Demonstration of Independent Roles of Proximal Tubular Reabsorption and Intratubular Load in the Phenomenon of Glomerulotubular Balance during Aortic Constriction in the Rat

WOLF E. BUENTIG and LAURENCE E. EARLEY

From the Department of Medicine and Cardiovascular Research Institute, University of California San Francisco, San Francisco, California 94122

ABSTRACT The mechanism of glomerulotubular balance was investigated by microperfusion of the rat proximal tubule at two different rates before and after contriction of the aorta sufficient to produce a 50% reduction in whole kidney filtration rate and plasma flow. At a perfusion rate of 28 nl/min the absolute rate of proximal tubular reabsorption averaged 4.80 ±0.28 nl/mm·min in the absence of aortic constriction. Reducing the perfusion rate by one-half resulted in only a 22% decrease in the absolute rate of reabsorption, and imbalance between load and reabsorption resulted as fractional reabsorption of the perfused volume increased from 0.56 to 0.83 at 3 mm length of perfused tubule. These observations support other studies indicating that changing the load presented to the individual proximal tubule does not change reabsorptive rate sufficiently to result in glomerulotubular balance. Aortic constriction decreased the absolute rate of proximal tubular reabsorption approximately 50%, resulting in imbalance between load and reabsorption at the higher perfusion rate (fractional reabsorption of the perfused volume fell to 0.23 at 3 mm). Thus, the decrease in proximal tubular reabsorption necessary for glomerulotubular balance will occur independent of a change in the load presented for reabsorption. Balance between load and reabsorption was produced artificially by combining aortic constriction and a reduction in perfusion rate proportional to the reduction in whole kidney filtration rate. Mathematical analysis of the data suggests that the absolute rate of reabsorption along the accessible length of the proximal tubule is constant and is not proportional to the volume of fluid reaching a given site. Thus, there appears to be no contribution to glomerulotubular balance of any intra- or extratubular mechanism directly coupling load and the rate of proximal tubular reabsorption. It is concluded that glomerulotubular balance during aortic constriction is a consequence of hemodynamic effects of the maneuver to decrease filtration rate and the rate of proximal tubular reabsorption independently but in an approximately proportional manner.

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INTRODUCTION

It is accepted generally that intrarenal mechanisms exist which couple the rate of proximal tubular reabsorption to the rate of glomerular filtration resulting in a somewhat imperfect, but nevertheless high degree of glomerulotubular balance when filtration rate is decreased experimentally. Despite extensive investigation into the nature of this phenomenon during the past several years the mechanisms responsible for near proportionality between the rates of filtration and proximal tubular reabsorption remain undefined. It has been thought that some effect of filtration rate per se may lead directly to an adjustment of proximal tubular reabsorption so as to effect the reabsorption of a constant fraction of the filtered load of sodium and water. Kelman suggested that proximal tubular reabsorption may change in a direct manner with changes in the linear velocity of the luminal fluid and the latter would be determined by the rate of filtration (1). Several lines of experimental evidence have been presented against this hypothesis (2–4). A different, but also nearly direct, mechanism for coupling proximal tubular reabsorption to the rate of filtration was suggested by Gertz, Mangos, Braun, and Pagel (5) who proposed, on the basis of their experimental observations, that a proportionality exists between luminal

volume and the rate of reabsorption in the proximal tubule. Therefore, glomerulotubular balance could result from a direct relationship between luminal volume and the rate of filtration. Although some studies have provided support for this hypothesis (2), the preponderance of recent evidence would seem to disprove a cause and effect relationship between intratubular volume and reabsorptive rate in the proximal tubule (3, 4, 6–8). Leyssac proposed that balance between filtration rate and proximal tubular reabsorption may result from the effect of an intrarenal hormonal mechanism (renin-angiotensin) which regulates proximal tubular reabsorption and which is controlled indirectly by the rate of filtration (9, 10). Although this general concept has not been disproved totally there is evidence that angiotensin has no effect to alter proximal tubular reabsorption directly (4, 11). Thus, several attractive hypotheses which could have accounted for the phenomenon of glomerulotubular balance have not withstood rigorous experimental testing.

