Physical Properties of Isolated Perfused Renal Tubules and Tubular Basement Membranes

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ABSTRACT To study the physical properties of renal tubular basement membranes directly, the epithelial layer of single isolated perfused rabbit proximal convoluted, proximal straight, and cortical collecting tubules was removed with sodium desoxycholate. Tubular segments were perfused using micropipets. The distal end of each segment was occluded in order to simplify the measurement of transmembrane water flow. The relation between outer tubular diameter and applied transmural pressure was identical in intact tubules and their respective isolated tubular basement membranes indicating that the basement membrane determines tubular distensibility. Young's modulus for basement membranes from all tubular segments corresponded to that of tendon collagen. Membrane hydraulic conductivity was measured in two ways: (*a*) from the rate of transmural flow in response to an applied difference in hydrostatic pressure and, (*b*) from the rate of transmural flow in response to a difference in colloid osmotic pressure. The hydraulic conductivity of tubular basement membranes was 300–800 times greater than that of the intact epithelial layer. Basement membrane hydraulic conductance was similar to that of peritubular and glomerular capillaries in vivo. The hydrostatic conductance of tubular basement membranes exceeded the osmotic conductance by 3–10-fold owing largely to the fact that the membranes were moderately permeable to the osmotic solute (albumin). In view of these findings we suggest that oncotic and hydrostatic pressure may play an important role in the movement of tubular absorbate from the epithelial compartment into the renal interstitium.

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INTRODUCTION

In contrast to its counterpart in the renal glomerulus little is known about the structure and function of the tubular basement membrane. From numerous morphological studies of normal and injured kidneys it has been inferred that the tubular basement membrane is relatively tough and probably serves principally as a structural support upon which the tubular epithelial cells rest. Recently, peritubular physical factors, most notably hydrostatic pressure and oncotic pressure, have been convincingly implicated in the control of fluid absorption in cortical nephrons in vivo (1–3). Clearly, the tubular basement membrane is permeable to relatively small solutes and water, as the glomerular membrane, since all tubular reabsorbate crosses the barrier before uptake by the peritubular vasculature. Definitive information is lacking, however, with regard to the hydraulic conductivity and permeability to serum proteins of tubular basement membranes. In the present study we have determined several physical properties of tubular basement membranes using a direct approach. Evidence was obtained to indicate that basement membranes provide the principal structural support of renal tubules and are barriers across which oncotic as well as hydrostatic pressure may play an important role in the movement of tubular absorbate.

METHODS

Female New Zealand white rabbits weighing 2–3 kg were maintained on water and regular rabbit chow before the experiments. They were lightly anesthetized with i.v. pentobarbital, the left kidney was removed, and several thin saggital sections of renal cortex were obtained. We used only kidneys free of obvious disease. For dissection of individual renal tubular segments the cortical slices were immersed in either rabbit serum (Kam Laboratories, Inc.,

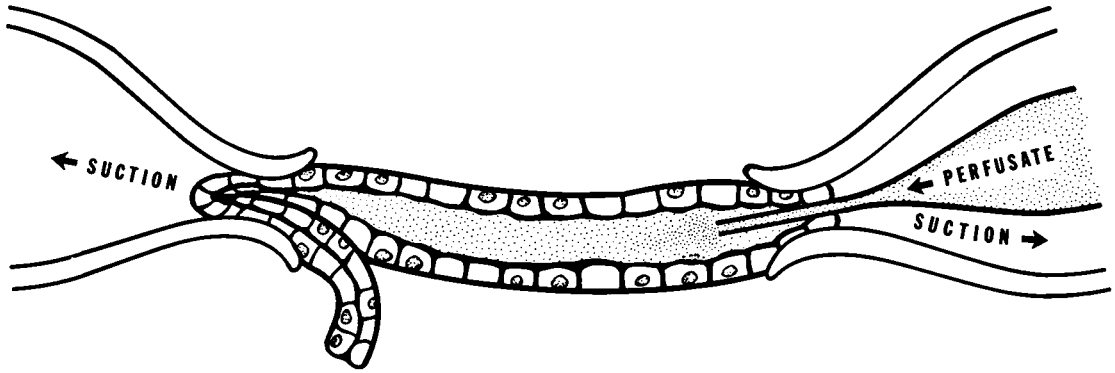

FIGURE 1 Arrangement for perfusing renal tubules.

Grandview, Mo.) (RS) [1] or in a standard medium (SM) containing NaCl, 115 mM; KCl, 5 mM; $NaHCO_3$, 25 mM; Na acetate, 10 mM; NaH_2PO_4, 1.2 mM; $MgSO_4$, 1.2 mM; CaCl, 1.0 mM; 5% calf serum (Kam Laboratories, Inc.); and dextrose, 5.5 mM. The commercial rabbit serum protein was 67% albumin and 33% globulin by electrophoretic analysis. The total protein concentration was 62 g/liter. All media were equilibrated with 95% O_2 and 5% CO_2 and the osmolality adjusted to 290±5 mOsm/kg. Dissection of nephron segments was performed under 10-90 × magnification using fine-tipped forceps with the media maintained at 4°C. Three anatomically distinct tubule portions were studied: the proximal convoluted (PCT), the proximal straight (PST), and cortical collecting tubules (CCT). Proximal convoluted tubules were obtained only from the midzone of the cortex. Proximal straight and collecting tubules were obtained from the mid- and juxta-medullary zones of the cortex. The length of individual tubule fragments ranged from 0.05 to 0.25 cm. We avoided crushing or stretching the tubules during dissection. Tubules with holes, an opulescent appearance, or swollen cells were rejected. Dissection time never exceeded 40 min. Single nephron fragments were transferred in fluid to a thermostatically controlled chamber containing 0.5 ml of SM and observed directly through an ultrathin coverslip in the floor of the chamber using an inverted microscope at 50 to 1,000 ×. Fluid in the chamber was oxygenated and vigorously stirred by bubbling with 95% O_2 and 5% CO_2 and was maintained at 28° or 37°C. A silicone antifoaming agent (Antifoam A Spray, Dow Corning Corp., Midland, Mich.) was lightly sprayed onto the surface of the bath. The specimens were photographed with a Polaroid or a 35 mm camera. In most experiments Polaroid photographs of the same portion of the tubule or isolated basement membrane were taken at 600 × to determine outer tubule diameter. By averaging the diameters measured at 10 equally spaced points on these photographs the tubule diameter could be determined repeatedly to within ± 0.3 μ. Tubule length was measured using a reticule in the microscope ocular. Because longitudinal stretching of the tubule decreased tubule diameter we measured diameter when the distance between the holding pipets was 10% less than the measured length of the tubule segment. In several experiments the tubule diameter at a specific point was measured using an Image Splitting Measuring Eyepiece (Vickers Instruments, Inc., Malden, Mass.). Average deviation using this instrument was ±0.3 μ.

The method of tubular perfusion is shown in Fig. 1. The perfusion pipet assembly is on the right. The tubules were secured by applying suction to the lumen of the outer pipet so that a concentric inner pipet, filled with perfusate, could be advanced 50–150 μ into the tubule lumen. After the initial delivery of perfusate had caused the tubule lumen to open, suction to the outer pipet was markedly increased in order to impact the tubule between the outer and perfusion pipets and prevent leakage retrograde at the perfused end. In all experiments the perfusate was identical to the SM except for the exclusion of calf serum and the addition of ^3H_2O (0.2 mCi/ml, International Chemical and Nuclear Corporation, Irvine, Calif.). The ^3H_2O was added in order to determine the rate of flow of perfusate out of the pipet.

