between the calculated and the experimental values for φ_{19} was even more pronounced. For PVP molecules with an equivalent radius between 23 and 35 Å, $A_p/\Delta x$ is constant. Again $A_p/\Delta x$ increases when the sieving coefficients for molecules larger than 35 Å are used in the calculations. This observation is difficult to explain. It may prove the presence of a small number of larger pores (1, 18). However it should be kept in mind that experimental errors are more likely to be greater in this range of molecular sizes (on account of the small excretory rates of these molecules). Finally the theoretical model may fail to be correct for these large molecules: for instance, the values for K_2 and K_1 are exact only for $a_s/r \leq 0.8$; this limit is reached for molecules with a radius of 39 Å.

DISCUSSION

A. *Assumptions of the mathematical model*

A Poiseuille flow inside the pores has been assumed. Indeed, the Reynolds number is very small on account of the low velocity of the fluid in the pores. According to Landau and Lifshitz (19) its value is:

$$R_e = \frac{2r \cdot \bar{v}_w}{\nu},$$

where ν is the kinematic viscosity (dynamic viscosity divided by density) and $\bar{v}_w$ the mean velocity of the fluid. R_e for the glomerular membrane is approximately 0.5×10^{-6}.[2] Simultaneously the particle Reynolds number has been calculated as (27):

$$R_{e_p} = \frac{a \left(\frac{a}{r}\right)^2 \bar{v}_w}{\nu}.$$

For molecular sizes of 19 and 37 Å, R_{e_p} is, respectively, 1.37×10^{-8} and 10×10^{-8}. According to Goldsmith and Mason there is no radial movement of rigid spheres in a cylindrical tube for R_{e_p} values lower than 10^{-6} (7). Therefore it seems justified to consider the molecular concentration identical at any point of the available area of the pore section. The word "concentration" deserves some explanation. Since tracer amounts of [125I]PVP are injected into the animals, a relatively small number of molecules are present in the membrane. It has been calculated that only 1 pore among 600 contains a molecule of PVP. Therefore concentration in a part of a pore means the probability for a PVP molecule to be localized at a given point of the available pore volume. Considering the whole set of pores, it represents the number of molecules located at homologous points per unit of volume.

In our calculations we assumed that K_1 and K_2 are constant over the cross section of the pore. This is certainly not exact. However experimental evidence shows that K_1 and K_2 are not very much different for off-axis motion. Francis has measured the terminal velocity of spheres falling in a vertical tube filled with a stationary viscous liquid. The values for K_1 did not differ significantly according to the position of the sphere with respect to the wall (6). Goldsmith and Mason have studied the movement of a sphere in suspension in a liquid undergoing laminar viscous flow. The translational velocity of the sphere is proportional to K_2/K_1. Its value has been measured experimentally for spheres moving at different distances off the axis (7). These meas-

[2] Such low values satisfy one of the assumptions of Haberman and Sayre and allow to omit the inertia terms in the Navier-Stokes equation from which start all calculations.

urements make it possible to calculate how K_2/K_1 varies as a function of the radial distance between the center of the sphere and the axis. If PVP molecules with an equivalent radius between 19 and 37 Å are distributed throughout the pore section area, the flow of solute differs by less than 10% from the value calculated using the axial values for K_2 and K_1.

The other assumptions on which the mathematical model is based will not be discussed in detail. Objections may be raised that the PVP molecules are probably neither spherical nor rigid (26).