Several micropuncture studies have shown that fractional reabsorption by the proximal tubule of the dog (12) and rat (13, 14) is depressed during volume expansion of the animal with isotonic saline. Thus, glomerular balance is disrupted when the excretion of sodium is augmented in response to infusion of saline. This disruption of glomerulotubular balance during volume expansion could result either from the superimposition of additional intrarenal or extrarenal factors that independently depress proximal tubular reabsorption or from a readjustment of the same intrarenal factors that determine glomerulotubular balance. Earley and associates (15–17) have presented evidence that depressed tubular reabsorption during volume expansion may be due, in large part, to a cause and effect relationship between peritubular capillary absorption and the rate of tubular reabsorption, and to an effect of volume expansion to decrease peritubular capillary absorption as a result of changes in hydrostatic and oncotic pressures across the capillary wall. Subsequently, it was suggested that these same hemodynamic and physical factors determining uptake by peritubular capillaries may be the forces determining glomerulotubular balance (18, 19). Lewy and Windhager observed in the rat that glomerulotubular balance was present when filtration rate was decreased by partial occlusion of the renal vein (19). Since increased renal venous pressure would be expected to increase peritubular capillary hydrostatic pressure, a change that should decrease capillary absorption, these authors (19) proposed this observation as evidence, in part, for the concept that glomerulotubular balance is a consequence of an adjustment of the peritubular capillary control of proximal tubular reabsorption. However, since glomerulotubular balance obtains when filtration rate is decreased during aortic or renal artery constriction (2, 3, 6, 7, 12), maneuvers that should decrease peritubular capillary hydrostatic pressure, and during ureteral constriction (7) which should result in a primary increase in intratubular hydrostatic pressure, the demonstration of glomerulotubular balance during renal venous constriction, in itself, provides little insight into the mechanism involved in the associated adjustment of proximal tubular reabsorption.

Despite extensive study of the phenomenon of glomerulotubular balance, only a few observations have been made which bear on the question of whether the changes in filtration and proximal tubular reabsorption are independent and only indirectly coupled processes. Morgan and Berliner (20) microperfused individual proximal tubules *in situ* at different rates and observed a constant absolute rate of proximal tubular reabsorption, indicating that balance between filtration and reabsorption does not follow when the load presented for reabsorption is the only factor changed in a single nephron under study. This observation is in agreement with the study of Burg and Orloff (4) who perfused isolated proximal tubules of the rabbit in vitro. This lack of a direct effect of changes in nephron perfusion rate, however, does not reveal whether glomerulotubular balance requires the combination of a change in nephron filtration rate and a change in other factors affecting tubular reabsorption. In striking contrast to the observations of Morgan and Berliner (20), Wiederholt, Hierholzer, Windhager, and Giebisch (21) used similar microperfusion techniques in the rat proximal tubule in vivo and observed a proportionality between nephron perfusion rate and the absolute rate of proximal tubular reabsorption. These latter authors concluded that changes in nephron filtration rate per se could result in proportional changes in the absolute rate of proximal tubular reabsorption. On the other hand, Lewy and Windhager (19) observed prolongation of the reabsorptive time in the rat proximal tubule during renal venous constriction sufficient to reduce whole kidney filtration rate and the reabsorptive rate of proximal tubules in filtering nephrons. Only this latter observation suggests that the reduction in reabsorptive rate necesary for balance when filtration rate is reduced experimentally occurs independently of a change in filtration in the nephron under study.

In the present study microperfusion techniques were utilized to determine the degree of independence of nephron filtration rate and proximal tubular reabsorption during aortic constriction, the maneuver which has been used most commonly to demonstrate glomerulotubular balance. The results indicate that the relationship between intratubular load and proximal tubular reabsorption can be uncoupled by either changing the load (nephron perfusion rate) or by changing the rate of tubu-

lar reabsorption (aortic constriction). Reducing load alone increased fractional reabsorption by the perfused nephron, and aortic constriction alone decreased fractional reabsorption by the perfused nephron. Balance between load and reabsorption was restored by combining a reduced perfusion rate (to decrease load) and aortic constriction (to decrease the rate of reabsorption). These observations are consistent with the view that glomerulotubular balance during aortic constriction is a consequence of hemodynamic effects of the maneuver to decrease filtration rate and the rate of proximal tubular reabsorption through different mechanisms.

METHODS

Male Sprague-Dawley rats weighing approximately 275 g were anesthetized by the intraperitoneal injection of Inactin, 12 mg/100 g body weight, and placed on a heated operating table that maintained body temperature at 37.5°C. An endotracheal tube was placed surgically, and polyethylene catheters were inserted into the right external jugular vein and the left carotid and right femoral arteries. The left kidney was exposed through a flank incision, stripped of its perirenal fat, and immobilized by placement in a Lucite cup which was attached firmly to the operating table. The left ureter was cannulated with plastic tubing near the renal pelvis. The capsule of the kidney was not removed, and the surface of the kidney was bathed continuously with mineral oil heated to 37.5°C. An adjustable snare was placed around the aorta above the left renal artery and usually below the right renal artery. During the surgical procedures the animals received an intravenous infusion of a modified Ringer's solution (Na, 142; K, 3.5; Cl, 125.5, and HCO_3, 20, mEq/liter, respectively), at a rate of 0.01 ml/100 g body weight per min. Approximately 1 hr prior to experimental measurements this infusion was replaced with the same Ringer's solution containing inulin and *p*-aminohippurate (PAH) in amounts appropriate for clearance measurements. Also, at this time tap water, 3 ml/100 g body weight, was injected directly into the stomach, and an infusion of 2.5 g/100 ml dextrose in water was begun at 0.02 ml/100 g body weight and continued throughout the experiment.