All intact tubules were perfused at 28°C or 37°C by a gravity flow apparatus in which the perfusion pipet was connected by plastic tubing to a movable fluid reservoir. By positioning the reservoir above or below the level of the pipet tip hydrostatic pressures of −10 to +90 cm H_2O could be applied. Zero pressure was determined to within 0.1 cm H_2O before the tubule was attached. After impaction on the perfusion pipet, the tubule segment was perfused for several minutes at a pressure slightly greater than that necessary to maintain a patent lumen. The distal end of the tubule was then crimped and occluded by applying suction to a second pipet, as shown on the left in Fig. 1. Studies were begun after the occluded tubule had remained at low applied pressure for an additional 5–10 min. The appearance of a typical perfused intact proximal convoluted tubule is shown in Fig. 2A.

We perfused isolated tubular basement membranes by constant pressure gravity flow or with a constant flow pump. To obtain basement membranes we first attached the intact tubular segments to the perfusion pipet and perfused them in 28° or 37°C SM at an applied pressure of 10 cm H_2O or at a pump flow rate of approximately 35 nl/min. In constant pressure perfusion experiments we at-

[1] *Abbreviations used in this paper:* A, outer tubular surface area; CCT, cortical collecting tubule; D, outer tubule diameter; F, steady-state net flow; F_m, net transmembrane flow; F_p, net flow from the perfusion pipet; F_{p+m}, net flow with pipet and tubule in series; L, tubular length; L_p, hydraulic conductivity; P_s, hydrostatic pressure applied to perfusion pipet; PCT, proximal convoluted tubule; P_m, transmembrane hydrostatic pressure; PST, proximal straight tubule; R_m, transmembrane resistance; R_p, resistance of pipet; R_{p+m}, resistance of pipet and tubule in series; RS, rabbit serum; SM, standard medium.

tempted to study the intact tubule first and then the basement membrane of the same tubule. After obtaining data from the intact tubule we released the distal occlusion and allowed perfusate to flow through the length of the tubular lumen. Three procedures were used to remove the epithelial cells and prepare the isolated basement membrane

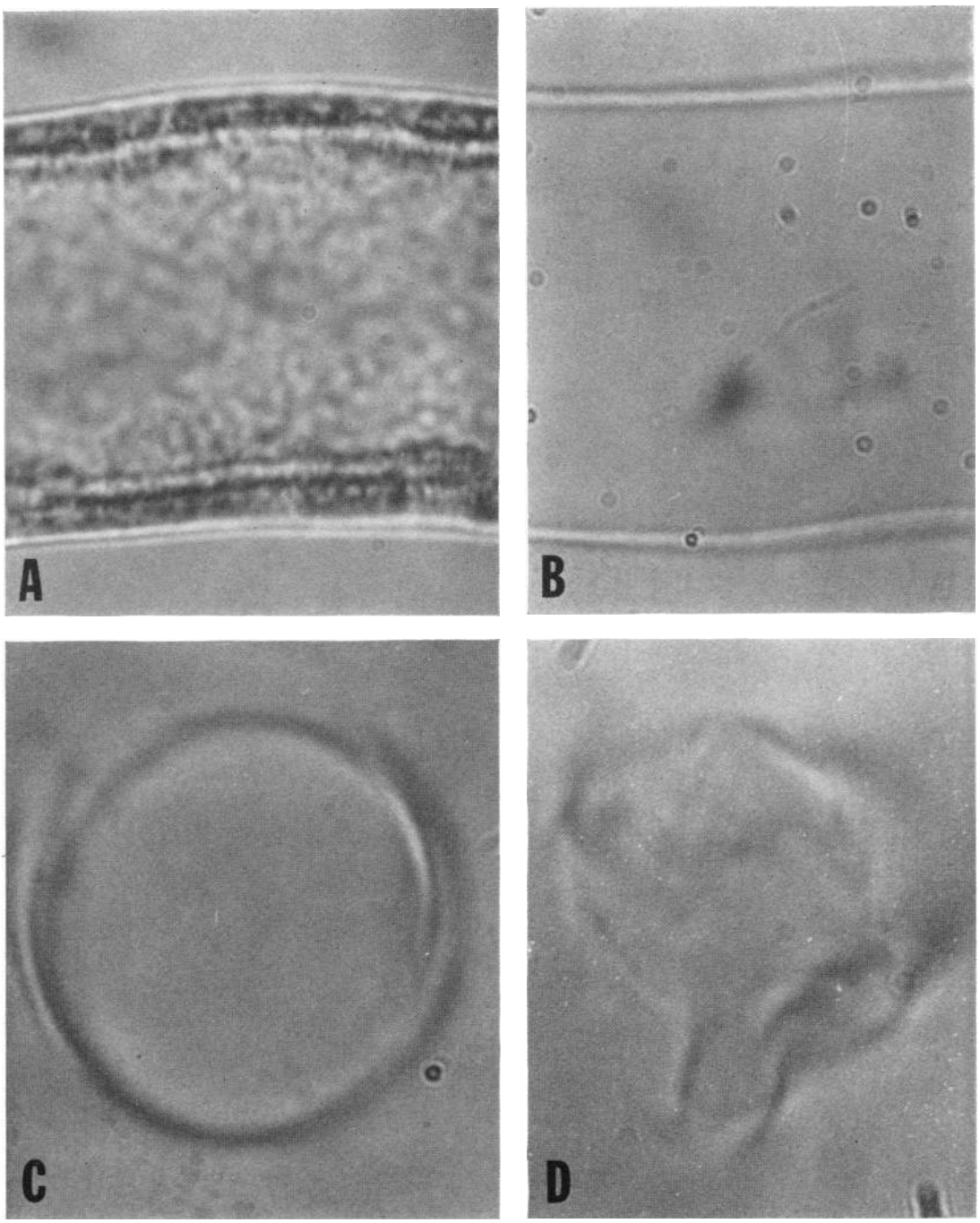

FIGURE 2 Appearance of intact tubules and isolated basement membranes. (A) Side view of an intact perfused PCT. Brush border is on the lumenal side of the cells. Outer diameter is 50 μ. (B) Side view of a perfused basement membrane from PCT. (C) End-on view of perfused basement membrane from PCT obtained by focusing on a vertically oriented loop of tubule. (D) End-on view of PCT basement membrane after collapse in 15% albumin bathing solution.

for study: (*a*) Sodium desoxycholate (1.5% in 0.15 M sodium chloride) was exchanged rapidly for the SM bath. The sodium desoxycholate caused immediate dissolution of the epithelial cells and flow of the amorphous debris out of the open distal end of the tubule. The basement membrane remained as a transparent tubular sheath as shown in Fig. 2B. In successful preparations there were no cellular remnants or other debris left in the tubule and the basement membrane was a continuous intact structure. (*b*) The non-ionic detergent Triton X-100 (Packard Instrument Company, Inc., Downers Grove, Ill.), 0.5% in 0.15 M sodium chloride, was exchanged rapidly for the SM bath. The cellular layer rapidly loosened from the basement membrane in most instances, leaving an intact basement membrane. Though the plasma membranes of epithelial cells appeared to be markedly disrupted by the Triton X-100 the cell nuclei remained relatively intact and could be identified in the debris flowing from the open end of the tubule. We had difficulty obtaining consistently clean basement membranes using the Triton X-100; however, in successful studies the resulting basement membranes appeared identical to those obtained with sodium desoxycholate. (*c*) When perfused cortical collecting tubules were incubated in distilled water the epithelial cells swelled markedly causing shearing of their basilar attachments to the basement membrane. By then increasing the perfusion pressure to 90 cm H_2O we were able to remove the entire epithelium from three cortical collecting tubules. The resulting basement membranes appeared identical to those prepared with desoxycholate or Triton X-100. Osmotic shock of proximal tubules did not yield a satisfactory basement membrane preparation.

