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Research Article

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Given this evidence of a causal relationship between postglomerular oncotic pressure and proximal reabsorption, we undertook to determine whether this relationship is responsible for the parallel adjustments in proximal reabsorption that follow changes in GFR (glomerulotubular balance). Using a separate group of hydropenic rats, proximal reabsorption was studied, initially during partial aortic constriction (during which renal […]

Postglomerular Vascular Protein Concentration: Evidence for a Causal Role in Governing Fluid Reabsorption and Glomerulotubular Balance by the Renal Proximal Tubule

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ABSTRACT We tested the relationship between postglomerular microvascular protein concentration and rates of sodium and water transfer by rat proximal tubules. Using recently described microperfusion techniques, efferent arterioles and branch peritubular capillaries of normal hydropenic rats were perfused with colloid-free Ringer's solution, and isoncotic (9.0–10.0 g/100 ml) and hyperoncotic (15 g/100 ml) albumin-Ringer's solutions. Reabsorption in adjacent proximal tubules was studied using free-flow techniques, with initial collections obtained during normal blood perfusion, recollections during experimental microperfusion, and in some tubules, repeat recollections after microperfusion and spontaneous resumption of blood perfusion. Colloid-free perfusion resulted in a uniform inhibition of proximal reabsorption (absolute and fractional). Despite identical techniques, substitution of isoncotic and hyperoncotic perfusates resulted, on average, in unchanged and increased rates of reabsorption, respectively. These findings of direct linear changes in reabsorption in response to changes in postglomerular protein concentrations usually occurred in the absence of significant changes in filtered load, and were nearly always found to be reversible within minutes of cessation of experimental perfusion.

Given this evidence of a causal relationship between postglomerular oncotic pressure and proximal reabsorption, we undertook to determine whether this relationship is responsible for the parallel adjustments in proximal reabsorption that follow changes in GFR (glomerulotubular balance). Using a separate group of hydropenic rats, proximal reabsorption was studied, initially during partial aortic constriction (during which renal perfusion pressure, single nephron GFR, absolute proximal reabsorption, and calculated filtration fraction all were reduced below levels prior to constriction), and again while adjacent efferent arteriolar and peritubular capillary protein concentrations, but not GFR, were restored to normal (preconstriction) levels by microperfusion with 9–10 g/100 ml albumin-Ringer's solution. During this dissociation of GFR and postglomerular protein concentration, absolute and fractional proximal reabsorption nearly always increased in parallel with the changes in the latter, thereby demonstrating that glomerulotubular balance is mediated, at least in part, by changes in postglomerular oncotic pressure brought about by changes in filtration fraction.

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INTRODUCTION

Despite the longstanding and intensive efforts of a number of investigators there is at present no evidence of a causal nature to explain the phenomenon of glomerulotubular balance, i.e., the parallel and more or less proportional adjustment in absolute proximal sodium reabsorption that takes place in response to variations in glomerular filtration rate. Two lines of evidence have recently accrued from these efforts, however, which appear to contribute to at least a partial understanding. The first of these, largely negative in nature, derives from attempts to demonstrate that the factor(s) responsible for mediating glomerulotubular balance is(are) localized within the lumen of, or intrinsic to, the epithelial cylinder of the proximal convoluted tubule. Evidence in support of such hypothetical mechanisms as luminal geometry, linear velocity of tubule fluid flow, and humoral influences exerted ultimately on active sodium transport sites within tubule cells either has not been forthcoming or has failed to withstand rigorous experimental scru-

tiny (1–8). Indeed, the tentative conclusion to be drawn from these studies is that neither the tubule nor its luminal boundary appear to be endowed with any intrinsic property of self-regulation of sodium reabsorption.

The second line of evidence, in part strengthened by the first, derives initially from observations in Earley's laboratory that led him and coworkers (9–11) to focus on the importance of the peritubular environment in the over-all regulation of sodium reabsorption. On the basis of clearance studies in the dog, these workers proposed that changes in renal vascular resistance, arterial pressure, and plasma oncotic pressure all serve to influence reabsorption, particularly proximal reabsorption, by determining the rate of uptake and removal of the epithelial reabsorbate into the peritubular capillary network. Lewy and Windhager reached much the same conclusions on the basis of recent micropuncture studies in the rat (12). The latter authors went on to integrate their observations into a detailed schema which emphasizes the functional and ultrastructural similarities between proximal tubules and other transporting epithelia (e.g. gall bladder), as well as incorporates the concepts of a middle compartment (13) and standing osmotic gradient (14).

In keeping with the predictions of the Lewy-Windhager proposal there has accumulated increasing, but for the most part indirect, evidence to support the view that proximal reabsorption can be influenced significantly by changes in peritubular capillary colloid osmotic pressure (15–28). Nevertheless, even for the most rigorous efforts to date (25–28), alternate interpretations cannot entirely be excluded. Largely this is because in each of these studies, whereas the experimental maneuvers produced the desired changes in peritubular capillary protein concentration, they also resulted in simultaneous and often sizable parallel changes in glomerular filtration rate (GFR). Thus, in these efforts to discern a causal link between postglomerular oncotic forces and proximal reabsorption, often it has not been possible to exclude the relative influences on reabsorption contributed by concomitant changes in filtration rate per se.

The present experiments were aimed at testing directly the relationship between changes in postglomerular protein concentration (achieved by microperfusion of surface efferent arterioles and branch peritubular capillaries with colloid-free, isoncotic, and hyperoncotic Ringer's solutions) and sodium and water reabsorption by the rat proximal tubule (measured simultaneously in adjacent surface proximal tubules bathed by these perfusion solutions) using free-flow recollection micropuncture techniques. The technique of microvascular perfusion is similar to that described by others (26, 29–31). In agreement with recent findings by Spitzer and Windhager (26), we found that absolute and fractional proximal reabsorption declined significantly during perfusion with non–colloid containing Ringer's solutions, and remained unchanged from control during perfusion with this same Ringer's solution to which albumin was added to make final concentrations (9.0–10.0 g/100 ml) equal to that normally measured at this site. In these same rats microperfusion with hyperoncotic (15 g/100 ml) albumin solutions resulted in a significant increase in proximal reabsorption (absolute and fractional). For each group the changes in reabsorption usually occurred in the absence of significant changes in glomerular filtration rate.

Having demonstrated a direct and linear relationship between postglomerular oncotic pressure and fluid reabsorption by the rat proximal tubule, we undertook as part of this study to determine whether this mechanism is responsible for the phenomenon of glomerulotubular balance. Proximal reabsorption was studied in a separate group of rats, initially during aortic constriction (when renal perfusion pressure, glomerular filtration rate, absolute reabsorptive rate, and postglomerular protein concentration all were measured to be reduced significantly below levels before constriction) and again (during continued and stable aortic constriction, when perfusion pressure and filtration rate were relatively constant at reduced levels) while efferent arterioles and branch peritubular capillaries were perfused with 9–10 g/100 ml albumin-Ringer's solution (i.e. the normal postglomerular protein concentration). Under these conditions absolute and fractional proximal reabsorption nearly always increased above preperfusion levels. These findings are taken as direct and strong support for the view that changes in filtration rate lead to concomitant and parallel changes in absolute reabsorption as a consequence of the resultant changes in filtration fraction and thereby in postglomerular protein concentration.

