Osmotic Volume Flow in the Proximal Tubule of *Necturus* Kidney

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ABSTRACT Volume changes due to osmotic flow in the distal portion of proximal tubules of *Necturi* were measured by the split oil drop technique. In agreement with previous findings no volume flow was induced by NaCl concentrations close to 60 mM. The tubule wall was found to be permeable to plasma electrolytes, which have an apparent reflection coefficient of 0.69. The mean apparent hydraulic conductivity was 0.33×10^{-11} cm^3/dyne sec, comparable with other epithelia. A number of lipid-insoluble nonelectrolytes of widely varying molecular size had apparent reflection coefficients of about 0.5. In view of the insensitivity to molecular size it seems likely that apparent reflection coefficients determined from tubular volume changes depend primarily on the porosity of the intercellular barrier closest to the lumen and give little information about the subsequent fate of the test substances.

The hydraulic conductivity of the proximal tubule of *Necturus* kidney may be determined by measuring the flow of solution induced by an osmotic pressure gradient. In previous experiments of this type we used mannitol to create the osmotic pressure difference since the rate of mannitol leakage out of the tubule had been found to be small (1, 2). These earlier results were based on sets of single observations since the stop flow perfusion method that was used yields only a single datum for each tubule. The subsequent development of the split oil drop technique for the measurement of fluid movement by Gertz (3) has made it possible to observe the time course of fluid movement and thus to make more accurate measurements. It was found that the isonatric point, at which there is no net Na movement, was 60 mM NaCl, in agreement with previous observations. The tubule was therefore perfused with 60 mM NaCl solutions, to which a series of lipid insoluble nonelectrolytes had been added, in order to

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determine apparent reflection coefficients by the zero time method of Gold-stein and Solomon (4).

EXPERIMENTAL METHODS

Experiments were performed on adult male *Necturi* (Lemberger and Co., Oshkosh, Wis.) between the months of March and October, 1965, and again during the same period in 1966. Tricaine methanesulfonate (2.0–2.5 g/liter) anesthesia was used in all experiments. Most of the osmotic flow experiments were performed in July, August, and September, 1966. Animals used during those months had been obtained fresh in May or June and then stored unfed at 4°C.

In preliminary experiments it was observed that water permeability was somewhat greater in segments of the proximal tubules close to the glomerulus than in the more distal portion; to allow comparison between animals, all studies in the present paper were done on the portion of the proximal tubule close to the thin segment between the proximal and distal tubules.

The technique for measuring rapid volume changes in the kidney tubule under stopped flow conditions has been described by Gertz in studies of the rat proximal tubule (3). The present application to the *Necturus* tubule has necessitated certain modifications. Stained mineral oil was injected into the glomerulus until the whole tubule was filled. During the course of the experiment clear mineral oil was periodically applied to the exposed kidney surface.

An approximately straight section of tubule was then selected and the oil column was split by means of a sharpened micropipette (5 to 10 μ tip diameter) containing the perfusion fluid. A series of experiments was then carried out in which 3 to 10 solutions of a single nonelectrolyte were injected in turn into the same animal, often into the same tubule. The concentration range was covered in three to five steps. After each measurement the remaining solution was removed by applying pressure through the glomerular pipette, thus forcing oil into the previously used experimental segment.

Whenever the concentration was altered a pretreatment perfusion was used to wash out the tubule. Usually it was injected through the same puncture; and after 2–3 min at the experimental site, the pretreatment fluid was in turn forced out of the tubule distally by applying pressure to the glomerular pipette, with the infusing pipette still in place. Pressure was then applied to the infusion pipette and a new portion of the test solution was forced into the experimental site. At the completion of this injection the stopwatch was started and the first measurement of length was recorded as soon as possible, usually within 0.2–0.5 min. Subsequent measurements were made as often as possible during the next 2 min, with less frequent measurement thereafter.

Most experiments on single animals were completed within 90 min after anesthesia was begun. Experiments were terminated if blood flow in the kidney was markedly reduced. After all the perfusions, 0.5–1.0 ml of blood was withdrawn from the inferior vena cava into a syringe containing so small a quantity of sodium heparin solution (Lilly Co., 200 milliosmols) as to be negligible with regard to the determination of blood osmolarity. Osmolarity was measured by freezing point depression (Os-

mometer model G-62, Fiske Associates, Inc., Bethel, Conn.) of the plasma after centrifugal separation of the cells.

Instead of rapid sequence photography, measurements of the tubular radius and the length of the oil column were made with a fixed 100 division micrometer disc placed in one of the eyepieces of the microscope (Zeiss dissection microscope). Calibrations were made periodically against a standard 1 mm length. Control experiments showed that the visual method gave the same results as were obtained by rapid sequence photography.

Solutions

Test solutions for osmotic flow experiments were prepared in the following manner. Solutions of polyethylene glycol of molecular weight 1000 and above, polyvinylpyrrolidone, and dextran were prepared in 60 mM NaCl and then dialyzed against 60 mM NaCl at about 4°C with periodic stirring and with two or three changes of the outside NaCl solution, for not less than 24 hr. After dialysis various dilutions of the sac contents were made with 60 mM NaCl.