After the tubule basement membrane had been prepared and all debris flushed from the lumen, the distal end was occluded in the crimping pipet and the SM bath replaced. Time from the addition of the desoxycholate or Triton X-100 and the final replacement of the SM was usually less than 2 min. The seal of the tubule around the perfusion pipet was tested by rapidly changing the pressure in the outer holding pipet and watching for abrupt changes in lumen diameter, indicative of leaks. Acceptable basement membranes had no obvious holes or leaks at either end when viewed at 1,000 × magnification.

In a few studies in which intact tubules were occluded at both ends before treatment with desoxycholate the addition of bile salt to the bath caused a marked increase in tubular volume. Although intratubular volume and transmembrane pressure were greatly increased, we observed no movement of cellular debris through the basement membrane, indicating that the basement membrane remained intact. In five studies, 1.3 μ polystyrene latex particles were included in the perfusate. These particles were easily seen as they entered the tubule lumen from the pipet but were never observed to pass through the basement membrane into the bath, or into either the proximal or distal holding pipets. In studies in which the external bath contained serum or albumin there was a dense shadow adjacent to the external surface of the basement membrane owing to the difference in refractive index of the absorbate as compared with the bath. We used this phenomenon to test for relatively large leaks in many of the basement membrane preparations. A few relatively large holes would be readily detected as scattered dense shadows adjacent to the membrane; only a diffuse shadow was seen in the studies reported here.

Basement membranes prepared using desoxycholate were imbedded in paraffin, cut, and stained with periodic acid Schiff reagent by standard methods. Isolated basement membranes were also fixed in glutaraldehyde and examined by electron microscopy.

The ^3H_2O in the perfusate was used as a volume marker to calibrate the perfusion pipet and to measure transmembrane flow of intact tubules or isolated basement membranes. From the amount of ^3H_2O (A*) appearing in the bath in a collection period (t) and the concentration of isotope in the perfusate (C*), the steady-state net flow (F) was calculated from the equation $F = A^*/C^*t$ (equation 1). With no tubular segment attached, F_p is the net flow from the pipet directly into the bath. With the intact tubule or basement membrane in place and distally occluded, F_{p+m} is net flow out of the pipet and through the tubule wall or basement membrane into the bath. Since the relatively large external bath was vigorously stirred, back diffusion of isotope from the bath into the pipet or tubule lumen could be neglected. At the completion of a collection period a total of 2.0 ml of SM was rinsed through the chamber and into a liquid scintillation vial. This removed more than 95% of the ^3H_2O from the bath chamber. 20 ml of scintillation solution containing 2,5-diphenoxyloxazole, 5.5 g/liter; 1,4-bis [2-(4-methyl-5-phenyloxazolyl)] benzene, 0.15 g/liter; Triton X-100, 330 ml/liter; and toluene, 670 ml/liter, were added to each vial. The radioactivity was counted in a three channel Nuclear-Chicago Model 6860 Liquid Scintillation Counter (Nuclear-Chicago, Des Plaines, Ill.).

Hydraulic conductivity was measured in two ways. In the first method, used for both intact tubules and isolated basement membranes, we determined the net transmembrane flow of fluid in response to hydrostatic pressure applied to the perfusion pipet, a measure of *hydrostatic* conductance. In the second method, used only for isolated basement membranes, we determined the net transmembrane fluid flow in response to a difference in transmural osmolality, a measure of *osmotic* conductance.

Hydrostatic conductance. When intact tubules or basement membranes are perfused at one end and the opposite end is occluded, the flow (F_m) of fluid from the perfusion pipet in response to an applied hydrostatic pressure is determined by the collective resistance of the perfusion pipet, tubular lumen, and tubular membrane. The lumen resistance can be calculated from Poiseuille's law, $R_\ell = 8\eta L/\pi r^4$, where r is lumenal radius, η is the viscosity of the perfusate, and L is tubular length. To evaluate the importance of R_ℓ we have chosen values for tubular dimensions from our experiments to give maximal estimates of R_ℓ, namely $r = 20 \times 10^{-4}$ cm, $L = 0.14$ cm, and $\eta = 8 \times 10^{-3}$ dyne sec/cm^2. R_ℓ is calculated to be maximally 3×10^3 cm $H_2O \cdot$min/cm^3 which is less than 1% of the average value for perfusion pipet resistance in these studies. Thus, lumenal resistance can be neglected. The resistances of the pipet, R_p, and the pipet and tubular segment in series, R_{p+m}, were determined separately by experiment. Transmembrane resistance, R_m, was derived from $R_m = R_{p+m} - R_p$ (equation 2). In the course of an experiment we first determined pipet resistance from P_s/F_p, where P_s is the difference in height between the fluid reservoir and the pipet tip and F_p is the resultant fluid flow. In some studies R_p was determined at several applied pressures to determine constancy of pipet resistance. When an intact tubule or basement membrane was attached to the pipet R_{p+m} was determined at several applied pressures from P_s/F_{p+m}, where F_{p+m} is the steady-state flow from the pipet across the tubular wall into the external bath. Membrane hydraulic conductance, then, is $1/R_m$ and transmural pressure can be calculated from $P_m = R_m/F_m$ at any transmembrane flow.

Osmotic conductance. The measurement of osmotic conductance differed significantly from that described for hydrostatic conductance. In these experiments a pump was connected to the perfusion pipet to deliver a constant rate of flow of perfusate into the lumen of the distally occluded tubule. Because of the low transmural conductivity of intact tubules and the inability of the pump to deliver flows less than 5 nl/min accurately, only isolated basement membranes could be studied by this technique. All studies were performed at 28°C. Since flow was held constant, the transmural hydrostatic pressure could be varied by imposing an osmotic force (π) across the basement membrane. Flow through the basement membrane, F_m, is in accordance with: $F_m = L_pA(P_m + \pi)$ (equation 3), where L_p is the hydraulic conductivity of the membrane. A is the surface area of the membrane, P_m is the transmural hydrostatic pressure difference, and π is the difference in transmural osmotic pressure. If F_m is held constant by perfusing the lumen with a pump and L_p and A are membrane constants, then an increase in π will cause P_m to decrease. When P_m is reduced to zero, $L_p = F_m/\pi A$. Operationally, we used a single concentration of osmotic solute and manually adjusted F_m to a point at which P_m was equal to or less than zero. We examined the osmotic effect of urea or dextrose, 10% in SM, and several concentrations of protein in SM. All solutions were equilibrated with 95% O_2 and 5% CO_2. For the protein solutions we used rabbit serum, bovine serum albumin (35% solution in saline, Sigma Chemical Co., St. Louis, Mo.) or bovine gamma globulin (dry powder, Schwarz/Mann Div., Becton-Dickinson & Co., Orangeburg, N. Y.). All stock solutions of albumin and globulin were dialyzed against 400 ml of the SM at 10°C for 18 hr and were found to give single peaks on electrophoretic analysis. The total protein content of the working solutions was adjusted with SM to a final concentration of 62, 100, or 150 g/liter using a refractometer.