METHODS

Male Sprague-Dawley rats weighing 270–330 g and allowed free access to a rat pellet diet and water were anesthetized with Inactin (100 mg/kg). They were placed on a heated micropuncture table and maintained at body temperatures of 36.5°–38.5°C. A tracheostomy was performed and indwelling polyethylene catheters were inserted into the left jugular vein for infusion of inulin and fluids and into the left femoral artery for periodic collection of blood and estimation of arterial pressure. In some rats a catheter also was inserted into the right jugular vein for periodic injections of Lissamine green. The changes in mean arterial pressure were monitored by means of a Statham strain gauge (model P23AA, Statham Instruments, Inc., Los Angeles, Calif.) connected to a Hewlett-Packard recorder (model 7712, Hewlett-Packard Co., Palo Alto, Calif.) The experimental kidney (left) was exposed by a subcostal incision and gently separated from its perirenal attachments. The kidney was suspended on a Lucite holder, its surface illuminated with a fiber-optic light source (American Optical Corp., Southbridge, Mass.) and bathed with paraffin oil maintained at 35°–37°C. Excessive fluid losses during preparative surgery

were carefully avoided; the small losses that were incurred were not replaced.

Efferent arteriolar microperfusion studies. The effects of microperfusion-induced variations in postglomerular capillary protein concentration on fractional and absolute sodium and water reabsorption in adjacent proximal tubules was studied in eight rats. Beginning 60 min before micropuncture each rat received an intravenous infusion of a solution of 10% inulin in isotonic NaCl given at the rate of 0.02 ml/min. Following this 60 min equilibration period a proximal tubule segment directly adjacent to a surface efferent arteriole was punctured. This method for proximal tubule localization (rather than the Lissamine green injection method) was chosen since Steinhausen, Eisenbach, and Galaske (32) have recently reported that proximal tubule segments so situated are statistically more likely to be late rather than early surface convolutions. After intratubular deposition of a column of polymer oil (Kel F polymer oil, Minnesota Mining and Manufacturing Co., St. Paul, Minn.) two to three tubule diameters in length, an exactly timed (1–2 min) sample of fluid was collected for determination of inulin concentration and flow rate. Sharpened micropipettes with external tip diameters of 10–12 μ were used. Using the fluid collection technique of controlled suction recently described for this laboratory (33), minimal changes were induced in tubule diameter and the position of the distal oil block. Following this initial tubule fluid collection, efferent arteriolar microperfusion was begun. A sharpened, internally siliconized micropipette (6–10 μ O.D.) containing either colloid-free Ringer's solution, isoncotic[1] (9.0–10.0 g/100 ml albumin), or hyperoncotic (15 g/100 ml albumin) Ringer's solution was inserted into the neighboring efferent arteriole. Using a microsyringe and pipette holder attached to a Sage perfusion pump (model 255-3, Sage Instruments, Inc., White Plains, N. Y.) it was possible to microperfuse an area of from 0.6 to 1.0 mm in diameter, a zone more than sufficient to encompass the surface convolutions of the proximal tubule under study. Lissamine green in a final concentration of 1.0% (w/v) was added to each perfusion solution to facilitate recognition of the precise zone being microperfused. During efferent arteriolar microperfusion, an exactly timed free-flow recollection of tubule fluid was obtained from the previous puncture site. These recollections were of at least 30 sec duration, and usually 1 min. Microvessel perfusion was carried out for periods of from 1 to 4 min before tubule fluid collections. The interval from initial tubule fluid collection to recollection was never more than 6 min. Following microperfusion, the pipette was withdrawn from the efferent arteriole. In several experiments a third timed collection (second recollection) of tubule fluid was obtained 3–5 min after the promptly noted spontaneous resumption of efferent arteriolar and branch peritubular capillary blood flow.

This protocol of tubule fluid collections, initially during normal blood perfusion and again during experimental microperfusion, was repeated four or five times in each rat. In this way it usually was possible to examine the influence of all three experimental perfusion solutions in the same rat. The compositions of the Ringer's perfusion solutions were: 60 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 10 mM sodium acetate, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 2.5 mM $CaCl_2$, to which was added sufficient crystalline Lissamine green[2] to yield a final concentration of 1%. Crystalline bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) was added where appropriate to make 9.0–10.0 g/100 ml and 15 g/100 ml solutions. The pH of all solutions was adjusted immediately before use and varied between 7.35 and 7.46 (mean = 7.41). In experiments 7 and 8, all perfusion solutions were bubbled with 95% O_2–5% CO_2 for 3 hr immediately before use. The range of final osmolalities and sodium concentrations was similar for all three perfusion solutions, for the entire group averaging 289.4 mOsm/kg (range: 277–312) and 151.9 mEq/liter (range 146–160), respectively. Potassium concentration averaged 4.6 mEq/liter.

In about 25% of these as well as the additional microvascular perfusion experiments to be described below, the operator (B.M.B.) inadvertently transfixed the efferent arteriole being perfused and entered the lumen of the adjacent experimental proximal tubule. Recognition of this complication was made easy by the presence of the Lissamine green dye which resulted in gross discoloration of the tubule fluid both *in situ* and in samples subsequently collected. Whenever green color was recognized to be present within the tubule lumen or in collected samples, the tubules were no longer studied and the samples discarded. All other samples were analyzed and the results recorded.

Efferent arteriolar microperfusion rate was kept constant in each rat, but was varied intentionally over a wide range from rat to rat (range: 260 nl/min to 590 nl/min). These flow rates were measured at the conclusion of each experiment using the same microperfusion pipette as was used during actual microvessel perfusion. The flow rate was measured by collecting the volume of fluid displaced by the pump per unit time into constant bore hollow glass capillary tubing of known internal diameters. Two or three consecutive collections (never less than 5 min each) were obtained. The results, which always agreed within 10%, were averaged.

Aortic constriction experiments. In the second part of this study we examined the effects on proximal reabsorption of reductions in glomerular filtration rate in the presence and absence of concurrent reductions in postglomerular protein concentration. The first six rats in this group were studied initially during normal hydropenia and again during partial aortic constriction. In another six similarly hydropenic rats, in addition to these same measurements, we studied a series of tubules initially during partial aortic constriction (when filtration rate and postglomerular protein concentration were reduced) and again (during sustained aortic constriction and continued reduction in filtration rate) but now while the ef-

[1] The term isoncotic here applies to the protein concentration of efferent arteriolar plasma, not systemic plasma. As a consequence of ultrafiltration of noncolloidal substances across the glomerular capillary wall, the concentration of protein in postglomerular (efferent) arterioles uniformly exceeds that of preglomerular blood, and as shown elsewhere in this and previous studies (25, 27, 28) averages between 9.0 and 10.0 g/ml. The Ringer's perfusion fluid chosen to replace normal efferent arteriolar blood contained crystalline bovine serum albumin in final concentrations of 9–10 g/100 ml. Whereas these values encompass the total protein concentration normally present at this site, since a pure albumin solution was used the effective oncotic pressure is slightly higher than for plasma (at most by some 12 mm Hg). Since mean efferent arteriolar hydrostatic pressure also must have been higher than usual during microperfusion, little alteration from control in net transcapillary dynamics would be expected.

[2] The sodium concentration in a 1% aqueous solution of Lissamine green is approximately 60 mEq/liter.

ierent arterioles and peritubular capillaries bathing the experimental tubules were being perfused with Ringer's solution containing a normal protein concentration of 9–10 g/100 ml.

After a 60 min equilibration period for the infusion of 10% inulin in isotonic NaCl (0.02 ml/min) samples of tubule fluid were obtained from late surface convolutions of one or two proximal tubules for determination of inulin concentration and flow rate, using techniques identical with those mentioned above. However, having found in the first group of rats in this study that the localization technique of Steinhausen et al. (32) does not regularly provide recognition of only late surface convolutions (it can be seen from the data presented below that TF/P inulin ratios using this approach varied far more than when the Lissamine green localization method was employed), during experiments in these 12 rats injection of Lissamine green (0.05 ml of a 10% solution) was the method used for the selection of late proximal convolutions. Samples of blood also were obtained at this time from superficial efferent arterioles for determination of protein concentration. The methods for selection of vessels for puncture, and preparation of sampling micropipettes have been described in detail elsewhere (25, 27). Simultaneous collections of small aliquots (75 μl) of femoral arterial blood were obtained for determination of plasma inulin and protein concentrations. Glomerular filtration rate then was reduced by means of partial constriction of the abdominal aorta, using techniques previously described (1). The degree of constriction was monitored by the change in mean arterial pressure in the left femoral artery, and by the stereo-microscopic appearance of the kidney. Following a 5–10 min stabilization period after partial aortic constriction, samples of tubule fluid were obtained from the previous puncture sites of each experimental tubule (recollection method) for repeat determination of inulin concentration and flow rate. Similarly, other efferent arterioles were punctured for repeat determination of protein concentration. Simultaneous collections of femoral arterial blood for determination of inulin and protein concentrations also were obtained.