Total osmolality of these solutions was determined by measuring freezing point depression calibrated against standard NaCl solutions. The osmolality due to the polymer was taken as the difference between the total osmolality and that of 60 mM NaCl. Since the osmolalities of the low molecular weight substances were within 2% of the calculated values, these substances were used without purification.

Chemicals

Raffinose, mannitol, and isobutanol (Distillation Products Industries, Rochester, N. Y.), glycerol (Merck and Co., Inc., Rahway, N. J.), erythritol (Calbiochem, Los Angeles, Calif.), diethylene glycol (Fisher Scientific Co., Fairlawn, N. J.), tetraethylene glycol (J. T. Baker Chemical Co., Phillipsburg, N. J.) were reagent grade. The polymers were as follows: dextran (Sigma Chemical Co., St. Louis, Mo.), polyvinylpyrrolidone (PVP) (Sigma Chemical Co., St. Louis, Mo. and General Aniline and Film Corp., New York, N. Y.), polyethylene glycol (PEG) (manufactured by Union Carbide Corp., New York, N. Y. and known as Carbowax). Molecular weights quoted by suppliers are given in Tables I and II. Olive oil was USP grade.

Determination of Partition Coefficients

The following labeled substances from New England Nuclear Corporation (Boston, Mass.) were used: inulin-carboxyl-^{14}C, mol wt 5000–5500 (2.3 mc/g); inulin-methoxy-^{3}H, in which about 3% of free hydroxyls are methylated (165 mc/g); and polyethylene glycol-^{14}C (polyethylene-1,2-^{14}C-glycol), mol wt 4000 (0.43 mc/g). The inulin-^{14}C was purified by taking the first portion of the eluate from a G25 Sephadex column. Aqueous phases were solutions of the labeled substances at concentrations ranging from 0.6 to 4.5 mg/ml in a solution containing 150 mM NaCl and 9 mM buffer (NaH$_2$PO$_4$ and Na$_2$HPO$_4$, pH 6.9).

Mixtures of roughly equal volumes of the aqueous solution and of olive oil or isobutanol were equilibrated by shaking in closed centrifuge tubes. They were then centrifuged until the radioactivity in the nonaqueous layer ceased to fall; in experi-

ments with olive oil at least 15 min centrifugation at about 9000 g was required. The experiments were done at room temperature, 20–24°C. After centrifugation samples of both phases were counted in a liquid scintillant on a counter (Nuclear Chicago Corp. Model 6807, Des Plaines, Ill.) with internal correction for quenching. In several experiments a second equilibration was done in the same tube after dilution of the aqueous phase by the addition of more buffer.

RESULTS AND DISCUSSION

Split Drop Method As an Index of Water Movement

Our previous studies of tubular fluid movement made by the split drop method indicated that there was not a one-to-one correspondence between such measurements at a tubular concentration of 100 mM NaCl and those made from the inulin concentration ratio (5). In view of this discrepancy it is important to show that the split drop method does indeed give a reliable estimate of the relation of water absorption to tubular NaCl concentration, which is a particularly distinctive feature of proximal tubular function. The results of 27 experiments performed by one of us (W.N.S.) using sequential photography in the summer of 1965 were recomputed to cast the data in a form comparable to the inulin experiments. In these photographic experiments, the measured lengths were translated into volumes using the measured tubular radius and applying the meniscus correction. Relative volumes (V/V_0) were plotted against time and the best straight line was drawn by eye. Four NaCl concentrations were used (100 mM [14 experiments]; 75 mM (three experiments); 62.5 mM [six experiments]; 50 mM [four experiments]) and the solutions were made isosmolar with mannitol. In order to make the comparison with the results of the inulin concentration experiments exact, V/V_0 at 20 min was determined from the graph, and water movement was computed from this figure. No correction was made for the delays occasionally observed in the onset of water movement, since such delays would have escaped detection by the inulin method. No correction was made for mannitol loss from the tubule, and the results have been compared with inulin data (2) before correction for mannitol loss. The data are shown in Fig. 1 and it will be seen that the NaCl concentration at which there is no salt-induced volume flow, the isonatric point, is close to 60 mM measured by either method.