We estimated the magnitude of osmotic conductance in two ways. The first method was used to quantify osmotic flow immediately after changing the bathing medium, before the solute had diffused into the tubular lumen appreciably. In the gravity flow perfusion experiments we observed that the lumen of a perfused tubular basement membrane collapsed when the applied hydrostatic pressure and transmural pressure was reduced to zero or negative values. Thus, when the tubule was pump perfused, lumenal collapse was used to indicate the point at which the tubular transmural pressure had been reduced to zero as a consequence of adding osmotically effective solute to the external bathing medium. To begin the study tubular basement membranes were bathed in SM at 28°C for 5 min and perfused at a flow rate unknown to the observer at the microscope. The SM was then changed to one of the test solutions. This caused turbulence about the perfused membrane, however, it was relatively easy to identify tubular collapse. The test solution was then replaced with the SM, the pump rate changed to a higher or lower value, and the sequence repeated. In this manner a flow rate was found above which the tubule did not collapse and below which collapse occurred. This flow rate, F_m, was used to calculate hydraulic conductivity from, $L_p = F_m/\Delta CA$ (equation 4) where ΔC is the molar concentration of the osmotic substance in the bath and A is the surface area of the membrane in SM. We assumed a molecular weight of 67,500 g/mole for bovine albumin and 150,000 g/mole for bovine gamma globulin. Calculated in this manner the dimensions of L_p are $cm^3/cm^2 \cdot min \cdot mole$ in contrast to the units of hydrostatically determined conductance, $cm^3/cm^2 \cdot min \cdot cm\ H_2O$. It is emphasized that in the osmotic studies we have not converted the chemical concentration gradient for albumin to units of osmotic pressure. This requires an accurate measure of oncotic pressure for each protein concentration used, an analytical method unavailable to us. Nor have we calculated the oncotic pressure of the different protein concentrations from empirical equations (4). Rather, to estimate the molar concentration we simply divided the protein concentration (grams per liter) by the molecular weight (grams per mole). Since the measured oncotic pressure of physiologic concentrations of albumin exceeds that calculated from $RT\Delta C$ by about 25% (5) to 50% (4, 6) our estimate of osmotic conductance is probably too high. This inaccuracy, however, does not invalidate the conclusions to be drawn from this study.

The second method estimated osmotic conductance several minutes after the osmotic solute had been added to the external bath. Individual basement membranes were perfused at three different calibrated flow rates using the pump. Bathing medium was infused and withdrawn continuously in a manner to cause turbulence about the membrane. Diameter was measured with the image-splitting device. At each of the three pump rates tubular diameter was measured initially in the SM, again after 5 min in 15% albumin in SM, and, finally, 5 min after the albumin had been removed. The presence of transmembrane osmotic flow was evaluated in individual tubules by determining the difference in tubular diameter in SM and 15% albumin at equal rates of transmembrane flow.

Estimation of basement membrane permeability to ^3H_2O and albumin-^{131}I. The purpose of these experiments was to determine to what extent ^3H_2O and albumin-^{131}I permeated the isolated PST basement membranes. A more conventional method of tubular perfusion was used (7). Perfusate containing ^3H_2O and albumin-^{131}I (Abbott Laboratories, North Chicago, Ill.) was infused into the tubule by gravity flow and the epithelial layer removed with sodium desoxycholate. To collect the lumenal perfusate the distal end of the tubular basement membrane was sucked into a collecting pipet as described previously (7). To determine the rate of fluid accumulation we collected the distal fluid under oil with a calibrated capillary pipet. The collected distal fluid and the external bath were quantitatively removed in two or three consecutive periods at intervals of 2–10 min. The ^3H_2O and ^{131}I content of the initial perfusate, the collected distal fluid and the external bath was determined by scintillation spectrometry using appropriate internal standards. Since both isotopes appeared in the bath and collected distal fluid, the perfusion rate was calculated by dividing the total amount of either isotope recovered by the isotope concentration of the initial perfusate. Equivalent results for perfusion rate were obtained with ^{131}I and ^3H_2O. This method was not satisfactory for measuring osmotic conductance in the manner described by Kokko, Burg, and Orloff (8) owing to the fact that transmembrane hydrostatic flow was invariably too high to permit reliable detection of relatively small increases in steady-state flow arising from osmosis.

Tubular distensibility. We determined the relationship between transmural pressure and outer tubule diameter (D) in intact tubules and in isolated basement membranes. For intact tubules we assumed that the applied and transmembrane pressures were equal since the low rate of fluid absorption resulted in an insignificant drop in pressure across the perfusion pipet tip. In basement membranes transmembrane pressure was calculated at each applied pressure. Diameter and length were measured 5 min after a change

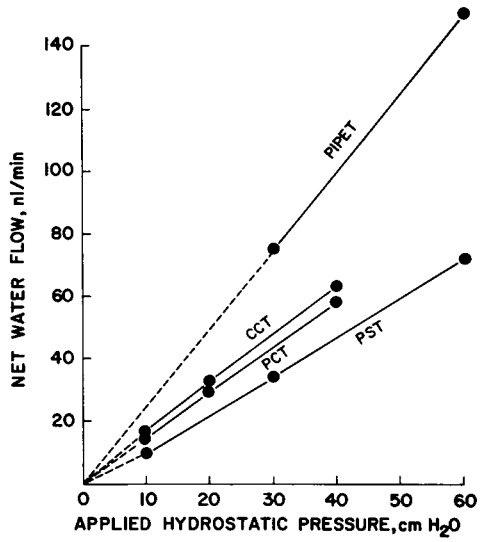

FIGURE 3 Relation between applied pressure and net water flow. The uppermost line labeled pipet depicts the relation between pressure and flow of 26 naked perfusion pipets selected at random. The lower lines were derived from the studies listed in Tables I, II, and III in which tubular basement membranes were attached to the perfusion pipets.

in pressure, and the pressure was always changed from lower to higher values.

RESULTS

Pipet conductance. Perfusion pipet conductance was determined at the beginning of every experiment. In the absence of an attached tubule the pipet conductance is equal to F/P_a, where F is net flow and P_a is the hydrostatic pressure applied to the pipet. In Fig. 3 the average flow from 26 pipets selected at random was determined at applied hydrostatic pressures of 30 and 60 cm H_2O. The extrapolated line intercepts at zero and the relationship between flow and pressure is linear. In 21 paired studies pipet conductances determined before tubules were attached and again after the tubule had been removed differed by less than 1%.