Micropuncture techniques. Perfusion of single superficial proximal tubules was performed essentially as described by Sonnenberg, Deetjen, and Hampel (22). Perfusion and injection pipettes were made on an automatic pipette puller from 1 mm glass capillary tubing, and the tips were ground to a bevel diameter of 10–12 μ. The perfusion pump was fabricated as follows. The end of a micropipette holder was permanently attached to the tip of a gas-tight 10 μl glass syringe (Hamilton Co., Whittier, Calif.) in such a way that the perfusion pipette became an extension of the syringe. The syringe with attached micropipette was fixed to the platform of a Leitz micromanipulator, and the end of the metal piston of the syringe was fitted against the advancing end of a screw-type micrometer caliper, which was also fixed to the micromanipulator. The drive of the micro-caliper was connected by a flexible metal cable to the shaft of an electric motor not attached to the micromanipulator. The electric motor contained a 1:1800 reduction gear system, and the speed of the motor was controlled by a highly stable electronic power supply. The speed of the motor was monitored by the output of a DC generator attached to one end of the armature. Once the speed of the motor was set there was no detectible variation in the output of the DC generator. The volume delivery of this perfusion system was calibrated by three different techniques several times during the course of these experiments, was found to be virtually identical on each occasion and related linearly to the DC output between delivery rates of 10 and 110 nl/min. The volume delivered by the pump was measured by collecting timed samples of mineral oil delivered through a perfusion pipette with a 30 μ tip, or water delivered through a pipette with a 9 μ tip, into a constant bore glass capillary. Corrections were made for evaporation of the water. The third method of calibration was to make timed collections of a perfusion solution containing iothalamate-^{125}I of known concentration of radioactivity.

The solution used to perfuse proximal tubules was made by adding Lissamine green (final concentration, 0.1 g/100 ml) and sodium iothalamate-^{125}I (final specificity activity, 10 μCi/ml) to an aqueous solution containing 145 mM Na, 5 mM K, 130 mM Cl, 20 mM HCO_3, 5 mM urea, and 2.5 mM glucose, respectively. The solution had a final osmolality of 292[1] and a pH of 8.1 in the absence of CO_2. The identity of clearances of iothalamate-^{125}I and inulin has been reported by others (23, 24). The use of iothalamate as a marker for reabsorption in the present study was examined in two ways. Single proximal tubules of the left kidney were perfused for 5 min at a rate of 20 or 40 nl/min, and urine was collected for the next 30 min from both the left and right kidneys. Small amounts of radioactivity were present in urine collected from the right kidney, and it was ascertained by other experiments that this was due to absorption of the perfusion fluid from the surface of the left kidney, presumably prior to entry of the perfusing pipette into the tubular lumen. Therefore, in the recovery experiments the amount of radioactivity appearing in the urine from the right kidney was subtracted from the amount excreted by the left (test) kidney. Urinary recovery of the isotope perfused during 5 min into a proximal tubule of the left kidney, calculated as described, was 99.4 ±2.3 (SE)% in 14 determinations in three rats. In an additional 14 perfusions of proximal tubules at 28 nl/min timed collections (2–5 min) were made through a micropipette inserted into the tubule downstream and just proximal to an oil block in the distal portion of the perfused tubule. Recovery of isotope determined in this manner averaged 98.2 ±1.1 (SE)% of the amount perfused, based on the in vitro calibration of the pump.

Experimental perfusions of the proximal tubule were performed as follows. A small drop of mineral oil was injected into a proximal tubule in order to determine the direction of flow through the surface convolutions. The perfusing pipette, with the pump operating, was then inserted slightly distal to the first pipette. An oil block was then injected through the first pipette proximal to the perfusing pipette. The first pipette was then removed leaving a punctured site between the glomerulus and the oil block proximal to the perfusion site. The perfused convolutions could be identified easily due to the presence of Lissamine green in the perfusion solution, and the most distal convolution was evident from the intensity of the color of the dye. After making a sketch of the perfused tubule, a collection site was

[1] Osmolality of collected fluid in 33 perfusions in five animals under the conditions of these experiments averaged 280 ±2 mOsm/kg yielding a ratio of 0.96 (collected fluid/perfusion fluid).