Computation of Apparent Hydraulic Conductivity and Apparent Reflection Coefficient for Salt

The volume, V, of the aqueous fluid between the two concave menisci is given by

$$V = \pi r^2 l + 2\pi r^3/3 \tag{1}$$

in which the menisci are assumed hemispherical. l is the length between menisci; and r, the tubular radius. Since all the computations of fluid move-

ment with which we are concerned depend on rates of volume change, and since our observations indicate that the tubular radius remains constant during the measurement:

$$dV/dt = \pi r^2(dl/dt)$$ (2)

The volume flow, J_v, which is normalized to unit area is given by:

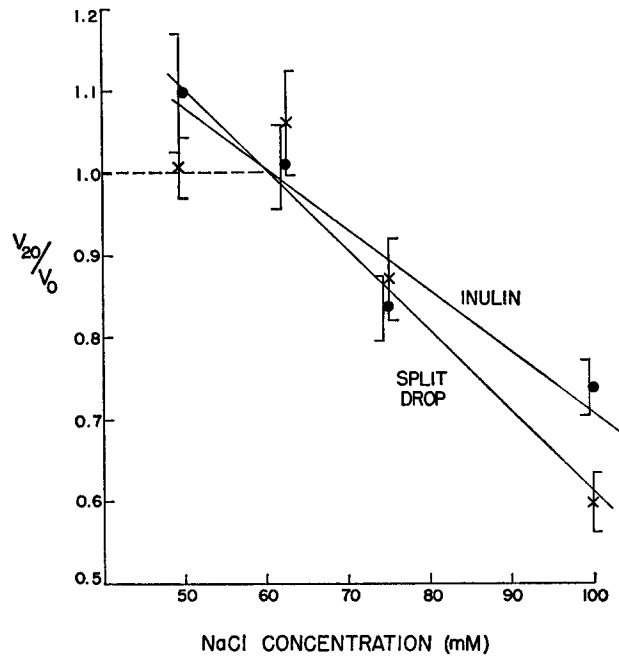$$J_v = \frac{1}{A}\frac{dV}{dt} = \frac{r}{2(l + 2r)}\frac{dl}{dt}$$ (3)

FIGURE 1. Comparison of water absorption at 20 min by inulin concentration ratio ($\times$) and photographic method (filled circles). Bars are SE.

At constant temperature and when there is no hydrostatic pressure difference, the volume flow induced by an osmotic pressure difference across a barrier permeant only to solute is given by:

$$J_v = L_{app}(\pi_{in} - \pi_{out})$$ (4)

in which $\pi_{in} = RT\sum_i \Phi_i c_i$ and $\pi_{out} = RT\sum_o \Phi_o c_o$, R and T have their usual meanings, Φ is the osmotic coefficient, and c is concentration. L_{app} is the apparent hydraulic permeability coefficient for the effective barrier between lumen and plasma. As will be shown below, this barrier does not behave as an ideal semipermeable membrane, but is permeable to ions and to a variety

of test molecules. Staverman (6) has pointed out that the osmotic pressure developed across a membrane that is partially permeable to the solute is less than theoretical. In the case of a single permeating species and a simple membrane the reflection coefficient, σ, is used to denote the ratio of the osmotic pressure actually developed by the solute to the ideal osmotic pressure, so that $\sigma = \pi^{obs}/\pi^{theor}$. An analogous situation exists across a complex barrier as in the present instance. In this case the reflection coefficient is not thermodynamically defined, and we will denote the *apparent* reflection coefficient by the symbol, s. Equation 4 then becomes:

$$J_v = L_{app}(\pi^{obs}_{in} - \pi^{obs}_{out})$$ (5)

in which $\pi^{obs}_{in} = RT \sum_i s_i \Phi_i c_i$ and $\pi^{obs}_{out} = RT \sum_o s_o \Phi_o c_o$

Thus the apparent reflection coefficient for each solute is explicitly introduced. Since NaCl will be shown to pass across the barrier, it is first necessary to determine s_s which is the mean apparent reflection coefficient for the plasma electrolytes, primarily NaCl. According to the method of Goldstein and Solomon (4) the zero time rate of volume flow can be used to obtain the reflection coefficient. This treatment was developed for a system in which the barrier was a single membrane, there was a single permeating solute, and the effect of the unstirred layer could be neglected. However, the method is also applicable to the determination of the apparent reflection coefficient of a solute in the lumen of the *Necturus* proximal tubule. The initial rate of volume flow is observed at a number of concentrations of the compound being studied. The initial rate of fluid movement, J_{v_0}, is obtained by extrapolation and is used because at that time the tubular concentration of the test solute is equal to its concentration in the perfusing solution, and no chemical analyses are required. A series of perfusing solutions are made up that are identical except for the concentration of the test solute which alone is varied. J_{v_0} is then plotted as a function of π_{in} and the concentration at which $J_{v_0} = 0$ is determined by interpolation. When $J_{v_0} = 0$, equation 5 leads to

$$\Sigma_i s_i \Phi_i c_i = \Sigma_o s_o \Phi_o c_o$$ (6)

An impermeant ($s_t = 1$) test molecule was used in the experiments to determine s_s. The following equation, applicable when $J_{v_0} = 0$, may be derived from equation 5 and the definition of π_{out}

$$s_s[(\pi_{out}/RT) - \Phi_{NaCl} c_{i\ NaCl}] = \Phi_t c_{it}$$ (7)