With the aorta still partially constricted (and with a stable but reduced renal perfusion pressure), samples of fluid were collected from late surface convolutions of one to five other proximal tubules for determination of inulin concentration and flow rate. Immediately (3–5 min) after each tubule fluid collection, the nearest efferent arteriole was punctured and perfused with an isotonic Ringer's solution (composition same as above) containing 9–10 g/100 ml crystalline bovine serum albumin and colored with 1% Lissamine green (mean osmolality = 280 mOsm/kg, range 266–291; pH range 7.40–7.41). Perfusion solutions were bubbled with 95% O_2–5% CO_2 immediately before use. Perfusion rates were constant in each rat and relatively similar from rat to rat, varying from 233 nl/min to 316 nl/min. With reduction in intrarenal arterial pressure induced by partial aortic constriction, remarkably large areas (at least 0.8 mm in diameter) were seen to be perfused at these relatively low rates.[3] Following a 1–3 min period of *in situ* perfusion, and while perfusion was continuing, fluid was recollected from the adjacent experimental proximal tubule segment. Fluid collections were usually of 1–2 min duration and never less than 30 sec. As in the protocol described earlier, if the contents of the tubule lumen or the collected fluid were found to be green in color it was assumed that the perfusion pipette inadvertently impaled the experimental tubule wall. The samples so collected (a total of six) were discarded and the tubules no longer studied. All other tubules and samples were analyzed and the results recorded. The total elapsed time from initial tubule fluid collection to recollection during perfusion was never more than 10 min. Immediately after tubule fluid collections, aliquots of femoral blood were obtained for determination of inulin concentration. In two tubules in rat 12, the sequence of collections was altered. In these instances, initial collections were obtained before partial aortic constriction. Recollections were obtained initially during aortic constriction (first recollections) and again during aortic constriction and efferent arteriolar perfusion (second recollection).

Analytical. The volume of tubule fluid collected from individual proximal tubules was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of inulin in tubule fluid was measured, nearly always in duplicate, by the microfluorescence method of Vurek and Pegram (34). Inulin concentrations in urine and plasma were determined by the anthrone method of Führ, Kaczmarczyk, and Krüttgen (35). Protein concentrations in efferent arteriolar and femoral arterial blood plasmas were determined, usually in duplicate, with an ultramicro-colorimeter (American Instrument Co., Inc., Silver Spring, Md.) using a recently described microadaptation (25) of the technique of Lowry, Rosebrough, Farr, and Randall (36).

Calculations. Glomerular filtration rate for single superficial nephrons (SNGFR) was calculated from the tubule fluid/plasma inulin $(TF/P)_{In}$ ratio and volume of tubule fluid (V_{TF}) collected per minute by means of the expression:

$$SNGFR = (TF/P)_{In} \cdot V_{TF} \quad (1)$$

The absolute rate of tubule fluid reabsorption to the site of puncture was calculated as the difference between SNGFR and V_{TF}.

The fraction of the SNGFR reabsorbed to the site of puncture was calculated using the expression:

$$\text{Fractional reabsorption} = 1 - \left(\frac{\text{Plasma}}{\text{Tubule Fluid}}\right)_{In} \quad (2)$$

Filtration fraction (FF) for superficial cortical glomeruli was estimated from simultaneous measurements of superficial efferent arteriolar (EA) and femoral arterial (FA) protein concentrations, using the expression given by Bresler (17):

$$FF = 1 - \frac{[\text{Protein}]\,FA}{[\text{Protein}]\,EA} \quad (3)$$

Student's t test was employed in the statistical analysis of all results.

[3] We have recently carried out precise measurements of blood flow for superficial efferent arterioles in the normal hydropenic rat and found an average value of 174 nl/min ±46 SD (33). Thus the perfusion rates employed in this study always exceeded average normal values. The term "relatively low rates" is intended only in the comparative sense in that in the absence of partial aortic constriction, we have noted, as have others (26), that adequate zones of perfusion require substantially greater perfusion rates than occur normally. With the reduction in intrarenal perfusion pressure resulting from partial aortic constriction, the flow rates could be lowered to values that more closely approximated the normal.

TABLE I

Effects of Variations in Peritubular Capillary Protein Concentration (Achieved by

	Colloid-free Ringer's perfusion										9–10g/100 ml albumin perfusion				
	$\left(\frac{\text{Tubule fluid}}{\text{Plasma}}\right)_{\text{Inulin}}$					Nephron GFR			Absolute reabsorbtion			$\left(\frac{\text{Tubule fluid}}{\text{Plasma}}\right)_{\text{Inulin}}$				
Exp. No.	C_1*	E*	C_2*	E/C_1	C_2/C_1	C_1	E	C_2	C_1	E	C_2	C_1	E	C_2	E/C_1	C_2/C_1
						nl/min			*nl/min*							
1	2.58	2.05		0.79		45.2	34.2		27.7	15.5		1.58	1.80	1.73	1.14	1.09
(440 nl/min)‡	1.35	1.20	1.39	0.89	1.03	29.4	23.3	33.6	7.7	3.8	9.5					
2	1.49	1.10		0.74		47.6	lost		15.7	lost		1.70	1.81		1.06	
(590 nl/min)												1.64	1.61	1.50	0.98	0.91
3	2.77	2.24		0.81		25.2	24.6		16.1	13.6						
(360 nl/min)	1.95	1.49		0.76		22.4	19.4		10.9	6.4						
	1.63	1.42	1.56	0.87	0.96	28.5	27.3	25.6	12.8	5.1	9.2					
4	2.59	1.56		0.60		25.4	lost		15.6	lost		1.88	2.19	2.01	1.16	1.07
(360 nl/min)	2.97	2.49	2.60	0.84	0.88	22.0	23.2	24.5	14.6	10.9	15.1	1.92	1.80		0.94	
	1.89	1.57		0.83		29.8	23.9		14.0	8.8						
5												2.10	2.06		0.98	
(260 nl/min)												2.06	2.16	2.09	1.05	1.01
												1.64	1.43		0.87	
6	1.36	1.21	1.16	0.89	0.85	22.3	18.5	25.6	5.9	3.2	3.5	1.81	1.87	1.81	1.03	1.00
(325 nl/min)	1.86	1.46	1.64	0.78	0.88	29.3	25.2	22.1	13.6	7.9	8.6					
7§	1.33	1.19		0.89		26.8	22.6		6.7	3.5		3.18	3.12		0.98	
(310 nl/min)	2.13	1.03		0.48		21.2	19.0		10.2	0.5		1.97	1.82		0.92	
												2.49	2.63		1.06	
8§	3.40	2.09		0.62		25.2	22.1		17.8	11.5						
(310 nl/min)																
Mean ±SE				0.77¶ ±0.03	0.92** ±0.04										1.01** ±0.02	1.02** ±0.04
% Δ from initial							−9.6‖ ±3.0	+1.1** ±7.9		−45.2¶ ±5.5	−15.7** ±12.5					

* C_1, E, and C_2 denote initial control (preperfusion), experimental perfusion, and second control (postperfusion) periods for each tubule.
‡ Numbers in brackets denote capillary perfusion rate for each rat.
§ In rats 7 and 8, perfusion fluids were bubbled with 95% O_2–5% CO_2 for 3 hr immediately before use.
‖ $P < 0.05$.
¶ $P < 0.001$.
** $P > 0.05$.