selected into which the collecting pipette was inserted. Depending on the rate of perfusion, collection periods varied from 5 to 20 min, and no attempt was made routinely to collect the total volume of fluid reaching the collection site. The volume of fluid collected was no more than approximately 25% of the perfused volume. Negative presrure in the collecting pipette was avoided by placing the oil-filled aspirating syringe just above the surface of the kidney. This permitted a slow rate of continuous collection. By avoiding negative pressure in the collecting syringe, and collecting fluid at a rate distinctly less than the rate at which fluid should have been reaching the collecting pipette, it is felt that retrograde contamination was minimized. After completing collections from a single proximal tubule the convolutions were filled with latex. At the completion of an entire study the kidney was macerated in 6 N HCl at 40°C, usually for 10–15 min. The latex casts of proximal convolutions were dissected free, and the perfusion and collection sites identified from the sketches. The distance between perfusion and collection sites was measured under the dissecting microscope by using an eyepiece micrometer. The lengths of proximal tubular segments perfused was varied intentionally and ranged between 0.2 and 3.0 mm.

Pressure in the carotid and femoral arteries was monitored throughout the experiment by a pressure transducer and a direct writing recorder (Hewlett-Packard). The aorta was constricted above the left renal artery to reduce femoral arterial pressure to values between 65 and 90 mm Hg, and at least 30 min were allowed after adjusting the aortic constriction before restarting experimental collections. Proximal convolutions were perfused at either 14 or 28 nl/min, both before and after constricting the aorta to reduce perfusion pressure to the left kidney. The transit time of Lissamine green was determined as described by Steinhausen (25) before and after aortic constriction. A total of 112 perfusions were performed in 21 animals, and the sequence of perfusion rates was alternated. In preliminary studies it was observed that reperfusion of the same tubule always resulted in a decrease in the calculated rate of reabsorption, and, therefore, reperfusion of the same tubular segment before or after aortic constriction was not attempted. Thus, each reported measurement usually represents a different proximal tubule. In a few instances up to three collections were made from the same proximal tubule by reinserting the collecting pipette at progressively shorter distances from the perfusing pipette. When a collection was completed the sample was sealed by aspirating a small amount of mineral oil into the tip of the collection pipette. Duplicate samples were then transferred under oil into a calibrated pipette (volume approximately 20 nl) and then expelled quantitatively under oil into a 20 μl volume of water. This final dilution of the collected sample was transferred quantitatively to a capillary tube for measurement of radioactivity in a Packard automatic gamma counter. Duplicate samples of the perfusion solution were handled in precisely the same manner as the samples collected from perfused tubules. Fractional reabsorption of the perfused fluid was calculated as $1-(PF/CF)_I$ where PF and CF are the total amounts of iothalamate-^{125}I in equal volumes, respectively, of perfusion fluid and collected fluid. The absolute rate of tubular reabsorption (C) was calculated as follows:

$$C(\text{nl/mm}\cdot\text{min}) = \frac{V_i[1 - (PF/CF)_I]}{L}$$

where V_i is the rate of perfusion of the tubule and L is the length of the tubule (in millimeters) between the perfusing and collecting pipettes.

Urine was collected from the left ureteral catheter directly into capillary micropipettes (Microcaps), and samples of arterial blood (0.1–0.2 ml) were withdrawn at the midpoint of urine collections. These clearance periods varied from 3.5 to 20 min, and two or three collections were made before and after constricting the aorta. Inulin, PAH, Na, K, and osmolality in plasma and urine were determined by techniques described previously (15).

RESULTS

In 14 animals prior to constriction of the aorta clearances of inulin and PAH (experimental kidney) averaged 1.08 ±0.07 (SD) and 3.79 ±0.17 ml/min, respectively, and the rate of sodium excretion averaged 555 ±72 nEq/min. These excretory data are based on a minimum of 43 separate collections, usually three consecutive clearance periods in each animal. 49 determinations of proximal tubular transit time of Lissamine green averaged 9.6 ±0.3 sec. A total of 37 proximal tubular perfusions were performed in 13 of these animals at a perfusion rate of 28 nl/min, and collections were made at distances ranging from 0.2 to 2.9 mm beyond the point of perfusion (Fig. 1). The absolute rate of volume reabsorption by proximal tubular segments, calculated from the perfusion rate $(CF/PF)_I$ and segment length averaged 4.80 ±0.28 nl/min·min (Table I). This average rate of reabsorption represented a calculated fractional reabsorption of 56% of the perfused volume at a proximal tubular segment length of 3 mm (Fig. 1, Table I). A total of 42 proximal tubular perfusions were performed in the 14 animals at a perfusion rate of 14 nl/min. At this lower perfusion rate the absolute rate of volume reabsorption by the proximal tubule averaged 3.73 ±0.23 nl/mm·min (Table I), which was significantly different ($P = < .001$)[2] than the rate of reabsorption observed at a perfusion rate of 28 nl/min (Fig. 2). However, since the decrease in reabsorptive rate was proportionately much less than the decrease in perfusion rate, fractional reabsorption of the perfused volume at 3 mm segment length increased to 83% at the lower perfusion rate.