π_{out} is determined from the freezing point depression of plasma and the subscript t refers to the test molecule. Equation 7 provides the definition for s_s which is a mean apparent reflection coefficient that includes contributions from the other components in the plasma. Once s_s has been measured, equa-

tion 8 serves to determine s_t for any single permeant test substance. When a permeant test substance is added to the perfusing solution, and the point at which $J_{v_0} = 0$ has been found

$$s_t \Phi_t c_t = s_s[\pi_{out}/RT - \Phi_{NaCl} c_{i NaCl}]$$ (8)

in which c_t has been used to replace c_{it}. In practice J_v is plotted against π_{in} and J_{v_0} obtained by interpolation. The same graph may also be used to determine L_{app}. The only variable responsible for changes in π_{in} is the test solute concentration, so equation 5 may be differentiated as follows, if s_t is assumed constant independent of changes of π_{in}
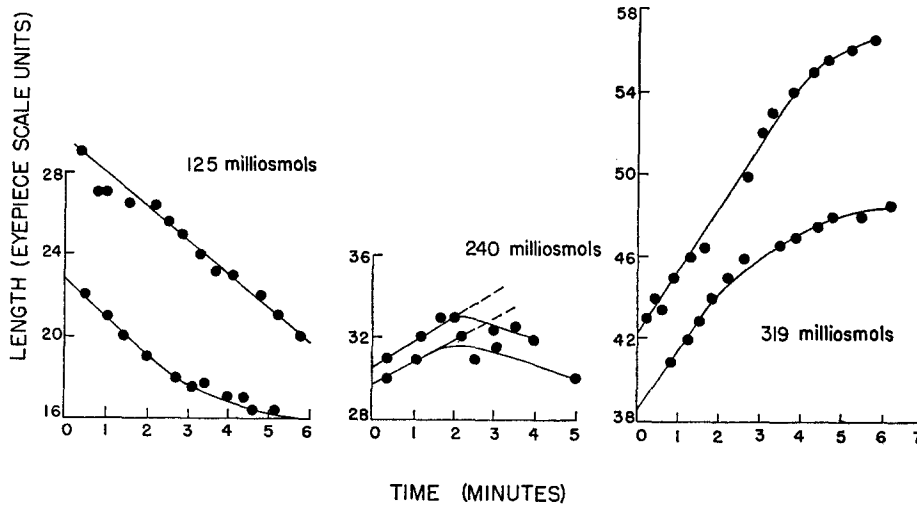

FIGURE 2. Time course of drop length (between menisci) in *Necturus* proximal tubule The experiments were done on two different tubules in the same animal, those at 125 and 240 milliosmols/kg on one tubule, and those at 319 milliosmols/kg on another tubule. Glycerol was the test substance. 32 length units = 1 mm.

$$\frac{dJ_{v_0}}{d\pi_{in}} = L_{app} \frac{d\pi_{in}^{obs}}{d\pi_{in}} = L_{app} s_t \frac{d\pi_{in}}{d\pi_{in}} = s_t L_{app}$$ (9)

so that L_{app} may be determined from the slope at the same time that s_t is determined from the interpolated point at which $J_{v_0} = 0$.

Fig. 2 illustrates the initial computation procedure in a characteristic experiment. All extrapolations were made by eye by one of us (M.D.) who did not perform the animal experiments, which was felt to reduce bias in this necessarily subjective procedure. Fortunately, as Fig. 2 shows, the slope remains linear over the first few minutes of the experiment and so problems in the exact determination of zero time are minimized. Fig. 3 shows the determination of s_t from the data obtained by this graphical procedure. The

straight lines in graphs like Fig. 3 have been drawn by eye, giving most weight to the points obtained from the most reliable determinations of J_{v_0} .

An alternative expression for L_{app} can be obtained by integrating equation 5 for an impermeant test solute. For this purpose it is desirable to express the left side in terms of dV/dt instead of J_v because a digital computer has been used to obtain the numerical solution and the equation has been fitted to the measured volume within the lumen rather than to volume flow. Equation 5 is
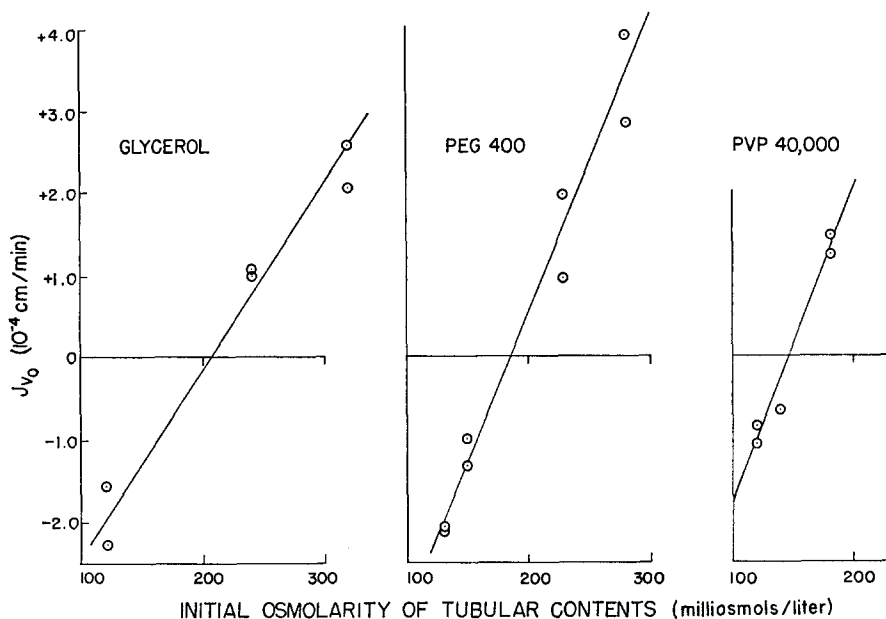