Hydrostatic conductance of intact tubules. Isolated intact tubules absorb salt and water by active transport at the 28°C temperature employed in these experiments. We assumed that the rate of active transport was unaffected by changes in tubular geometry (9). Hydraulic conductivity was calculated from the change in transmembrane flow occurring in response to a change in applied hydrostatic pressure. Tubular area was measured at an applied pressure of 10 cm H_2O. For 13 proximal straight, 9 proximal convoluted, and 8 cortical collecting tubules, L_p was 9±6, 7±2, and 8±5 × 10^{-6} $cm^3/cm^2 \cdot min \cdot cm\ H_2O$, respectively (Table IV). There was considerable variability in the results obtained using this method owing to the exceedingly low transmural conductances, and only the value for proximal convoluted tubules differed significantly from zero ($P < 0.05$). Nevertheless, the present values for all tubular segments may be considered maximal estimates and are adequate for comparing the relative conductances of intact tubules and their isolated basement membranes.

Hydrostatic conductance of basement membranes. We used the hydrostatic pressure method to determine the relationship between applied pressure and transmembrane water flow in 15 proximal straight, 6 proximal convo-

TABLE I
Proximal Straight Tubular Basement Membranes

Experiment	P_a	F_p	F_{p+m}	P_m	D	L
	cm H_2O	*nl/min*		*cm H_2O*	*μ*	*μ*
19	10	38	13	6.5	50.6	505
	30	110	46	17.5	53.3	555
	60		109	30.3	55.4	606
22	10	20	16	2.1	43.8	970
	30	59	48	6.2	45.0	1010
	60	121	100	10.4	48.1	1040
24	10	19	12	3.2	46.1	566
	30		40	7.4	47.8	626
	60	106	82	13.6	49.1	667
25	10	19	13	2.6	41.8	465
	30		41	6.8	44.7	465
	60	106	84	12.5	45.7	465
27	10	25	10	6.0	46.1	1131
	30	75	39	14.4	49.6	1172
	60	150	86	25.6	51.5	1273
30	10	11	8	1.7	45.5	1030
	30		22	7.2	48.8	1071
	60	58	46	12.4	51.0	1111
32	10		9	1.6	54.4	889
	30	32	27	4.7	56.4	929
	60	64	55	8.4	58.3	990
34	10		8	4.1	48.1	869
	30	40	25	11.5	51.3	909
	60	81	53	20.7	52.6	949
35	10		3	7.8	42.7	990
	30	40	15	18.9	45.1	1010
	60	81	32	36.3	46.4	1050
36	10		4	4.5	44.5	889
	30		13	12.3	46.7	929
	60	44	27	23.2	48.2	949
37	10		5	3.2	41.0	1030
	30		15	9.5	46.0	1071
	60	44	29	20.5	47.6	1091
38	10		11	4.7	46.8	1232
	30		38	11.8	51.1	1293
	60	125	83	20.2	53.0	1353
39	10		1	9.7	48.8	343
	30		23	23.9	55.0	384
	60	225	67	42.1	57.3	434
40	10		4	8.6	48.6	525
	30		22	22.4	51.7	556
	60	174	62	38.6	53.8	606
42	10		28	5.7	60.5	990
	30		83	17.3	63.8	1030
	60	392	159	35.7	63.5	1091

TABLE II

Proximal Convoluted Tubular Basement Membranes

Experiment	P_a	F_p*	F_{p+m}	P_m	D	L
	cm H_2O	*nl/min*		*cm H_2O*	*μ*	*μ*
82	10		12	2.9	55.4	465
	20	(101)	24	5.8	56.5	485
	40		49	11.1	58.4	505
84	10		14	4.8	52.8	470
	20	(162)	30	8.9	53.5	500
	40		58	18.5	54.6	540
85	10		19	1.6	64.6	550
	20	(137)	35	4.6	66.2	580
	40		66	11.0	68.1	600
87	10		17	3.9	59.7	440
	20	(167)	36	7.0	62.3	450
	40		71	14.4	63.0	490
88	10		14	5.0	62.7	330
	20	(167)	25	11.0	65.6	370
	40		53	20.9	69.8	380
91	10		10	4.3	50.8	440
	20	(106)	24	6.3	53.1	450
	40		50	11.5	52.6	450

* All flows determined at 60 cm H_2O applied pressure.

TABLE III

Cortical Collecting Tubular Basement Membranes

Experiment	P_a	F_p*	F_{p+m}	P_m	D	L
	cm H_2O	*nl/min*		*cm H_2O*	*μ*	*μ*
96	10		16	3.8	44.9	460
	20	(154)	31	7.9	45.4	490
	40		60	16.6	46.2	480
99	10		16	2.5	42.7	840
	20	(127)	31	5.4	43.7	830
	40		65	9.4	44.7	700
100	10		21	3.1	43.2	720
	20	(184)	43	5.8	43.4	740
	40		83	12.6	44.2	750
102	10		30	2.3	46.5	920
	20	(234)	57	5.4	48.2	940
	40		103	13.6	49.1	940
103	10		8	1.8	38.3	530
	20	(59)	14	5.7	38.8	560
	40		28	11.4	40.0	560
104	10		12	2.2	45.3	690
	20	(93)	24	4.4	46.1	710
	40		50	7.5	47.4	750
105	10		12	2.2	46.6	750
	20	(93)	25	3.7	47.7	770
	40		51	6.8	49.3	780

* All flows determined at 60 cm H_2O applied pressure.

luted, and 7 cortical collecting tubule basement membranes prepared with desoxycholate (Tables I, II, and III). The conductance of each tubular segment remained constant throughout the range of applied pressures as illustrated by the relatively straight lines in Fig. 3. When expressed in units of membrane area, however, the calculated hydraulic conductivity tended to decrease at higher pressures owing to the nonlinear relationship between transtubular pressure and surface area (vide infra). Since the physiologic transtubular hydrostatic pressure is probably in the neighborhood of 5 mm Hg (10) we calculated the L_p of basement membranes at an arbitrarily selected applied pressure of 10 cm H_2O, as summarized in Table IV.

The mean L_p's of four PST basement membranes prepared with Triton X-100 and of 3 CCT membranes prepared by "osmotic shock" were not different from the respective membranes prepared with desoxycholate (Table IV). In three PST basement membranes incubation in desoxycholate for 25 min had no effect on L_p. Increasing the temperature from 28°C to 37°C had no effect on the L_p of PST basement membranes prepared with desoxycholate in six paired studies (mean change 0.02±0.38 SEM).

Osmotic conductance of basement membranes. Osmotic conductance at 28°C was estimated in pump perfused desoxycholate prepared basement membranes using two methods.

In the first method we estimated osmotic conductance coincident with the addition of solute to the external bath. In this manner dissipation of the osmotic gradient owing to diffusion of solute into the tubular lumen should be minimized. Membranes 0.05–0.15 cm in length at an

TABLE IV

Hydraulic Conductance of Intact Tubules and Basement Membranes Using Applied Hydrostatic Pressure Method

	PST	PCT	CCT
	$cm^3/cm^2 \cdot min \cdot cm\ H_2O \times 10^3$		
Intact tubules	0.009±0.006 (13)	0.007±0.002 (9)	0.008±0.005 (8)
Basement membranes			
Desoxycholate	2.5 ±0.6 (15)	5.3 ±1.0 (6)	6.6 ±0.5 (7)
Triton X-100	3.0 ±0.7 (4)		
Osmotic shock			6.0 ±0.7 (3)

Mean values ±SEM with number of experiments in parenthesis.