In 12 animals the aorta was constricted above the level of the left renal artery to reduce pressure in the aorta below the constriction to 65–90 mm Hg. Once the aortic constriction had been adjusted to achieve the desired reduction in pressure no further adjustments were required to maintain the lower pressure at a stable value. Aortic constriction resulted in a fall in clearances of inulin and PAH to 0.52 ±0.06 and 1.73 ±0.18 ml/min, respectively, and a fall in the rate of excretion of sodium to 107 ±9 nEq/min (averages of 24 clearance collections). 24 determinations of proximal tubular transit

[2] Determined from Student's t test for unpaired data.

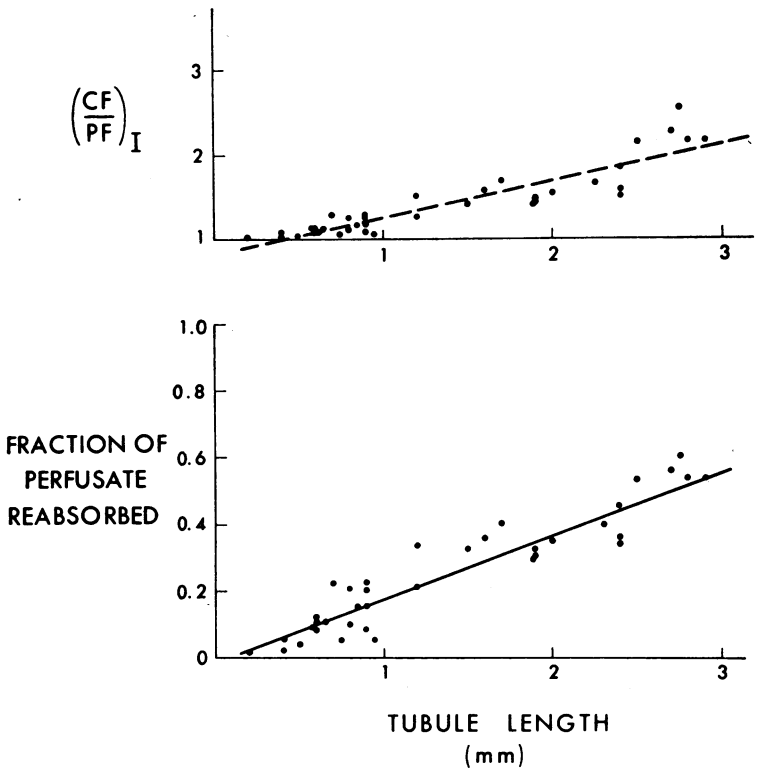

FIGURE 1 Proximal tubular reabsorption by control kidneys (absence of aortic constriction) at a perfusion rate of 28 nl/min. The results of individual perfusions of proximal tubules are shown. The upper panel indicates the concentration ratio of iothalamate-^{125}I (collected fluid/perfusion fluid) at various lengths of proximal tubule. The lower panel gives the same data calculated as the fraction of perfused fluid reabsorbed. The lines are least squares regressions.

time of lissamine green averaged 23.5 ±1.5 sec during aortic constriction. 12 proximal tubular perfusions in six of these animals were performed at a perfusion rate of 28 nl/min, and collections were made 0.4–2.9 mm beyond the site of perfusion. During aortic constriction the absolute rate of reabsorption of the perfused fluid at a perfusion rate of 28 nl/min averaged 2.57 ±0.46 ml/mm·min (Table I). This rate was significantly different from that observed at a perfusion rate of 28 nl/min in the absence of aortic constriction ($P = < 0.001$). Fractional reabsorption of the perfused fluid at a perfusion rate of 28 nl/min was decreased during aortic constriction to 23% at 3 mm segment length (from a value of 56 per cent in the absence of aortic constriction). 21 perfusions were performed in seven of these animals at a perfusion rate of 14 nl/min during aortic constriction. Collections were made over tubular segment lengths ranging from 0.3 to 3.0 mm (Fig. 3). The absolute rate of reabsorption in these experiments (perfusion rate 14 nl/min in the presence of aortic constriction) averaged 2.50 ±0.38 nl/mm·min. This was not significantly different from the rate of reabsorption observed at a perfusion rate of 28 nl/min in the presence of aortic constriction but was significantly different ($P = < 0.001$) from the rates of reabsorption observed at a perfusion rate of either 28 or 14 nl/min in the absence of aortic constriction (Table I). The effects of aortic constriction on individual measurements at a perfusion rate of 14 nl/min are shown in Fig. 3, and it can be seen that the individual measurements were clearly displaced below regression lines describing $(CF/PF)_I$ and fractional reabsorption at a perfusioin arte of 14 nl/min in the absence of aortic constriction. In Fig. 4 these same experimental points during aortic constriction at a perfusioin rate of 14 nl/min are superimposed on the regression lines describing the experimental observations at a perfusion rate of 28 nl/min in the absence of aortic constriction, and it can be seen that fractional reabsorption and $(CF/PF)_I$ were similar at any length under the two different experimental conditions. Thus, in the absence of aortic constriction, reducing the perfusion rate 50% was associated with an average decrease of 22% in the absolute