FIGURE 3. Relationship of J_{v_0} to π_{in} in *Necturus* proximal tubule for three different solutes. The glycerol data are taken from the experiment illustrated in Fig. 2. As discussed in the text, s_t may be determined from the condition $J_{v_0} = 0$ and L_{app} from the slope of the line.

equivalent to:

$$\frac{dV}{dt} = L_{app}\, RT \left(\frac{2V}{r} + \frac{8\pi r^2}{3}\right)\left[\frac{(s_s c_{s0} + c_{t0})V_o}{V} - s_s c_m\right] \qquad (10)$$

in which the subscript 0 refers to initial conditions. $s_s c_m = \pi_{out}^{obs}/RT$ and c_s is the luminal NaCl concentration. Since the test solute is impermeant, $s_t = 1$.

Customarily, experiments were initiated at the isonatric point, that is with the luminal concentration of NaCl at 60 mM, so that pump and leak balanced and there was no net movement of NaCl or water caused by the sodium pump. However, as water enters the tubule due to osmotic gradients, the system moves off the balance point because the tubular Na concentration decreases and the pump no longer balances the leak. Fortunately water flux induced by

the Na pump is relatively slow, compared to the osmotic flow, and can be neglected both with respect to water and Na movement.[1] Under these conditions the solution of equation 10 is

$$\ln\left[\frac{\gamma V^2 + \beta V + \alpha}{\gamma V_0^2 + \beta V_0 + \alpha}\right] - \frac{\beta}{\sqrt{q}}\ln\left[\frac{(2\gamma V + \beta - \sqrt{q})(2\gamma V_0 + \beta + \sqrt{q})}{(2\gamma V + \beta + \sqrt{q})(2\gamma V_0 + \beta - \sqrt{q})}\right] \quad (11)$$

$$= 4s_s c_m RTL_{app}t/r$$

in which

$$\alpha = \tfrac{4}{3}\pi r^3(s_s c_{s_0} + c_{t_0})V_0$$

$$\beta = [(s_s c_{s_0} + c_{t_0})V_0 - \tfrac{4}{3}\pi r^3 s_s c_m]$$

$$\gamma = -s_s c_m$$

$$q = \beta^2 - 4\alpha\gamma$$

The effect of the unstirred layer must be examined critically. As has been described, each introduction of a new solution into the tubular lumen was preceded by a 2–3 min pretreatment in which the tubule was washed out with the solution about to be introduced. Thus there should have been essentially no unstirred layer at $t = 0$. As soon as the experiment begins there is a flow of water which should make the concentration at the tubular wall somewhat different from the average for the entire drop. The thickness of this layer must have been minimal at zero time, so that the usual extrapolation to obtain J_{v_0} would minimize the unstirred layer. Furthermore, as Fig. 3 shows, the product $s_i L_{app}$ is independent of osmolarity, and the straight lines observed are entirely consonant with the absence of an effective unstirred layer. Finally, as Dainty (7) has already pointed out, the effect of the unstirred layer is of much less importance in studies of hydraulic conductivity than in measurements of the solute permeability coefficient.

Measurement of the Apparent Reflection Coefficient for Electrolytes and L_{app}

All the zero time data from experiments using test substances of molecular weight of 10^4 or greater were averaged to evaluate s_s. The results, given in Table I, show that $s_s = 0.69$.

Using this value of s_s, L_{app} was then determined according to equation 9, remembering that the permeant species is the salt. The mean value for L_{app}

[1] The suggestion has been made that the presence of PVP or dextran might shift the system away from the isonatric point. These two solutes are chemically dissimilar and the molecular weights range from 10×10^3 to $(60–90) \times 10^3$. Table I indicates that there is no systematic difference over this range either in L_{app} or σ. Furthermore, as stated above, the Na pump-induced water flux is small compared to the osmotic fluxes. Thus it appears that any effect of PVP or dextran on the Na pump would have little influence on the parameters that have been measured.

is 0.33×10^{-11} cm³/dyne sec. L_{app} has also been determined by fitting the data of the experiments in Table I to equation 11. As an example, the fit of the experimental points to the curve generated by the computer is shown in Fig. 4. The mean L_{app} is 0.26×10^{-11} cm³/dyne sec in satisfactory agreement with the figure obtained from J_{v_0}. When s_i has been obtained for all the permeant molecules, L_{app} may also be computed for each of these molecules by equation 9. As shown in Table II, L_{app} is 0.33×10^{-11} cm³/dyne sec for the polyethylene glycols (nine experiments) and 0.34×10^{-11} cm³/dyne sec for all the other solutes. The grand average value for all the J_{v_0} experiments is $0.33 \pm 0.02 \times 10^{-11}$ cm³/dyne sec.