TABLE V

Osmotic Conductance of Basement Membranes

	Experiment	F_m*	A	$C_{albumin}$	L_p
		nl/min	*cm² × 10⁻⁴*	*g/liter*	*cm³/cm²·min·mole*
PST	68	20	8.6	98	16
	69	83	17.0	98	34
	70	78	16.0	100	33
	71	68	12.7	100	36
	72	24	14.5	98	12
	73	31	9.6	98	23
	74	16	8.2	99	13
	75	37	12.4	99	20
	Mean				23±3
PCT	82	7	6.9	87	8
	85	24	10.1	87	18
	86	13	9.9	85	13
	88	9	6.9	91	10
	91	18	7.5	87	18
	92	22	8.3	87	20
	93	23	10.1	87	17
	94	13	5.4	87	19
	95	23	10.5	87	17
	Mean				16±1
CCT	96	22	8.2	100	18
	100	39	9.9	100	27
	102	27	12.8	100	14
	103	18	6.6	100	18
	104	12	9.5	100	9
	106	10	5.2	100	13
	107	20	8.0	100	17
	108	13	5.8	100	15
	109	24	8.1	100	20
	Mean				17±2

* F_m is the transmembrane water flow at the end point. A was determined at F_m in the absence of albumin.

initial pump rate of 5 nl/min were clearly tubular shaped, as shown in Figs. 2B and 2C, when SM was the bathing medium. The SM bath was then exchanged rapidly for SM solutions containing urea, dextrose, bovine albumin, or bovine globulin in final concentrations ranging up to 15%. In five studies we never detected an effect of urea or dextrose on tubule diameter, even when the perfusion rate was reduced further to approximately 1 nl/min. In contrast, solutions containing albumin or globulin always caused instantaneous collapse of the entire tubular segment (Fig. 2D). In most instances when the perfusion rate was 10 nl/min or less the lumen remained totally collapsed for 3–5 sec after the addition of the protein, and then gradually reopened. To calculate L_p according to equation 4 we chose as our end point (F_m) the pump rate above which the tubular lumen did not collapse, and below which collapse occurred. This end point could be determined repeatedly to within a few nanoliters per minute. Average values for osmotic conductance in eight proximal straight, nine proximal convoluted, and nine cortical collecting tubule basement membranes were 23±3, 16±1, and 17±2 cm³/cm²·min·mole, respectively (Table V). To compare the osmotic conductance with the hydrostatic conductance of basement membranes we converted the mean values in Table IV to equivalent units of driving force. In these terms the hydrostatic conductances of proximal straight, proximal convoluted, and cortical collecting tubules are 64±14, 137±24, and 169±14 cm³/cm²·min·mole, respectively.

To estimate the relative osmotic effectiveness of the dominant serum proteins we bathed other PST basement membranes in rabbit serum, albumin, and globulin solutions, each having a total protein concentration of 62 g/liter. As shown in Table VI more fluid was osmotically abstracted by albumin than by an equivalent amount of globulin. If, however, we compare the ratio of changes in net flow due to albumin and globulin (18/8.7 = 2.1) with the ratio of the approximate molecular weights of the respective proteins (150,000/67,500 = 2.2), it is clear that globulin was as osmotically effective as albumin. That whole serum was only slightly less effective osmotically than pure albumin is consistent with the fact that rabbit serum protein is two-thirds albumin.

Three observations point to the fact that isolated basement membranes are permeable to albumin. First, when a perfused segment was rapidly bathed in albumin solution the immediate total collapse was only transient. Secondly, if perfusate flow was totally stopped and then albumin added to the bath, the lumen collapsed markedly and remained collapsed. Upon rinsing the tubule with SM the lumen of the nonperfused segment opened rapidly, indicating the presence of osmotic material, presumably albumin, within the tubular lumen. Third, when tracer amounts of ^{131}I-labeled albumin were perfused into the lumens of tubular basement membranes we detected significant amounts of tracer in the external bath indicating a finite permeability to albumin (vide infra).

Since the basement membrane is permeable to albumin it is important to determine if the protein is osmotically effective in the steady state when the lumenal compartment is continuously perfused with a protein-free solution. We used a second indirect approach to test for osmotic conductance in the steady state after addition of

TABLE VI

Relative Osmotic Effectiveness of Serum Proteins in Proximal Straight Tubular Basement Membranes

Experiment	I Albumin, 6.2%	II Globulin, 6.2%	III Serum protein, 6.2%
		F, nl/min	
124	22	9	20
125	15	9	12
126	21	8	19
127	14	9	14
Mean	18.0	8.7	16.3

albumin to the external bath. Proximal straight tubular basement membranes prepared with desoxycholate were perfused at approximately 10, 35, and 70 nl/min and the tubular diameter was measured in SM and after 5 min of incubation in 15% albumin solution. The relationship between flow and diameter of four membranes in the different bathing media is summarized in Fig. 4. At each perfusion rate the outer tubular diameter was always less when albumin was in the external bath. Since tubular diameter is a direct reflection of transmural hydrostatic pressure (vide infra) we conclude that the reduction in diameter in the albumin medium is a reflection of the osmotic pressure of the protein exerted across the tubular wall. Since the difference in flow between the albumin solution and SM at equivalent tubular diameter is due to osmosis, the osmotic conductance can be estimated simply from $L_p = F_2\text{-}F_1/\Delta CA$, where F_2 is the flow in albumin and F_1 the flow in SM. L_p calculated in this manner at a tubular diameter of 47 μ (Fig. 4) was 13 $cm^3/cm^2 \cdot min \cdot mole$, a value about one-half of that determined for PST basement membranes using the non-steady-state method. Presumably, steady-state L_p would approach the non-steady-state value as the perfusion rate was increased further and the lumenal concentration of albumin was decreased. These considerations support the view that significant amounts of fluid can be abstracted osmotically in the steady state provided that the lumenal compartment is continuously perfused with protein-free solution.

In Table VII are the results of three studies of PST basement membranes in which we estimated the relative transmembrane permeability to ^3H_2O and albumin-^{131}I. In these continuous perfusion studies a significant volume of perfusate entered the external bath as a consequence of the transmembrane hydrostatic pressure difference. In each study the concentration of ^3H_2O in the collected distal fluid was much less than that of the initial perfusate indicating a high diffusional permeability to water. In contrast, the concentration of albumin-^{131}I in the distal fluid exceeded that of the initial perfusate indicating restricted transmembrane albumin

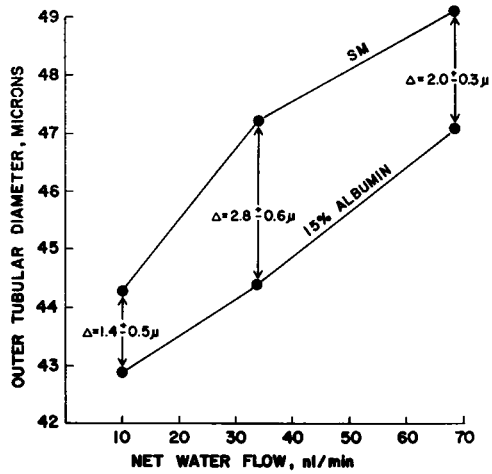

FIGURE 4 Evidence for steady-state osmotic water flow in PST basement membranes. Transmural net water flow, on the abscissa, was adjusted using a pump. The vertical lines depict the difference in outer tubular diameter (mean differences ±SEM) in the standard medium and medium containing 15% albumin.

movement. From the data in Table VII we have estimated the minimum value for the fraction of the filtrate entering the bath through channels sufficiently small to retard the movement of albumin, from: $(V_t[C_t/C_0 - 1]/V_0 - V_t) \times 100$ (equation 5). Viewed in this context it is estimated that in these studies minimally 22–44% of the filtrate entered the bath through channels sufficiently small to retard the movement of albumin.