rate of reabsorption and an increase in the fraction of perfused fluid reabsorbed at 3 mm from 56 to 83%. Constricting the aorta to produce an average fall in whole kidney filtration rate of 52% resulted in a decrease in the absolute rate of proximal tubular reabsorption to 54% and 67% of the rates observed at 28 and 14 nl/min, respectively, in the absence of aortic constriction. The effects in individual animals of the two different perfusion rates, and of aortic constriction at the same perfusion rate, on the absolute rate of proximal tubular reabsorption are summarized in Fig. 5.

The relationship between the length of proximal tubule perfused and $(CF/PF)_I$ (concentration ratio of iothalamate-^{125}I collected fluid/perfused fluid) and the fraction of perfused fluid reabsorbed are shown for individual measurements in Figs. 1–3. At a perfusion rate of 28 nl/min a good fit of the points was obtained for straight line regressions of either $(CF/PF)_I$ or fractional reabsorption against segment length ($r = 0.91$ and 0.94, respectively), Fig. 1. However, mathematically both functions cannot be linear. If fractional reabsorption increases linearly with tubular length then $(CF/PF)_I$ must increase hyperbolically, and if $(CF/PF)_I$ increases linearly then fractional reabsorption approaches 100% as an asymptote. Below $(CF/PF)_I$ of 2.0, fractional reabsorption of 50%) the data are inadequate to distinguish between these two possibilities. At a perfusion rate of 14 nl/min in the absence of aortic constriction $(CF/PF)_I$ increased to values between 2 and 5 at tubular segment lengths between 2 and 3 mm. A least squares linear regression calculated for these points ($r = 0.87$) has an origin displaced a considerable positive distance along the abscissa (tubule segment length) with points at either end falling away from the regression line (Fig. 6). Furthermore, when this regression line is plotted as fractional reabsorption (lower panel of Fig. 6, broken line) there is a poor visual fit of the experimental points. On the other hand, the linear regression of fractional reabsorption on tubule segment length shows a good fit of the experimental points ($r = 0.93$) and an intercept very close to zero on both axes (lower panel of Fig. 6, solid line). When this latter regression formula was plotted as $(CF/PF)_I$ an excellent visual fit of the experimental points was obtained (upper panel of Fig. 6, solid line). For these reasons we conclude that the present data best fit a linear increase in fractional reabsorption and a hyperbolic increase in $(CF/PF)_I$ with increasing tubule segment length.

DISCUSSION

In the present study the absolute rate of proximal tubular reabsorption did not decrease in proportion to an experimentally produced decrease in nephron perfusion rate. When the rate of perfusion of the individual tubule was reduced by one-half the average rate of reabsorption

TABLE I

*Independent Effects of Aortic Constriction and Nephron Perfusion Rate on Reabsorption by the Rat Proximal Tubule**

V_i	C	TT	Fractional reabsorption‡	Aortic pressure	GFR	C_{PAH}	$U_{Na}V$
nl/min	*nl/min·min*	*sec*		*mm Hg*	*ml/min*	*ml/min*	*nEq/min*
Control ($n_1 = 20$)							
28	4.80	9.6	0.56	133	1.08	3.79	555
($n_2 = 37$)	±0.28	±0.3	±0.03	±3	±0.07	±0.17	±72
14	3.73		0.83				
($n_2 = 42$)	±0.23		±0.05				
Aortic constriction ($n_1 = 12$)							
28	2.57	23.5	0.23	82	0.52	1.73	107
($n_2 = 12$)	±0.46	±1.5	±0.05	±2	±0.06	±0.18	±9
14	2.50		0.52				
($n_2 = 21$)	±0.38		±0.08				

Abbreviations are as follows: V_i = rate of pump perfusion of proximal tubule; C = rate of proximal tubular reabsorption of perfusate; TT = proximal tubular transit time of lissamine green; GFR = clearance of inulin by experimental kidney; C_{PAH} = clearance of *p*-aminohippurate by experimental kidney; $U_{Na}V$ = rate of excretion of sodium by experimental kidney; n_1 = number of animals studied under the respective conditions; n_2 = number of proximal tubular perfusions under the respective conditions.

* Values are means and standard deviations.