When Whittembury, Oken, Windhager, and Solomon (1) measured the hydraulic conductivity of the *Necturus* proximal tubule they added mannitol

TABLE I

Substance	Molecular weight	Molecular radius*	No. of experiments	Apparent reflection coefficient for salt, s_g	Apparent hydraulic conductivity, L_{app}	
					Equation 9	Equation 8
		A			*cm³/dyne sec* $\times$ *10¹¹*	
Dextran	$(60-90)\times10^3$	700×100	2	0.70	0.31	0.29
PVP	40×10^3	60	4	0.75	0.35	0.29
PVP	10×10^3	29	3	0.60	0.29	0.20
Mean				0.69	0.33	0.26
SE					0.04	

* The molecular radii for dextran are the axes of equivalent hydrodynamic ellipsoids as calculated by interpolation from data of Ogston and Woods (8). The molecular radius for PVP is the root mean square unperturbed radius of gyration calculated from viscometric data (9).

to an isonatric solution to produce the osmotic gradient. In those experiments only a single experimental point could be taken so the time course of the fluid flow could not be measured. They obtained a mean value of 0.15×10^{-11} cm³/dyne sec and assigned an upper value of 0.3×10^{-11} cm³/dyne sec because of the uncertainty in the time course. They argued that the reflection coefficients for both NaCl and mannitol were nearly equal to one another, and that any large difference would have resulted in a detectable osmotic flow. As will be shown below, our present results indicate that $s_{mannitol} = 0.79$, reasonably close to $s_s = 0.69$. Consequently the major cause of the difference between the present figure and the earlier one is the uncertainty of the time course. In the *Necturus* distal tubule, Maude, Shehadeh, and Solomon (13) obtained a value of 0.041×10^{-11} cm³/dyne sec for L_{app} and pointed out that this was very much smaller than the permeability coefficient of the proximal tubule, a conclusion which the present study reinforces. The value of L_{app} for the proximal tubule is comparable to that in many other epithelia, being

0.4 × 10⁻¹¹ cm³/dyne sec for toad skin (14) and 0.3 × 10⁻¹² cm³/dyne sec for toad bladder (15). The apparent hydraulic conductivity of the rat proximal tubule is about 50 times greater than that for *Necturus*, being 18.1 × 10⁻¹¹ cm³/dyne sec as measured by Ullrich, Rumrich, and Fuchs (16).
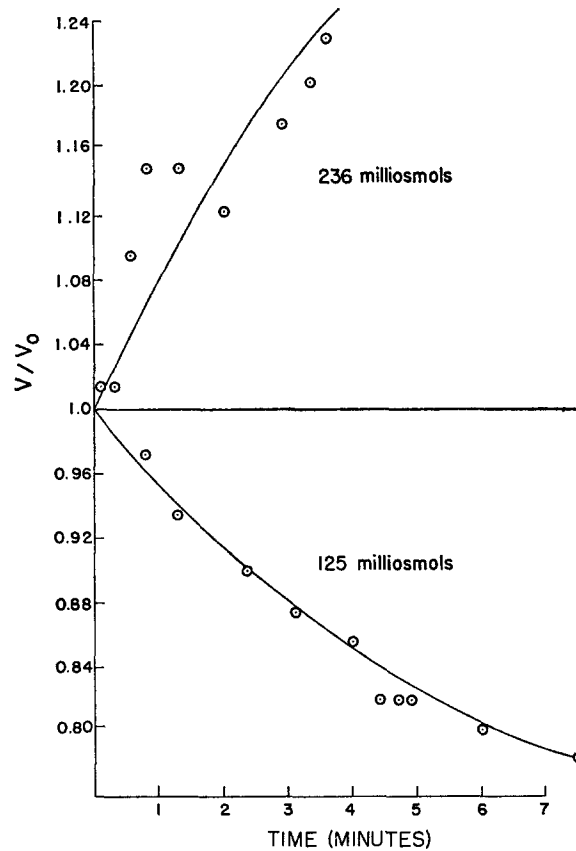

FIGURE 4. Time course of drop volume in an experiment with PVP 10,000 as the test solute. The points are the experimental data and the curves have been obtained by fitting equation 11 to the data by least squares using a digital computer. For the upper curve, L_{app} = 0.19 × 10⁻¹¹ cm³/dyne sec; for the lower curve L_{app} = 0.31 × 10⁻¹¹ cm³/dyne sec.