RESULTS

Efferent arteriolar microperfusion studies. The effects of microperfusion-induced variations in efferent arteriolar and branch peritubular capillary protein concentration on proximal sodium reabsorption was studied in eight rats. Mean arterial pressure averaged 124 mm Hg (range: 110–140 mm Hg). 14 proximal tubules in seven rats were studied before and during microperfusion with colloid-free Ringer's solution. The results are summarized in the left hand portion of Table I.[4] Displacement of normal blood in efferent arterioles and peritubular capillaries by colloid-free isotonic Ringer's solution resulted in a fall in TF/P inulin ratios in each of the 14 tubules studied. Recollection/initial collection inulin ratios ranged from 0.48 to 0.89 and averaged 0.77 ±0.03 SE, a highly significant difference from unity ($P < 0.001$).

[4] The tubule segments selected for micropuncture in these rats where chosen using a method suggested from observations recently reported by Steinhausen et al. (32). These authors noted that for the majority of nephrons late surface convolutions of proximal tubules are distributed in close apposition to efferent arterioles. It can be seen in Table I that an unusually wide scatter in TF/P inulin ratios obtained when segments most closely bordering efferent arterioles (hence the presumed late segments) were punctured. TF/P inulin ratios below 1.5 (an exceptionally low ratio for late proximal segments in hydropenia) were found in 4/14 instances, compared with 1/19 in a separate group of hydropenic rats in this report prepared in identical fashion except that late surface convolutions were localized by the Lissamine green injection technique (see below and Table II, first section). Another feature of interest is that values for single nephron glomerular filtration rate in rats not exposed to Lissamine green (Table I) tended to be lower (mean for all nephrons in these eight rats = 27.5 nl/min ±1.2 SE [$n = 32$]) than other similarly hydropenic rats in this study prepared in identical fashion except that injections of Lissamine green were given (mean = 40.5 nl/min ±1.3 SE [n = 19]). Since whole kidney filtration rates were not measured in these two groups of rats, we do not yet know whether comparable differences also exist in this measure.

Capillary Microperfusion) on Several Measures of Individual Nephron Function

9–10 g/100 ml albumin perfusion						15 g/100 ml albumin perfusion										
Nephron GFR			Absolute reabsorption			$\left(\frac{\text{Tubule fluid}}{\text{plasma}}\right)_{\text{Inulin}}$					Nephron GFR			Absolute reabsorption		
C_1	E	C_2	C_1	E	C_2	C_1	E	C_2	E/C_1	C_2/C_1	C_1	E	C_2	C_1	E	C_2
	nl/min			*nl/min*								*nl/min*			*nl/min*	
29.8	28.5	30.6	10.9	12.7	12.9											
24.6	28.6		10.1	12.8												
31.5	31.2	33.0	12.3	11.9	11.0											
						1.61	1.92		1.19		28.7	26.6		10.9	12.8	
						1.31	1.55	1.44	1.18	1.08	33.6	33.6	30.3	7.9	11.9	9.3
35.8	35.0	27.0	16.7	19.0	13.6	1.26	1.64	1.32	1.30	1.05	27.9	23.3	28.3	5.7	9.1	6.9
21.9	31.3		10.5	13.9												
23.5	18.3		12.3	9.4		1.57	1.62		1.03		23.9	27.0		8.7	10.3	
22.3	23.7	26.7	11.5	12.7	14.1	1.69	2.21	1.61	1.31	0.95	26.6	29.6	24.6	10.9	16.3	9.3
28.8	29.5		11.2	15.8												
30.1	28.9	30.1	13.5	13.4	13.5											
33.3	30.5		22.8	20.7		1.89	2.08		1.10		17.4	21.9		8.2	11.4	
12.4	14.4		6.1	6.5												
26.6	32.3		15.9	16.1												
									1.19‖ ±0.04	1.03** ±0.04						
	+5.3** ±4.9	+0.5** ±7.2		+9.6** ±5.2	+2.5** ±8.0							+4.4** ±6.3	−5.3** ±3.4		+41.0‖ ±6.3	+8.0** ±9.3

The fall in fractional reabsorption ranged from 9.1% to 94.3% and averaged 34.9% ±6.1 SE. The simultaneous changes in calculated values for SNGFR and absolute fluid reabsorption are also indicated in Table I. SNGFR tended to change relatively little during microperfusion, falling by more than 20% in but one instance (− 24.3%), by less than 20% in eight, and remaining unchanged (±5%) in three. In one instance SNGFR increased slightly (+ 13.4%). In the presence of these relatively small changes in SNGFR (which averaged − 9.6% ±3.0 SE) we noted uniform reductions in absolute reabsorption. For paired data from each tubule the changes in this measure ranged from − 15.5% to − 92.4%, and averaged − 45.2% ±5.5 SE ($P < 0.001$). In two tubules, recollection estimates of SNGFR were lost. Of the remaining 12, the decline in absolute reabsorption in 9 was greater than could be accounted for by the simultaneous changes in SNGFR. Note in Table I that these uniform reductions in fractional and absolute proximal sodium and water reabsorption occurred irrespective of the exact rates of efferent arteriolar microperfusion (see values in brackets, column 1).[5] In five tubules in this group, repeat collections (second re-collections) were obtained within 3.5 min of cessation of microperfusion and spontaneous resumption of blood perfusion. $(TF/P)_{In}$ ratios returned

[5] One possible explanation to account for this depression in reabsorption might be that inulin-free perfusate also flowed in retrograde fashion back to the glomerular capillary bed. Were this to occur, the inulin concentration in glomerular plasma would be lowered, resulting in artefactual reductions in tubule fluid inulin concentrations, and therefore, in $(TF/P)_{In}$ ratios. To test this possibility we perfused 10 efferent arterioles in two separate rats with isotonic, colloid-free Ringer's solution containing inulin and Lissamine green in concentrations of 300 mg/100 ml, and 2%, respectively. No inulin or Lissamine green was given systemically. Analysis of tubule fluid from 10 adjacent proximal tubules revealed each sample to be colorless and to contain no detectable inulin.

TABLE II

Comparison of Effects of Reduced Nephron GFR on Absolute Reabsorptive Rate in the