‡ Fractional reabsorption of the perfused solution was calculated from the mean C as that which would obtain at a proximal tubular segment length of 3 mm.

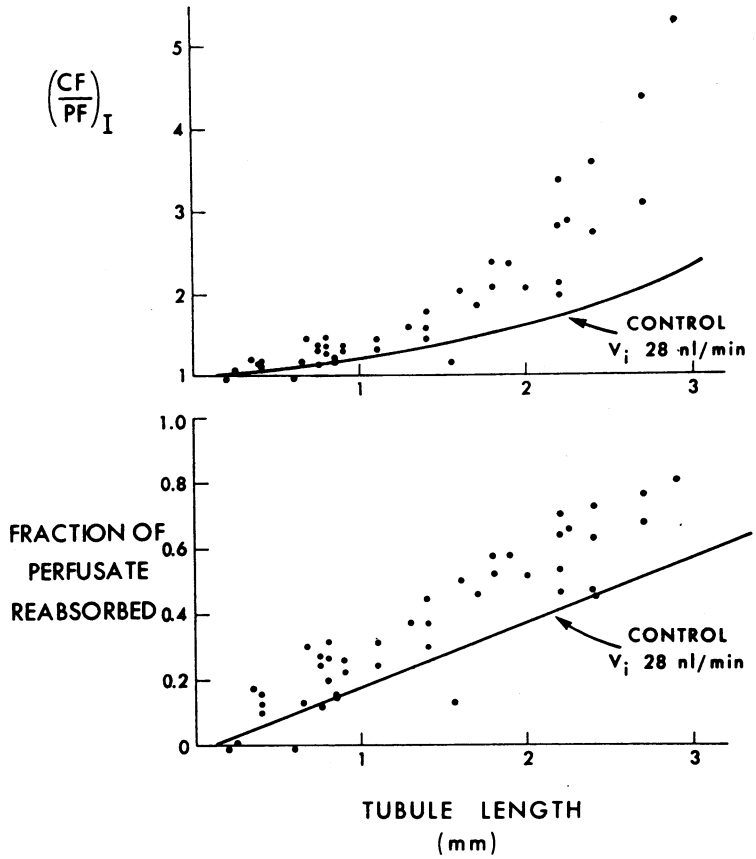

FIGURE 2 Proximal tubular reabsorption by control kidneys (absence of aortic constriction) at a perfusion rate of 14 nl/min. Each point represents a single proximal tubular perfusion as in Fig. 1. The lines are the least squares regression of fractional reabsorption at a perfusion rate of 28 nl/min (lower panel) and the mathematical conversion of this regression to CF/PF (upper panel). As shown, the ratio $(CF/PF)_I$ and the fraction of perfusate reabsorbed at a perfusion rate of 14 nl/min were greater than observed at a perfusion rate of 28 nl/min.

decreased only 22%. This resulted in imbalance between the load presented and the rate of proximal tubular reabsorption as the fraction of perfused fluid reabsorbed by a 3 mm segment of tubule increased from 56 to 83%. This is in contrast to the observations of Wiederholt and associates (21) who reported near proportionality between the rate of proximal tubular reabsorption and rates of proximal tubular perfusion through the same range of perfusion rates as those employed in the present study. Our results are in closer qualitative agreement with those of Morgan and Berliner (20) obtained from in vivo perfusion of the rat proximal tubule, and those of Burg and Orloff (4), obtained from in vitro perfusion of the isolated rabbit proximal tubule. Thus, the present studies support the conclusion made earlier by others (4, 8, 19, 20) that there is no direct intrinsic coupling between the load or volume presented to the proximal tubule and the rate of proximal tubular reabsorption, which could account entirely for the phenomenon of glomerulotubular balance.

The rate of proximal tubular reabsorption observed in this study at the higher rate of nephron perfusion is approximately twice that reported by Morgan and Berliner (20) and is close to that observed by Widerholt and associates (21) at a similar nephron perfusion rate. The reasons for the difference in the rate of absorption observed by Morgan and Berliner (20) and that observed in the present study and by Wiederholt and associates (21) is not clear. Nephrons were perfused with a Ringer's solution in the latter two studies, whereas Morgan and Berliner (20) perfused with 0.9% saline. If the filtration rate of individual superficial rat nephrons is as great as 40 nl/min (3, 6, 7, 26), the reabsorptive rate observed in the present study would result in the reabsorption of about 60% of the filtrate at a distance of 5 mm along the proximal tubule. Free flow micro-