Distensibility of intact tubules and basement membranes. We determined the relationship between transmural pressure and the outer diameter of intact tubules and basement membranes. At zero applied pressure the epithelial cell surfaces of intact tubules were closely apposed and no lumen was visible at 600 × magnification. As the applied pressure was increased in small increments the lumen remained collapsed and the outer tubular diameter relatively constant until a pressure of approximately 5 cm H_2O (range 4–7 cm H_2O) was reached. At this point the lumen opened and the diameter

TABLE VII

Recovery of ^3H_2O and Albumin-^{131}I in Collected Perfusate of PST Basement Membranes

Experiment	L	P_a	V_0	V_t	V_0/V_t	Tritiated water			Albumin-^{131}I		
						C_0	C_t	C_t/C_0	C_0	C_t	C_t/C_0
	cm	*cm H_2O*	*nl/min*			*dpm/nl*			*dpm/nl*		
52	0.15	30	99	35	2.8	535	4	0.01	22.4	31.5	1.4
54	0.09	10	49	8	6.1	413	15	0.04	8.9	22.5	2.5
56	0.15	20	273	197	1.4	397	75	0.19	6.5	7.6	1.2

L, tubular length; P_a, applied pressure; V_0 and V_t, volume flow rate from perfusion pipet and rate of distal collection, respectively; C_0 and C_t, concentration of isotope in perfusate and collected fluid, respectively.

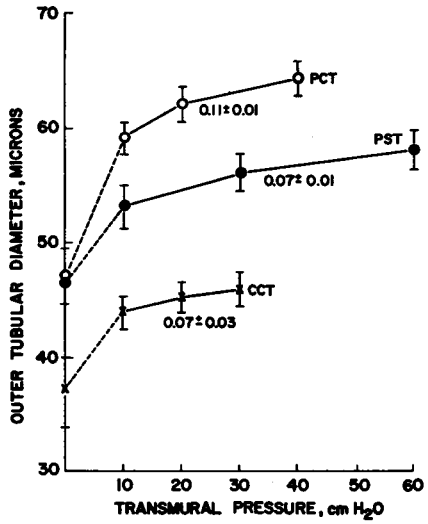

FIGURE 5 Relation between transmural pressure and outer diameter of perfused nephrons. Slopes (μ/cm H_2O, mean ±SEM) are indicated in the highest range of pressure for each segment.

increased abruptly. Because of the tendency for intact tubules to detach from the perfusion pipet at high pressure we did not extend the pressure above 30 cm H_2O for cortical collecting, 40 cm H_2O for proximal convoluted, and 60 cm H_2O for proximal straight intact tubules.

An opening phenomenon was not observed in perfused basement membranes. At zero applied pressure the lumens of basement membranes were collapsed and the walls were irregular in transverse outline and longitudinally wrinkled. At pressures slightly above zero, however, all basement membranes were tubular in shape.

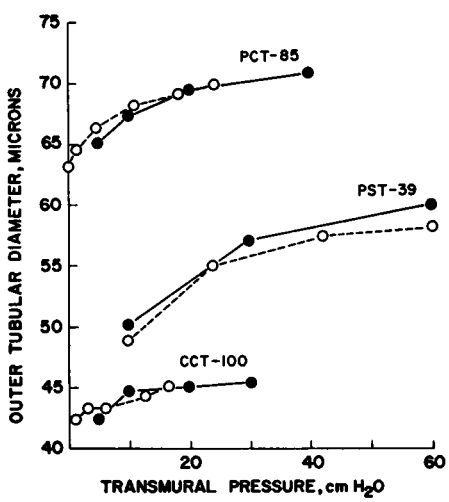

FIGURE 6 Relation between pressure and outer diameter of intact tubules and their isolated basement membranes. Basement membranes are depicted by the open circles. Representative studies are shown from three nephron segments listed in Tables I, II, and III.

The relationship between pressure and diameter in intact tubules is summarized in Fig. 5. Above the opening pressure the slopes of the pressure-diameter relationship could be determined accurately. Included in Fig. 5 are the slopes of seven proximal straight, eight proximal convoluted, and five cortical collecting intact tubules in the maximal ranges of applied pressure. From the progressive decrease in slope as pressure was increased it is inferred that the tubule wall becomes increasingly stiff as it is stretched. In several experiments at constant high pressure the tubule diameter remained constant for periods as long as 180 min indicating the absence of viscoelasticity.

We compared the distensibility of intact tubules and their isolated perfused basement membranes in eight proximal straight, six proximal convoluted, and six cortical collecting tubules. Representative studies are shown in Fig. 6. Above 10 cm H_2O transmural pressure the relationship between transmural pressure and diameter of basement membranes was similar to that of the intact tubules indicating that the relation between pressure and diameter in intact tubules reflects the elastic properties of the basement membrane, primarily.

Young's modulus (E) for tubular basement membranes was calculated using an equation derived for thin wall tubules reported by Fung, Zweifach, and Intaglietta (11): $E = (R/h)\ (R)\ (dP_m/dR)$ (equation 6), where R is the mean radius of the tubule in the range of applied pressure, h is the thickness of the wall, and dP_m/dR is the derivative of transmural pressure with respect to radius. Our data from intact tubules are more complete than from the studies of isolated basement membranes in the range of maximal wall stiffness, thus, dP_m/dR was computed for each of the intact tubules used to construct Fig. 5. Since the epithelium of the intact tubules does not contribute significantly to tubular distensibility in the range of maximal stretching, h is the thickness of the basement membrane. Basement membrane thickness is approximately 0.26 μ in intact perfused rabbit proximal (12) and collecting tubules (13). Derived in this manner, Young's modulus for basement membrane of proximal straight, proximal convoluted, and cortical collecting tubules is 1.0±0.1, 0.8±0.2, and 0.7±0.1 × 10^8 dyne/cm^2, respectively.

DISCUSSION

To obtain direct information about the physical properties of the basement membrane, we removed the cellular layer from intact nephron segments using two classes of detergents known to lyse plasma membrane, and by mechanically loosening the cells by osmotic shock. Sodium desoxycholate consistently yielded tubular basement

membrane fragments entirely free of adherent cellular debris and, therefore, was used as the standard preparatory agent in these studies. Several observations support the view that desoxycholate does not appreciably alter the physical properties of basement membrane: (*a*) When the cells were removed with a non-ionic detergent, Triton X-100, the hydraulic conductivity of the resultant basement membrane did not differ significantly from the conductance determined in other proximal tubule membranes prepared with desoxycholate. (*b*) We extruded cells from collecting tubules by osmotic disruption in distilled water and the L_p of the resulting membranes did not differ from those prepared in desoxycholate. (*c*) Prolonged incubation of basement membranes in desoxycholate had no effect on L_p. (*d*) Sodium desoxycholate did not significantly alter the mechanical properties of the basilar lamina since the response of tubular diameter to changes in transmembrane pressure in intact tubules and their isolated tubular basement membrane was virtually identical. (*e*) Basement membranes isolated in sodium desoxycholate appeared morphologically indistinguishable from untreated membranes when examined by electron microscopy and stained positively with the periodic acid Schiff reagent indicating preservation of the carbohydrate moities in the membrane.