	Normal hydropenia						Aortic constriction (recollection)						
		$\left(\frac{TF}{P}\right)_{In}$		Abs.	[Protein]			$\left(\frac{TF}{P}\right)_{In}$			Abs.	[Protein]	
Exp. No.	B.P.	(a)	SNGFR*	Reab.	EA‡	FA‡	B.P.	(b)	b/a	SNGFR	Reab.	EA	FA
	mm Hg		*nl/min*	*nl/min*	*g/100 ml*		*mm Hg*			*nl/min*	*nl/min*	*g/100 ml*	
1.	115	1.73	42.4	18.9	9.1	6.2	77	2.39	1.38	12.7	7.4	7.2	5.7
		1.75	35.9	15.3				3.50	2.00	7.0	5.0		
2.	130	2.06	40.4	20.8	8.6	6.0	75	2.28	1.11	26.9	15.1	7.6	6.0
3.	120	1.45	32.5	10.1	7.9	5.7	85	1.22	.84	22.4	4.0	6.6	5.3
4.	120	1.61	35.6	13.4	9.2	6.4	70	2.74	1.70	16.9	10.7	7.1	5.5
5.	100	2.79	43.3	27.8	8.9	6.3	75	3.83	1.32	29.0	21.5	8.3	6.4
6.	135	1.83	37.7	17.7	9.2	5.5	90	2.65	1.45	23.5	14.6	6.7	5.1
7.	128	2.19	37.2	20.2	10.5	6.1	88	2.40	1.10	23.4	13.6	7.6	6.2
		2.72	35.3	22.4				3.32	1.22	24.8	17.3		
8.	125	1.98	41.3	20.4	8.6	5.6	90	3.34	1.69	15.6	10.9	7.3	5.8
9.	142	2.64	59.7	37.1	8.8	6.3	100	2.55	.96	34.5	19.8	7.8	6.1
		2.67	44.3	27.7				2.87	1.07	15.4	10.0		
10.	140	1.67	37.4	15.0	10.4	7.3	85	1.87	1.12	26.0	12.1	7.9	6.1
		1.84	39.7	18.2				2.22	1.21	29.0	15.9		
11.	140	2.72	38.6	24.4			90	2.94	1.08	29.2	19.3		
		2.02	40.6	20.5				2.39	1.18	24.9	14.5		
12.	120	1.98	46.3	23.0			100	2.11	1.06	33.1	17.7		
		2.11	43.1	22.6				2.77	1.30	26.3	16.9		
		2.28	38.3	22.8				2.63	1.15	27.6	17.1		
Mean	126	2.11	40.5	20.9	9.1	6.1	85	2.63‖	1.26¶	23.6¶	13.9¶	7.4	5.8
±SE	3.5	0.10	1.3	1.4	0.2	0.2	2.8	0.14	0.06	1.6	1.1	0.2	0.1
Mean Δ from initial (%)								+15.6§‖		−41.5¶	−33.7¶	−18.0¶	−4.9**
±SE								5.6		3.8	4.1	2.3	2.5

* SNGFR, single nephron glomerular filtration rate.
‡ EA and FA refer to efferent arteriolar and femoral arterial protein concentration, respectively.
§ Refers to mean changes in fractional reabsorption.
‖ $P < 0.05$.
¶ $P < 0.001$.
** $P > 0.05$.

to or toward control levels in four, the mean recollection/initial collection ratio averaging 0.92 ±0.04 SE, a value not significantly different from unity ($P > 0.05$). This tendency to reversibility in fractional reabsorption occurred in the absence of significant changes in SNGFR and was accompanied by similar and parallel changes in absolute reabsorption.

Despite identical methods of microperfusion and tubule fluid collection, addition of 9.0–10.0 g/100 ml crystalline bovine serum albumin to the Ringer's perfusion solution in six of these same rats resulted in no mean change in recollection/initial TF/P inulin ratios (1.01 ±0.02 SE), and therefore in fractional reabsorption (+ 0.1% ±2.9 SE, for each $P > 0.5$). Values for all 12 tubules were distributed randomly about unity. Once again the simultaneous changes in calculated values for SNGFR during microperfusion were small and random in direction. Absolute reabsorption therefore changed little from control, the mean value of + 9.6% ±5.2 SE not differing significantly from unity ($P > 0.05$). Second recollections in five tubules after cessation of microperfusion and resumption of efferent arteriolar blood flow likewise revealed small and insignificant changes in fractional and absolute reabsorption and SNGFR. It is apparent from these measurements during, and following, isoncotic Ringer's perfusion that the microperfusion technique per se exerts no untoward or detrimental effects on the various functions measured in these single nephrons. Moreover this absence of any systematic influence on reabsorption during isoncotic microperfusion was demonstrable irrespective of (*a*) the wide range of perfusion rates employed (260 nl/min to 590 nl/min) and (*b*) the absence of the normal concentration of

Presence and Absence of Reduction in Postglomerular Protein Concentration

Aortic constriction (initial collections)				Aortic constriction and capillary perfusion (recollection)					
B.P.	$\left(\frac{TF}{P}\right)_{In}$ (a)	SNGFR	Abs. Reab.	Perfusion rate	B.P.	$\left(\frac{TF}{P}\right)_{In}$ (b or c)	b/a or c/b	SNGFR	Abs. Reab.
mm Hg		*nl/min*	*nl/min*	*nl/min*	*mm Hg*			*nl/min*	*nl/min*
90	3.02	11.8	7.9	312	90	5.26	1.74	12.9	10.5
	3.00	8.8	5.9			3.94	1.31	17.5	13.1
90	4.10	15.7	11.9	285	91	5.26	1.28	20.4	16.5
	1.89	21.9	10.3			2.51	1.33	19.5	15.7
100	1.76	29.9	12.9	315	95	2.02	1.15	26.4	18.3
	2.03	27.5	13.9			2.75	1.35	22.0	14.0
85	2.38	21.7	12.6	270	85	2.91	1.22	27.0	17.7
	2.63	17.2	10.6			3.25	1.24	19.8	13.6
	2.22	20.2	11.1			2.94	1.32	23.2	15.3
	2.09	18.9	9.9			2.00	.96	15.8	7.9
	2.14	22.3	11.9			2.37	1.11	22.5	13.0
100	3.92	20.4	15.2	233	105	4.04	1.03	26.6	20.0
100	2.12	28.4	15.0	315	95	2.95	1.39	37.5	24.8
	3.01	29.3	19.6			3.82	1.27	28.5	21.3
						5.15	1.84	30.9	24.9
						3.41	1.30	26.1	20.2
94	2.59	21.0	12.0	289	93	3.41‖	1.30¶	23.5**	16.7‖
2.7	0.20	1.7	0.9	13.4	2.7	0.27	0.06	1.5	1.2
						+14.6§¶		+12.8**	+34.7¶
						2.3		7.1	7.9

erythrocytes contained in efferent arteriolar and peritubular capillary fluid.

In four rats in this same group the influence of hyperoncotic (15 g/100 ml) albumin perfusion solutions on nephron function also was studied. $(TF/P)_{In}$ ratios increased by at least 10% in 5/6 tubules, the recollection ratio for the group averaging 1.19 ±0.04 SE, a value significantly greater than unity ($P < 0.01$). The corresponding change in fractional reabsorption averaged + 34.7% ±11.9 SE ($P < 0.05$). SNGFR remained little changed during microperfusion relative to preperfusion values, indicating that the mean (+ 41.0% ±6.3 SE, $P <$ 0.005) as well as the individual changes in absolute reabsorption (in five of the six tubules studied) could not be attributed simply to simultaneous changes in filtered load. Third fluid collections (second recollections) following microperfusion in three tubules demonstrated a uniform return to control levels of values for absolute and fractional sodium reabsorption. Once again these changes occurred in the absence of significant changes in SNGFR.

Fig. 1 summarizes the mean percentage changes (relative to preperfusion control values) in absolute and fractional proximal reabsorption and SNGFR for each group of tubules in these rats during microperfusion with colloid-free, isoncotic, and hyperoncotic Ringer's solutions.