Likewise the glomerular basement membrane is much more likely a fibrillar network than an idealized isoporous membrane with cylindrical pores. However it is uncertain that the basement membrane is the ultimate structure responsible for the molecular sieving in the range of molecular sizes here con-
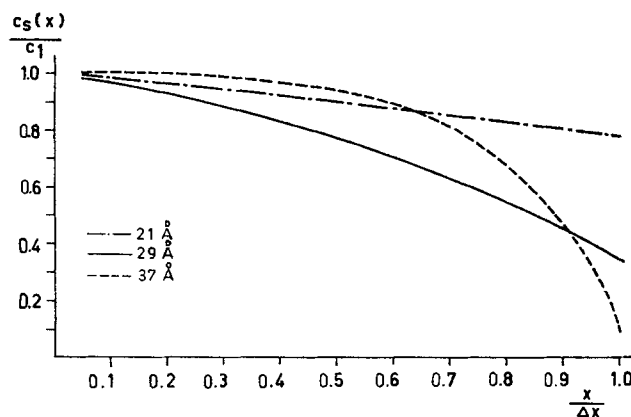

FIGURE 4. Decrease of the normalized [125I]PVP concentration $c_s(x)/c_1$ along the pore for different molecular sizes.

sidered (11, 29). It is therefore difficult to avoid making major simplifications since experimental evidence is lacking concerning the structure of the sieving membrane and the physical characteristics of the PVP molecules.

B. *Concentration of PVP molecules inside the pores and the respective contributions of convection and diffusion*

The concentration of PVP molecules (with the meaning defined above) along the pores has been calculated according to Eq. 18 for three molecular sizes (21, 29, and 37 Å) using Q_f = 0.709 ml s^{-1} (mean value for GFR of 23 normal dogs) and the values for r and $A_p/\Delta x$ given in Table I. The concentration decreases curvilinearly as shown in Fig. 4. If diffusion alone were responsible for the transport of solute, the concentration would decrease linearly. As the relative part of convection increases, the curve separates more and more from the straight line (3, 4).

The parts taken by convection and diffusion in the transport of PVP molecules are shown in Table II. Convective flow prevails at all the molecular sizes, especially for the smallest and the largest molecules in the range here considered. The relative part of diffusion is maximum at intermediate molecular sizes (27 Å). For larger molecules it falls rapidly since the decrease of $1/K_1$ is more rapid than that of K_2/K_1.

This explains the intersection of the curves representing the concentration of PVP molecules in relation with distance inside the pores. Table II also shows the contribution of the third term present in Eq. 17 $(1/K_1)(\bar{V}_s\bar{c}_s/F^{\circ}_{sw}) \times (\Delta P/\Delta x)S_D$. The values obtained are low enough to be neglected in the range of molecular sizes under consideration.

The mean concentrations inside the pores $\bar{c}_s$ have been calculated using Eq. 19. They have been normalized with respect to c_1 (Table III). The arithmetical means $(c_1 + c_2)/2c_1$ are given for comparison. Both mean values

TABLE II

CONVECTION AND DIFFUSION IN THE TRANSPORT OF PVP MOLECULES AND THE CONTRIBUTION OF THIRD TERM OF EQ. 17

a_s	Diffusive flow	Convective flow	$\dfrac{\text{Diffusion}}{\text{diffusion + convection}}$	$\dfrac{1}{K_1}\dfrac{\bar{V}_sD}{R\ T}\Delta P\dfrac{A_p}{\Delta x}S_D\dfrac{\bar{c}_s}{c_1}$
$\mathring{A}$				
19	0.2290	0.3756	0.379	
21	0.2305	0.3195	0.419	0.0061
23	0.2146	0.2635	0.449	
25	0.1882	0.2150	0.467	0.0027
27	0.1472	0.1719	0.461	
29	0.1064	0.1372	0.437	
31	0.0709	0.1093	0.394	0.0007
33	0.0426	0.0881	0.326	
35	0.0243	0.0710	0.255	0.0002
37	0.0122	0.0566	0.177	