We had previously computed that the protein osmotic pressure was too small by a factor of 60 to provide the driving force for the measured water absorption from *Necturus* proximal tubule (1). This conclusion was confirmed experimentally, by showing that net water absorption continued even when the tubule contained albumin at an osmotic pressure greater than that of the plasma proteins. The present measurements do not alter the situation; the factor becomes about 30 rather than 60. Ullrich, Rumrich, and Fuchs (16)

reached a similar conclusion with respect to water absorption in rat proximal tubule.

Apparent Reflection Coefficient for Other Solutes

The top section of Table II gives values for the apparent reflection coefficient for a number of smaller molecules. We have not included an estimate of the standard error for these points because of the subjective nature of the measure-

TABLE II

APPARENT REFLECTION COEFFICIENTS
AND HYDRAULIC CONDUCTIVITY MEASURED
WITH SMALL MOLECULES

Substance	Molecular weight	Molecular radius*	No. of experiments	Apparent reflection coefficient	Apparent hydraulic conductivity
		A			$cm^3/dyne\ sec \times 10^{7}$
Glycerol	92	2.78	3	0.38	0.32
Diethylene glycol	106	2.73	3	0.47	0.23
Erythritol	122	2.89	4	0.50	0.28
Mannitol	182	3.15	3	0.79	0.40
Tetraethylene glycol	194	3.78	3	0.51	0.33
Raffinose	594	4.62	4	0.51	0.44
Mean					0.34
		Molecular radius‡			
Polyethylene glycol	400	6.1	2	0.56	0.32
Polyethylene glycol	1000	9.7	4	0.56	0.43
Polyethylene glycol	4000	19.4	1	0.52	0.32
Polyethylene glycol	6000	23.7	3	0.71	0.21
Mean					0.33
Grand mean					0.33
SE					0.02

* Half the diameter of a cylinder in average configuration (see Soll, 11) from Stuart and Briegleb models $\times$ 1.05 to simulate a viscometric value (cf. Schultz and Solomon, 12).
‡ Rms unperturbed radius of gyration (9) from viscometric data (10).

ments; a complete set made by one of us (C. J. B.) agreed within about 10% with the values given in Table II. Though this is somewhat greater than the standard error of the individual averages, we would consider 10% to be a reasonable estimate of the possible error. The molecular radii for these molecules have been obtained from Stuart and Briegleb models in average configurations. As Soll (11) has shown, the most important molecular parameter in equivalent pore radius studies is the diameter of the equivalent cylinder, which we have obtained by measurement of molecular models. Ullrich,

Rumrich, and Schmidt-Nielsen (17) have calculated a reflection coefficient of 0.69 for NaCl in rat proximal tubule, virtually equivalent to our values for *Necturus*. However, their reflection coefficient for erythritol is 0.89, very much higher than our figure of 0.50 for the apparent reflection coefficient of this compound.

It might be possible for the permeant molecules in the upper half of Table II to pass through the fabric of the membrane using channels not available for solvent, as would be the case for lipid-soluble molecules. Under these conditions there is no simple relation between the reflection coefficient and molecular dimensions of the solute as Dainty and Ginzburg have pointed out (18). The dimensionless coefficient, $\omega_t \bar{V}_t / L_{app}$, is a measure of the ratio of the apparent permeability coefficient, ω_t, for the test molecule and the apparent hydraulic permeability coefficient. $\bar{V}_t$ is the partial molal volume of the test solute. Although no explicit studies of ω have been made, it is possible to make a rough estimate from the measurement of mannitol leakage by Wind-hager et al. (2). These authors found that about 20% of the mannitol diffused out of the tubule in 20 min when J_v was close to zero. This corresponds to an $\omega_{mannitol}$ of 2.4×10^{-17} mol/dyne sec. Taking 120 cm³/mol (from the crystal density) for $\bar{V}_{mannitol}$, $\omega_t \bar{V}_t / L_{app}$ is 10^{-3} and may be neglected. Since the other solutes also have low lipid solubilities we have assumed that passage through the membrane fabric is unimportant for the molecules in the upper half of Table II.

The lower part of Table II gives the data for the polyethylene glycol polymers for which the molecular radius has been taken as the root mean square "unperturbed" radius of gyration calculated from viscometric data (9, 10). These radii are not directly comparable with the ones in the upper section of the table since they are obtained by a completely different method.

The behavior of the PEG polymers is unusual since the apparent reflection coefficient appears to be independent of chain length from an average molecular weight of 400 to one of 4000. Over this range, the unperturbed radius of gyration goes from 6.1 to 19.4 A. Even the polymer with a molecular weight of 6000 and a radius of 23.7 has a reflection coefficient of 0.71. Though these molecules are considered to behave in general as flexible spheres, the apparent reflection coefficients in the proximal tubule are essentially independent of molecular radius. Such a result would suggest either that the polyethylene glycols did not penetrate the barrier in a spherical form, or that the barrier is complex so that a simple dependence of the apparent reflection coefficient on molecular size would not be exhibited.