Our studies indicate that the tubular basement membrane is a relatively tough, elastic structure. On distending the lumen of an intact tubule the outer diameter increased steeply as pressure was raised from 0 to 10 cm H_2O. Above 10 cm H_2O there was a marked reduction in the increase of diameter as pressure was elevated further in all tubular segments, indicating that tubular rigidity increased with increasing stretch. In the present study we present evidence to suggest that the basement membrane is the principal anatomic structure limiting distensibility of intact renal tubules studied in vitro. To determine the relative stiffness of the basement membrane we calculated Young's modulus for each tubular segment in the range of maximal transmural pressure. Young's modulus ranged between 0.7 to 1.0×10^8 dynes/cm^2, a value close to that of pure tendon collagen ($1–10 \times 10^8$ dynes/cm^2) (14). It is of interest that studies of amino acid composition indicate the presence of two carbohydrate-rich proteins, one of which is a collagen, in glomerular basement membranes (15). Thus, it is highly probable that an important structural component of renal basement membranes is a protein resembling collagen.

In spite of their relative thickness and toughness in comparison to cellular plasma membranes, the tubular basement membranes are highly permeable to water. Two of our observations indicate that a significant portion of the permeating channels are sufficiently small to retard the movement of a molecule the size of albumin. First, introduction of serum proteins in the external bath induced substantial transmembrane water flow. Second, the concentration of tracer ^{131}I-labeled albumin increased in the lumenal fluid as a consequence of hydrostatically induced transmembrane water flow. We have not determined at this juncture whether the permeating channels are uniformly somewhat larger than the albumin molecule, or whether the membrane channels vary widely in size.

It is notable that the hydraulic conductivities of the different tubular basement membranes determined using an applied hydrostatic force were several-fold greater than those obtained using a colloid osmotic force. This discrepancy is related, in part, to the fact that the isolated membranes are moderately permeable to albumin, in which case the transmembrane osmotic driving force would be less than the theoretical maximum. Owing to the fact that our method of determining osmotic conductance is indirect and is probably a high estimate we do not feel that a meaningful reflection coefficient can be derived from these data.

It is of interest to compare the hydrostatic conductance of isolated tubular basement membranes to other highly permeable structures in the kidney. The renal peritubular capillary is characterized anatomically by a fenestrated endothelial layer and contiguous basement membrane (16). In a recent report Anagnostopoulos and Windhager (17) found the hydraulic conductance of rat peritubular capillaries to be 3×10^{-3} cm^3/cm^3·min·cm H_2O, a value similar to those reported here for isolated basement membranes. The renal glomerular capillary is a structure in which the basement membrane is prominent anatomically. Based on direct measurements of glomerular capillary pressure, Brenner, Troy, and Daugharty (18) have recently determined the average hydraulic conductance to be 1.4×10^{-3} cm^3/cm^3·min·cm H_2O, a value only slightly less than that observed for isolated basement membranes. Thus, in these renal vascular structures in which the endothelial and epithelial cells are intact the basement membranes may be even more permeable to water than tubular basement membranes.

We now consider the findings of this study in relation to net fluid absorption in the proximal tubule. In the course of transepithelial fluid flow it is generally held that the absorbate crosses the epithelial barrier owing to the active transport of solute and passive flow of water into an extracellular region on the lumenal side of the basement membrane. The basement membrane is obviously in a strategic location, since all of the solute and water that is transported from the tubular lumen into the interstitium passes through this barrier. The force, either hydrostatic or oncotic, needed to drive the absorbate through the basement membrane can be esti-

mated on the basis of our present results. The magnitude of the force depends on the area of basement membrane through which the absorbate flows, and the rate of net fluid transport. The absorbate may flow through the total area of the basement membrane, or the absorbate may flow through narrow slits at the base of the lateral intercellular spaces and basilar infoldings, a view predicated on studies of the gallbladder (19, 20), and widely held by renal physiologists. For intact proximal convoluted tubules 45 μ in outer diameter with a basement membrane L_p of 5.4×10^{-8} $cm^3/cm^2 \cdot min \cdot cm$ H_2O and net fluid absorption of 10^{-6} $cm^3/min \cdot mm$ tubule length (9), a pressure of only 0.13 cm H_2O would be required to force fluid into the interstitium, provided that the absorbate flowed evenly through 100% of the basement membrane area. If, however, the path of absorbate flow is intercellular then more hydrostatic pressure per unit volume flow would be required to force the fluid through the restricted membrane area at the base of the channels. From electron microscope studies of proximal tubules (reference 12 and unpublished electron micrographs of isolated perfused PCT's supplied by Dr. John Tormey and Dr. Charles Ganote) we estimate that maximally only about 10% of the basement membrane surface area is exposed to the lateral intercellular and basilar labyrinthine channels. If flow is restricted to these paths then the required driving force across the basement membrane would be of the order of 1–2 cm H_2O.

The transbasement membrane driving force could be generated in several ways. First, if the plasma membranes of the cells are sufficiently rigid then local hydrostatic force could be generated within the hypothetical intercellular transport path as water enters in response to active solute transport. Against this possibility is the fact that proximal tubular plasma membranes are extremely deformable (21). A pressure of only 1–2 cm H_2O would markedly distend an interepithelial transport path resulting in dilations that could be seen by electron microscopy, as in the gallbladder (19, 20) and cortical collecting tubule (13, 22, 23). Morphologic confirmation is lacking, however, in the mammalian proximal tubule.

Secondly, a tubular hydrostatic pressure difference might aid movement of absorbate through the basement-membrane. The magnitude of the transtubular pressure in vivo in not known with certainty. Gottschalk and Mylle (24) were unable to detect a significant difference in pressure between the lumens of proximal tubules and peritubular capillaries, whereas in recent studies of Falchuk and Berliner (10) a difference of 5 mm Hg was found. Falchuk and Berliner inferred that there was a difference in pressure between the tubular lumen and interstitium of an undetermined magnitude, an interpretation which is consonant with the present observations that isolated renal tubules have an opening pressure and are elastic. The highly deformable cells are probably compressed against the basement membrane so that the postulated drop in transtubular hydrostatic pressure is across the basement membrane, primarily. Thus, a hydrostatic pressure difference in vivo of only a few centimeters of water may be a highly significant factor in the flow of absorbate into the renal interstitium.

Osmosis, due to a difference in oncotic pressure, is a third possible mechanism for transporting absorbate through the basement membrane. In the present studies we have demonstrated a significant oncotic effect of serum proteins on flow through the basement membrane. In this regard it should be noted that the concentration of serum proteins in renal lymph, a derivative of renal interstitial fluid, is about one-half that of blood serum (25). Thus, conditions are such that osmosis could contribute to the movement of tubular absorbate from the epithelial compartment into the renal interstitium. A report by Imai and Kokko (26) indicates that removal of serum protein from the fluid bathing isolated perfused tubules significantly depresses the rate of tubular fluid absorption, a finding corroborated in our laboratory (27). The mechanism of this effect of reducing the protein concentration of the peritubular fluid on active solute transport, the importance of the tubular basement membrane in mediating the effect of protein removal and the significance of changes in protein concentration at the peritubular surface in relation to the control of proximal tubular fluid absorption in vivo remain to be determined.

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