TABLE III

MEAN CONCENTRATIONS INSIDE THE PORES $\bar{c}_s$ NORMALIZED WITH RESPECT TO c_1

a_s	φ calculated	K_1	K_2	$\bar{c}_s/c_1$	$(c_1 + c_2)/2\,c_1$
19	0.850	3.173	2.879	0.928	0.925
21	0.774	3.784	3.358	0.893	0.887
23	0.669	4.725	4.084	0.847	0.834
25	0.560	5.668	4.786	0.803	0.780
27	0.446	7.189	5.888	0.762	0.723
29	0.341	9.226	7.332	0.733	0.670
31	0.253	12.080	9.257	0.722	0.627
33	0.184	16.670	12.330	0.736	0.592
35	0.134	23.010	16.440	0.770	0.567
37	0.097	34.430	23.685	0.828	0.548

decrease with increasing molecular sizes up to 31 Å. The differences are negligible for small molecules but increase rapidly for molecules greater than 27 Å. For molecular radii greater than 31 Å, $\bar{c}_s/c_1$ increases with molecular size, illustrating the greater part of convection in the total transport of the largest molecules.

To conclude, the biomathematical model used to study the permeability of a porous membrane to macromolecules such as [^{125}I]PVP (actually the glomerular membrane) has been modified on the basis of theoretical studies calculating the drag of a sphere moving in a viscous liquid undergoing a Poiseuillian flow inside a cylinder. The new sieving equations differ essentially from those proposed in a previous study by the value given to the wall correction factor used to calculate the contribution of convective flow. They provide a better agreement than the older formulas between the calculated and the experimental values for φ, the sieving coefficients of PVP fractions with a mean equivalent radius between 19 and 37 Å (sieving coefficients varying between 0.9 and 0.1).

The mean effective glomerular filtration pressure has been calculated from r and $A_p/\Delta x$ the parameters describing the permeability of the equivalent isoporous membrane, applying Poiseuille's law. A value of 15.4 mm Hg was derived from the mean sieving curve obtained from 23 experiments performed on normal anesthetized dogs.

LIST OF SYMBOLS

a_s	Radius of solute molecules ($\mathring{A} = 10^{-8}$ cm)
a_w	Radius of water molecules ($\mathring{A} = 10^{-8}$ cm)
A_p	Total pore area (cm^2)
c_1	Solute concentration in filtrand (mol ml^{-1})
c_2	Solute concentration in filtrate (mol ml^{-1})
c_s	Solute concentration in the pores (mol ml^{-1})
$\bar{c}_s$	Mean solute concentration in the pores (mol ml^{-1})
D	Free diffusion coefficient of solute in water (cm^2 s^{-1})
F°_{sw}	Molar frictional coefficient in infinite medium (dyn s cm^{-1} mole^{-1})
j_s	Solute flow per unit time and area (mol cm^{-2} s^{-1})
J_s	Mean solute flow (mol cm^{-2} s^{-1})
J_v	Water volume flow (ml cm^{-2} s^{-1})
K_1, K_2	Wall correction factors (dimensionless)
K	$= (K_2 Q_f S_F/DA_p S_D)\Delta x$
K'	$= [(K_2 Q_f S_F/DA_p S_D)\Delta x] + \bar{V}_s \Delta P/F^\circ_{sw}D$
ΔP, GFP_e	Effective filtration pressure (dyn cm^{-2})
Q_f	Filtration rate (ml s^{-1})
r	Radius of the pores (Å)
$\bar{r}$	Mean radius of the pores (Å)

S_D, S_F	Steric hindrance restriction factors (dimensionless)
v_s	Velocity of the solute (cm s^{-1})
v_w	Velocity of the water (cm s^{-1})
V	Axial velocity of water in Poiseuille flow (cm s^{-1})
$\bar{V}_s$	Partial molar volume of solute (ml)
Δx	Length of the pores (cm)
ΣE	Sum of weighted quadratic errors
η	Dynamic viscosity (P = dyn s cm^{-2})
φ	Sieving coefficient (dimensionless)

ADDENDUM

C. P. Bean has independently reached the same conclusion concerning wall correction factors. (1972. *In* Membranes. G. Eisenman, editor. Marcel Dekker Inc., New York. **1:** 32.)

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