Aortic constriction studies. Having demonstrated that when filtered load is relatively constant, absolute and fractional proximal sodium reabsorption vary directly with peritubular capillary protein concentration, we next sought to determine whether the fall in absolute reabsorption that accompanies reductions in filtration rate (glomerulotubular balance) is the consequence of the recently described accompanying fall in postglomerular

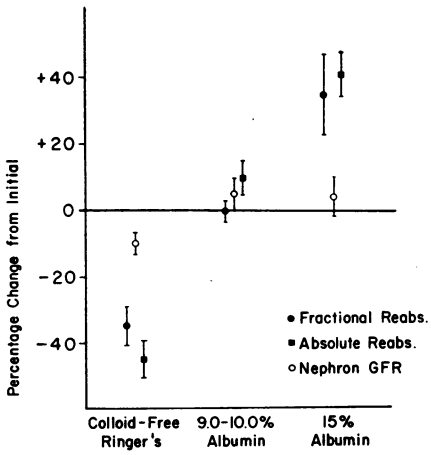

FIGURE 1 Comparison of mean changes in fractional and absolute proximal reabsorption and single nephron glomerular filtration rates induced by each postglomerular arteriolar perfusion solution. Preperfusion measurements during normal blood perfusion represent the control situation, from which the changes for each tubule were calculated. Vertical bars denote ±1 SE.

protein concentration (28, 37) or the result of some other factor(s) which also change(s) as a consequence of the reduction in GFR. To distinguish between these possibilities, proximal sodium reabsorption was studied during reductions in GFR induced by partial aortic constriction, initially while postglomerular protein concentration was likewise lower than normal and again (during sustained reductions in GFR) when the efferent arterioles and peritubular capillaries surrounding the experimental tubules were perfused with Ringer's solution containing 9–10 g/100 ml albumin, a concentration equal to that found in these postglomerular vessels in the absence of partial aortic constriction (see below and reference 27). For comparative purposes, other tubules in these same, as well as other, rats were studied before and during partial aortic constriction. A summary of individual and mean values for normal hydropenia (first section), and recollection data for these same tubules during partial aortic constriction (second section) is shown in Table II. The third section summarizes the results of nephron function in a separate group of tubules studied for the first time during aortic constriction, and restudied using recollection techniques (fourth section) during sustained aortic constriction but now while vessels were being perfused with 9–10% albumin solutions. Values for $(TF/P)_{IN}$ ratios, SNGFR, and absolute reabsorption on a given line in first and second sections represent results from the same tubule studied before and during aortic constriction, respectively. Likewise values on the same line in third and fourth sections reflect the initial and recollection findings for the same tubule. This pattern differs only for the last two tubules in experiment 12 in which second recollections (fourth section) were obtained during microperfusion and compared to the values from these same tubules prior to (first section) and during aortic constriction (second section).

As shown in previous studies in the rat (1, 4, 28, 37, 38) in response to reductions in renal perfusion pressure, there takes place a uniform and parallel decline in SNGFR and absolute reabsorption (Table II, first and second sections). The extent of the decline in the latter was nearly always less than that in SNGFR, resulting in the familiar (1, 3, 37, 38, 39) modest increase in $(TF/P)_{IN}$ ratios and fractional reabsorption. Efferent arteriolar protein concentration likewise declined in uniform fashion, and without any accompanying mean change in systemic (femoral) arterial protein concentration. Filtration fractions across these superficial cortical glomeruli (estimated using equation 3) declined from an average of 0.32 ±0.015 SE during normal hydropenia to 0.22 ±0.01 SE during partial aortic constriction (mean change = −31.5% ±3.3 SE, $P < 0.001$). A summary of the percentage changes in SNGFR, absolute and fractional sodium reabsorption, and efferent arteriolar

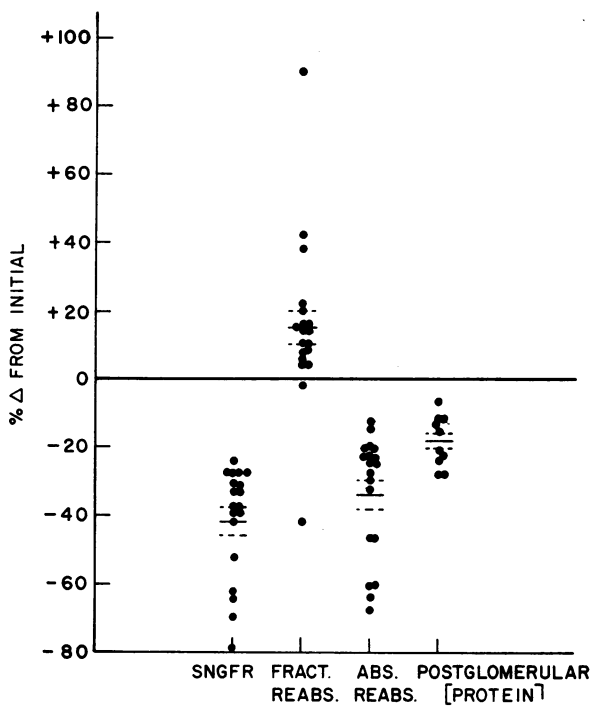

FIGURE 2 Summary of individual and mean changes in single nephron glomerular filtration rate, fractional and absolute proximal sodium reabsorption, and postglomerular arteriolar protein concentration induced by partial aortic constriction, relative to preconstriction levels. For all but the measurements of protein concentration, the recollection technique was employed; hence each tubule served as its own control. Horizontal lines denote means (solid bar) ±1 SE (broken lines).

protein concentration that occurred during partial aortic constriction is given in Fig. 2.

In six of these rats, while renal perfusion pressure remained stable at reduced levels during partial aortic constriction, initial collections of tubule fluid were obtained from 14 other late proximal tubules (Table II, third section). $(TF/P)_{In}$ ratios and calculated values for absolute reabsorption were similar to values from other tubules from these same animals (second section). Similarly the values for SNGFR were reduced comparably in these initially punctured tubules. Within 3–5 min following these initial fluid collections during partial aortic constriction, the efferent arteriole and branch peritubular capillaries adjacent to each experimental tubule were perfused with 9–10% albumin in isotonic Ringer's solution. Perfusion rates were constant for all tubules in a given rat, and (as shown in Table II, fourth section) varied relatively little from rat to rat. During microperfusion, despite absence of significant changes in mean arterial pressure, and modest if any, changes in SNGFR, recollection $(TF/P)_{In}$ ratios increased by at least 10% in 14/16 tubules, with recollection/initial collection $(TF/P)_{In}$ ratios for all averaging 1.30 ±0.06 SE. This difference as well as the corresponding mean change in fractional reabsorption of +14.6% ±2.3 SE were highly significant ($P < 0.001$). Absolute reabsorptive rates likewise increased in 14/16 tubules; in 11 of these, the changes exceeded any influence that could be attributed solely to the simultaneous changes in SNGFR. For all tubules the change in absolute reabsorption of +34.7% ±7.9 SE was highly significant ($P < 0.001$). Fig. 3 summarizes the percentage changes in SNGFR and proximal reabsorption observed during stable aortic constriction and microperfusion, relative to values measured within the prior 10 min period during aortic constriction alone. Note that despite the occurrence of random changes in SNGFR, absolute and fractional reabsorption increased in nearly every instance.[6]

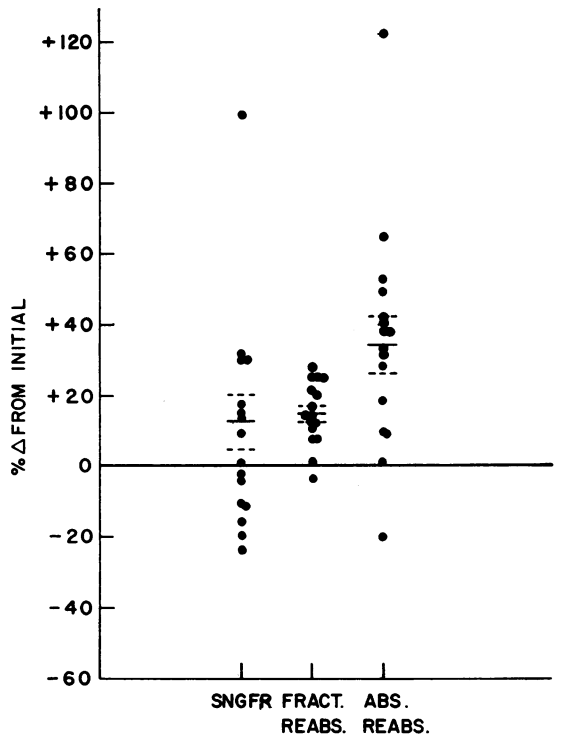

FIGURE 3 Summary of individual and mean changes in single nephron glomerular filtration rate and proximal reabsorption, measured initially during aortic constriction, and again (during stable reductions in renal perfusion pressure) while adjacent efferent arterioles and branch peritubular capillaries were microperfused with 9-10 g/100 ml albumin-Ringer's solution. Horizontal lines denote means (solid bars) ±1 SE (broken lines).