We therefore investigated the possibility that the polyethylene glycols could permeate the membrane in a mode other than as equivalent spheres through aqueous channels. No data were available for the partition coefficients of these molecules so experiments were carried out as described in the Methods sec-

tion. Table III shows the partition coefficients for polyethylene glycol 4000 against olive oil measured at three different concentrations in the aqueous layer. In similar experiments the apparent partition coefficient for inulin was about 2×10^{-4} but it was not possible to reequilibrate the inulin after dilution of the aqueous phase. Consequently the partition coefficient of inulin was compared with that for polyethylene glycol in isobutanol, in which the partition coefficient was 1.9×10^{-3} for inulin-^{14}C; 1.8×10^{-3} for inulin-^{3}H; and 1.2×10^{-3} for polyethylele glycol-^{14}C. Wartiovaara and Collander (19) have reviewed evidence which suggests that the partition coefficient in a two phase system, such as isobutanol and water, is related to the permeability of the cell membrane to the substance. We conclude that the aberrant apparent reflection coefficients for the polyethylene glycol polymers may not be attributed to membrane solubility.

TABLE III

PARTITION OF POLYETHYLENE GLYCOL 4000
BETWEEN OLIVE OIL AND BUFFER

Concentration in buffer	Partition coefficient olive oil/buffer
mg/ml	$\times 10^4$
4.5	0.61
2.4*	0.79
1.5	0.51

* By dilution of the aqueous phase after the equilibration at 4.5 mg/ml.

Since polyethylene glycol is a flexible linear polymer it seemed possible that it might pass through small circular apertures by unwinding and passing through in a more extended conformation than that assumed in the calculation of the unperturbed radius of gyration from viscometric data. Davies[2] has therefore compared the rates of dialysis from cellophane sacs of polyethylene glycol 6000 (radius of gyration 23.7 A) and recrystallized inulin, which is a fairly rigid rod-shaped polymer with dimensions about 12×100 A and molecular weight about 5600 (20). Since the escape of inulin from the cellophane bags was substantially greater than that of polyethylene glycol 6000, it appears that the low apparent reflection coefficient for the polyethylene glycols is probably not to be attributed to diffusion in an extended conformation through membrane channels. Soll (11) has shown that measurements by the method of Goldstein and Solomon are correlated much more closely with the diameter of the probing molecule than with the radius of an equivalent sphere, whereas in diffusion through a red cell membrane, the correlation is with the length of the equivalent cylinder. Thus the experiments of Davies do not entirely rule out the possibility that the polyethylene glycol

[2] Davies, M. Private communication.

polymers may flow in a nonspherical conformation when they cross a bio-
logical membrane under an osmotic pressure gradient. However, the generally
accepted conclusion among polymer chemists that these molecules behave
primarily as spheres makes such behavior unlikely.

This would lead to the conclusion that the barrier between tubular lumen
and plasma is unable to discriminate among molecules such as the polyeth-
ylene glycols solely on the basis of molecular radius. With the exception of
mannitol and glycerol, a similar conclusion may be drawn for the molecules
whose apparent reflection coefficients are given in the top half of Table II.
Diethylene glycol with a molecular radius of 2.73 A has an apparent reflec-
tion coefficient undistinguishable from that of raffinose with a molecular
radius of 4.62. In the case of the rat proximal tubule, Gertz (3) has also not
been able to discriminate among the permeability of sorbose (mol wt 180),
maltose (mol wt 360), and raffinose (mol wt 594). We would conclude, there-
fore, that the barrier is complex rather than simple, and that it has large
apertures leading from the lumen of the tubule. The zero time method for the
determination of the apparent reflection coefficient would seem to be sensitive
in the first instance to the porosity of the barrier closest to the lumen, and to
give virtually no information about the subsequent fate of the test substances.

It seems quite clear that the apertures, whatever their size, are too large to
maintain normal cellular composition. This leads us to conclude that the
luminal face of the tubular cells is not the barrier whose properties are being
measured in these experiments. We infer, therefore, that the paths for these
molecules of intermediate size are between the cells. Since the polyethylene
glycols diffuse out of cellophane bags much more slowly than inulin, it would
seem that the initial barrier is not tight enough to exclude inulin unless the
behavior of the polyethylene glycols is aberrant. These results, therefore, sug-
gest that the inulin barrier is located beyond the initial barrier. Nonetheless,
there is a barrier at the tubular surface, since the studies with the larger
molecules in Table I are consistent with a firm barrier for these molecules at
the luminal face.

These studies lead to the conclusion that the *Necturus* proximal tubule has
large apertures in the wall. Unless the subsequent barrier or barriers are un-
expectedly tight, NaCl and glucose could each leave the tubule by diffusion.
This view is consistent with the observation of Oken, Whittembury, Wind-
hager, and Solomon (21) that the apparent efflux of Na from the tubule is
accompanied by a large apparent Na influx, the ratio of the apparent fluxes
being 5/4. Windhager and colleagues (22, 23) have also shown that there is an
intercellular passive conductance channel in *Necturus* proximal tubules which
fits very well with the present findings.

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