DISCUSSION

The results in the first portion of this study demonstrate that perfusion of efferent arterioles and branch peritubular capillaries with 9–10 g/100 ml (isoncotic) albumin-Ringer's solution exerts no systematic influence, relative to pre- and postperfusion values, on the rate of glomerular filtration or values for absolute and fractional proximal sodium and water reabsorption. In contrast, perfusion with colloid-free Ringer's solution in these same rats results in uniform and marked inhibition of absolute and fractional reabsorption, averaging 45.2% ±5.5 SE and 34.9% ±6.1 SE, respectively. These observations are in accord with the recent findings by Spitzer and Windhager (26) who noted a fall in absolute reabsorption (measured using free-flow and shrinking drop techniques) of about 50% when proximal tubules, studied initially during normal blood perfusion, were restudied during capillary perfusion with non–colloid-containing Ringer's solution. While the argument in the latter authors' study was weakened somewhat by the relatively frequent occurrence of simultaneous reductions in GFR during capillary perfusion, the finding in the present study that inhibition in reabsorption was, at least in the majority of instances, independent of changes in filtration rate, lends strong support to their conclusion that a direct and causal relationship exists between postglomerular capillary protein concentration and absolute sodium reabsorption by the proximal tubule. This conclusion is further strengthened by the finding in the present study that during perfusion with hyperoncotic (15 g/100 ml) albumin-Ringer's solution, we measured

[6] Even if the change in absolute reabsorption of +122% noted in one tubule is excluded (and it should be noted that it is from this same tubule that the change in SNGFR of nearly 100% was seen) the change in absolute reabsorption for the remaining population still remains highly significant (+28.9% ±7.8 SE, $P < 0.005$).

significant and reversible increases in absolute and fractional reabsorption, averaging 41.0% ±6.3 SE and 34.7% ±11.9 SE, respectively. Again these changes in reabsorption in the majority of instances were independent of variations in filtered load.

From a technical standpoint the present study offers three advantages in experimental design over that employed by Spitzer and Windhager (26). Firstly, the influences of each of the various perfusion solutions usually were examined in the same rather than separate animals.[7] Secondly, by using micropipettes which had been internally coated with silicone, it was possible to perfuse wide areas of superficial cortex at rates often only slightly in excess of normal, rather than at the considerably higher rates (400–800 nl/min) regularly found necessary by these workers. Finally, albumin (normally the principal determinant of intracapillary oncotic pressure) rather than dextran served as the experimental colloid in this study.

The conclusion, based on these, as well as the findings of Spitzer and Windhager (26), that proximal reabsorption varies as a direct function of the postglomerular oncotic pressure has not regularly been reached by others. Thus, although Rumrich and Ullrich (29) calculated an average fall in proximal sodium transport (based on shrinking drop measurements) of 18% when capillaries were perfused with moderately reduced (6 g/100 ml) albumin-Ringer's solutions, they noted no further inhibition when colloid-free Ringer's was employed. Of interest however, is that these Ringer's solutions contained $NaHCO_3$ in concentrations of 35 rather than the more physiological 25 mmoles/liter. When a colloid-free Ringer's solution with the latter bicarbonate concentration was used proximal sodium transport fell an average of 47%. More recently however, using a batch of dextran supplied to them by Spitzer and Windhager (26), Rumrich and Ullrich did detect a significant influence of changes in peritubular capillary oncotic pressure on proximal reabsorption (see citation of this work on p. 255 of reference 40). Of interest in these latter studies is that 25 mM $NaHCO_3$ was used. Also using a colloid-free perfusate which contained 35 mM $NaHCO_3$, Lowitz, Stumpe, and Ochwadt (31) failed to detect significant changes in reabsorptive half-times of shrinking drops in segments of proximal (and distal) tubules, relative to preperfusion values. Unfortunately, tubule radius, necessary to insure that tubule volume remained constant during these maneuvers if transport rates are to be calculated from $t_{\frac{1}{2}}$ values, was not measured. This factor, together with the higher $NaHCO_3$ concentration, may explain their negative findings.

The present findings constitute evidence of a causal nature in support of at least one factor (postglomerular oncotic pressure) extrinsic to the epithelium of the proximal tubule as being a major determinant of isotonic reabsorption by this nephron segment. While nothing in the experimental design in this study excludes any simultaneous contribution by factors of intrinsic origin, there remains at present little evidence supporting a predominant role for such mechanisms (1–8). Nevertheless, we sought as part of the present study to test directly whether the parallel and roughly proportional adjustment in proximal reabsorption that takes place in response to alterations in glomerular filtration rate (glomerulotubular balance) is a consequence of some influence intrinsic to the tubule brought about by these concurrent alterations in filtered load, or results by virtue of accompanying parallel changes in filtration fraction (17, 28, 37) from direct and causal influences exerted by changes in postglomerular oncotic pressure. Precisely these parallel relationships between changes in GFR, absolute proximal reabsorption, filtration fraction, and postglomerular oncotic pressure have been observed during aortic constriction, renal venous occlusion, and carotid occlusion (28, 37), as well as during acute hemorrhage and volume expansion with isotonic saline (unpublished observations). From such observations however, one can only conclude that, for each of the conditions indicated, postglomerular oncotic pressure changes in the direction required by the hypothesis that the over-all regulation of proximal reabsorption is governed by the rate of uptake and removal of the epithelial reabsorbate by the postglomerular microcirculation. Remaining to be determined is whether these changes in postglomerular oncotic pressure are related in a causal way to the changes in reabsorption, or whether the changes measured in the former represent fortuitous parallel adjustments, and that the changes in reabsorption remain dependent on some as yet undefined influence initiated by these (thus far, undissociated) concomitant and parallel variations in glomerular filtration rate.

To test these possibilities, proximal reabsorption was studied initially during periods of reduced but stable

[7] As already discussed, in about 25% of instances during arteriolar perfusion, the perfusion pipette inadvertently entered the lumen of the proximal tubule under study. Recognition of this error was facilitated by the addition of Lissamine green to all perfusates, so that collected samples and involved tubules noted to be discolored were excluded from consideration. To the extent that lesser degrees of contamination may have occurred and gone unrecognized, the effect contributed by the addition of unknown volumes of these non-inulin-containing perfusates to the collected samples would be expected to spuriously lower our estimates of reabsorption. While this error would be in the appropriate direction to explain the observed changes in reabsorption during colloid-free Ringer's perfusion, the finding of no mean change during isoncotic perfusion and enhanced reabsorption during isoncotic perfusion makes highly unlikely the possibility that this could have been a frequent and systematic occurrence.

levels of renal perfusion pressure (achieved by partial aortic constriction). This maneuver produced significant parallel reductions in glomerular filtration rate and absolute proximal reabsorption of single nephrons, as well as concomitant reductions in postglomerular arteriolar but not systemic protein concentrations. Filtration fraction therefore declined. Postglomerular arteriolar and branch peritubular capillary protein concentration alone, and not glomerular filtration rate then was restored to preconstriction levels by means of microperfusion with 9–10 g/100 ml albumin-Ringer's solution. Under these conditions, absolute reabsorption nearly always paralleled the change in postglomerular protein concentration, rising, despite relatively constant reductions in filtration rate, to or toward preconstriction values. Fractional reabsorption therefore often reached remarkably high levels. Thus, in dissociating the simultaneously occurring changes in postglomerular oncotic pressure and filtration rate seen in glomerulotubular balance, we found absolute reabsorption to be influenced predominantly by the former. It should be noted that whereas absolute reabsorption was restored to preconstriction control levels in one rat (No. 12) during microperfusion, values for all rats increased, on average, to 52% of control. We view this restoration as a minimum value for the following reasons. (*a*) Microperfusion was carried out at rates slightly in excess of that normally measured for postglomerular blood flow (and clearly therefore, at rates higher than would be expected to obtain when renal perfusion pressure is reduced by aortic constriction) in order to insure adequate zones of perfusion. Were hydrostatic pressure in the vessels being perfused not higher than in adjacent vessels, perfusate would not displace the blood normally contained therein. (*b*) Not all convolutions of a given cortical proximal tubule reside on the surface, so that perfusate very likely failed to surround all portions of the tubule under study. (*c*) While the gross appearance of green color in the tubule lumen or collected samples (contributed by the Lissamine green marker) led us in six instances to recognize that the perfusion pipette entered the lumen of the experimental tubule, we had no sure way of excluding lesser degrees of contamination (which failed to produce significant discoloration). Since perfusate contamination could only be expected to lower estimates of reabsorption (perfusate contained no inulin) the extent to which reabsorption increased in this group may have been a minimum estimate.

Based on the foregoing, we believe it reasonable to conclude that glomerulotubular balance is causally mediated, at least in part, by adjustments in postglomerular oncotic pressure initiated as a result of changes in filtration fraction. That this is not the sole mechanism is suggested from the recent studies by Burg and Orloff

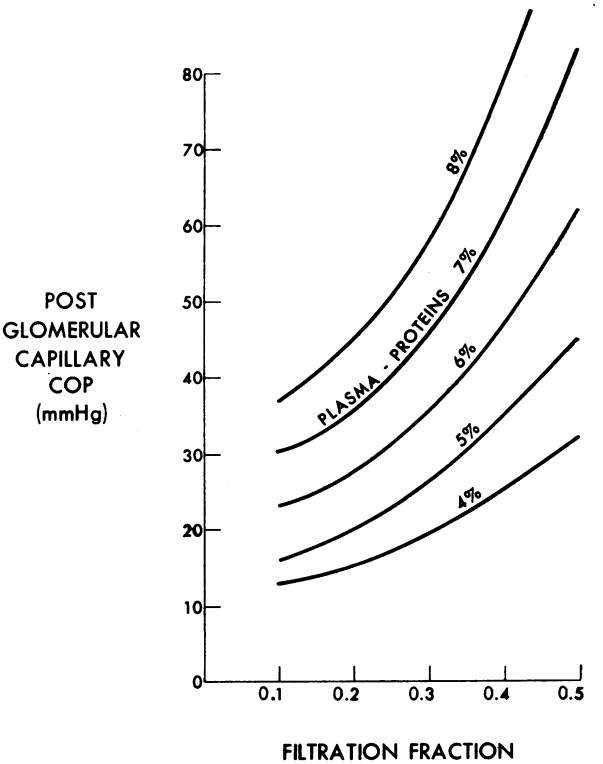

FIGURE 4 The interrelationships among pre- and postglomerular protein concentrations, filtration fraction (calculated using equation 3), and postglomerular colloid osmotic pressure (calculated from these postglomerular protein concentrations using the formula of Landis and Pappenheimer [41]) for different values of systemic plasma protein concentration. See text for a detailed discussion.

(2) and Buentig and Earley (8) who showed that, whereas absolute proximal reabsorption failed to undergo proportional changes in response to microperfusion-induced variations in load, there nevertheless appeared to be a minor adjustment, perhaps of intrinsic origin, which for each study averaged approximataely 20%. Also remaining to be explored in similar direct fashion as in the present study is the precise contribution, if any, to this phenomenon initiated by changes in transcapillary gradients of hydrostatic pressure.

At first glance it might appear somewhat surprising that changes in reabsorption of the magnitude shown could be the consequence of the relatively small measured changes in postglomerular arteriolar protein concentration. It should be recalled however, that aqueous solutions of proteins, unlike solutions of ideal solutes (electrolytes, for example) display nonlinear osmotic properties. In other words, because the osmotic pressure of protein solutions, but not of ideal solutions, depends in part on ionic strength, net charge, pH, and other factors (41), it tends to deviate from the linear Van't Hoff relationship with concentration otherwise true for ideal solutions.

Instead protein, or more generally, colloid osmotic pressure increases disproportionately with increasing concentration (41). Given this nonlinear osmotic profile of protein solutions, it seems of value to consider the extent to which glomerulotubular balance might be influenced by changes other than simply in filtration fraction. The complex interrelationships among pre- and postglomerular protein concentrations, filtration fraction and postglomerular colloid osmotic pressure (COP) are examined in Fig. 4. As an example, consider the situation in which preglomerular (i.e. systemic) plasma protein concentration exists at the normal value of 7.0 g/100 ml. The curve so designated in Fig. 4 describes the relationship between variations in filtration fraction and corresponding variations in postglomerular COP. Thus for filtration fractions ranging from 0.1 to 0.5, the resulting postglomerular protein concentrations (calculated from equation 3) increase from 6.3 to 14.0 g/100 ml. As shown, postglomerular COP, calculated from these latter values using the empirical formula of Landis and Pappenheimer (41), increases disproportionately with increasing protein concentration. During normal hydropenia in the rat in which filtration fraction averages about 0.35 (27), values for postglomerular oncotic pressure typically occur in the exponential or steep portion of the curve. Small changes in postglomerular protein concentration (±20%) therefore tend to exert rather large changes in COP, and are considerably in excess of simultaneously measured values for intratubular and intracapillary hydrostatic pressures (28, 37).

Following volume expansion with isotonic saline glomerulotubular balance still obtains (1, 3) but the mechanism of its "resetting" has not been established. A possible explanation is suggested from these interrelationships. As shown in Fig. 4, with progressive dilution of the plasma protein concentration the oncotic profile of the postglomerular blood is shifted toward the right so that when systemic protein concentration falls to levels of about 4 g/100 ml, postglomerular COP likewise is reduced and the curve remains relatively flat throughout the range of physiologically attainable filtration fractions. Thus for identical changes in GFR in hydropenia and saline-loaded animals, because of the lower and relatively flat profile of postglomerular COP for the latter, the magnitude of the adjustment in absolute reabsorption, as previously demonstrated (1, 3), is less than in hydropenia. Although thus far not tested, the opposite would be expected to obtain during contraction of extracellular volume. For any given level of filtration fraction under this condition, since systemic protein concentration would be expected to increase, postglomerular protein concentration (COP) would likewise increase. This would have the effect of displacing the curve to the left, thereby insuring that the steep portion encompasses almost the full range of physiologically attainable filtration fractions. The enhanced proximal reabsorption that would be expected to follow would thereby tend to restore extracellular volume to normal.

Of related interest is the finding that under normal hydropenic conditions values for postglomerular hematocrit are also found to fit the exponential portion of the similarly nonlinear viscosity-erythrocyte concentration curve (27, 33). The effects that changes in filtration fraction (glomerulotubular balance) and extracellular volume, by influencing the shape of this curve, might likewise have on pressure-flow relationships in the glomerular and postglomerular microvasculature have recently been discussed (27).

Windhager and associates (12, 26) have reviewed and discussed in detail the possible mechanisms whereby changes in postglomerular oncotic pressure are ultimately translated into changes in sodium and water transport by the proximal convolution. It has been a concern of the present authors and others (1, 25, 28) that these hypothetical and theoretical analyses were somewhat premature, in that evidence of a causal nature relating the control of proximal reabsorption to changes in transcapillary oncotic gradients had yet to be provided unequivocally. Given the findings in the present study, the timeliness of these theoretical inquiries perhaps no longer need be of concern.

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