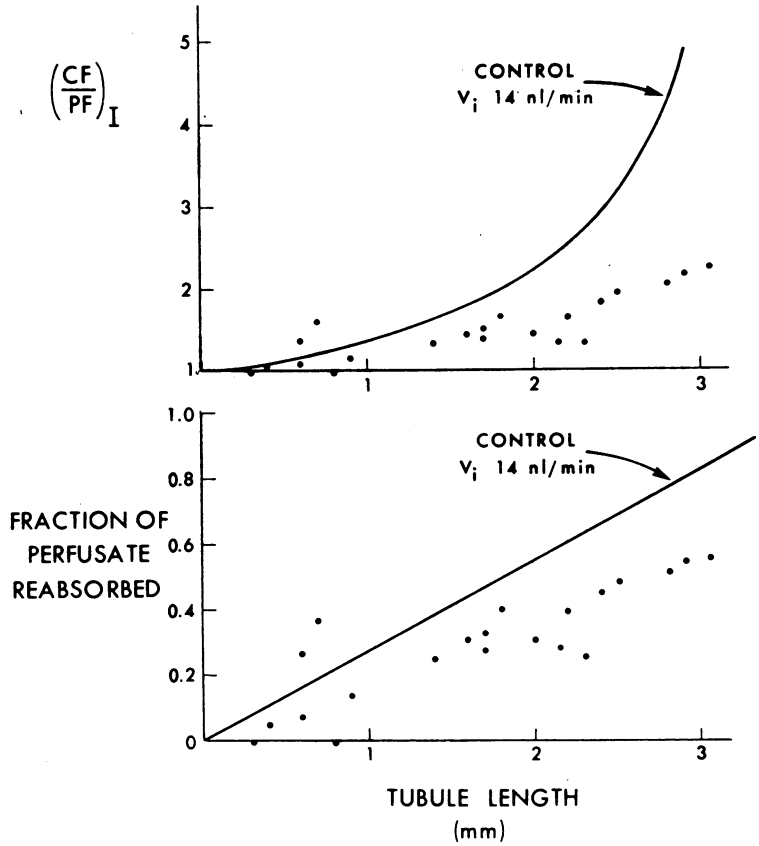

FIGURE 3 Proximal tubular reabsorption during aortic constriction at a perfusion rate of 14 nl/min. The lines are the least squares regression of fractional reabsorption of perfusate at a perfusion rate of 14 nl/min in the absence of aortic constriction (lower panel) and the conversion of this regression to CF/PF (upper panel). As seen, the points during aortic constriction fall considerably below the mean values in the absence of aortic constriction, indicating a decrease in the rate of reabsorption of perfused fluid.

puncture studies in the rat indicate that approximately 50% of the filtrate is reabsorbed at a distance of 50–60% of the length of the superficial rat proximal tubule (13, 27). Assuming a proximal tubule length of 9 mm (3, 28) and a filtration rate of 40 nl/min this would indicate a reabsorptive rate of 3.7–4.4 nl/mm·min, a rate close to that observed in the present studies. The possibility that some retrograde collection of perfusing fluid contributed to the calculated reabsorptive rate in the present study cannot be eliminated, but for the reasons discussed in Methods this seems unlikely.

The present experiments were extended to demonstrate that the relationship between intratubular load and proximal tubular reabsorption may be uncoupled in the absence of a change in nephron perfusion rate as a consequence of decreased tubular reabsorption resulting from aortic constriction. Constriction of the aorta sufficient to decrease whole kidney filtration rate by one-half resulted in approximately a 50% decrease in the absolute rate of reabsorption by the perfused proximal tubule. At the higher perfusion rate aortic constriction resulted in imbalance between perfusion and reabsorptive rates as the fraction of perfused fluid reabsorbed by a 3 mm segment of proximal tubule fell from 56 to 23%. This degree of aortic constriction has been shown to be associated with a high degree of glomerulotubular balance in the dog (12) and rat (2, 3, 6, 26) during free flow micropuncture studies. Our findings demonstrate, therefore, that the reduction in proximal tubular reabsorption accompanying glomerulotubular balance during aortic constriction will occur independent of a simultaneous reduction in filtration rate by single nephrons under study. This conclusion is in substantial agreement with the observation of Lewy and Windhager (19) that the reabsorptive rate of isolated droplets in the rat proximal tubule (shrinking drop technique) was decreased during

renal venous constriction sufficient to reduce whole kidney filtration rate by approximately one-half.

The imbalance between nephron perfusion rate and proximal tubular reabsorption observed during reduction of perfusion rate or constriction of the aorta alone was restored to balance by combining the two maneuvers. When the perfusion rate during aortic constriction was decreased by one-half, approximately the same degree to which whole kidney filtration rate was decreased, the fraction of perfused fluid reabsorbed by a 3 mm segment of proximal tubule was restored to nearly the same value as that observed at the higher perfusion rate in the absence of aortic constriction. This observation supports a major conclusion of the present study, viz, that glomerulotubular balance during aortic constriction is the consequence of two independent effects of the maneuver—one to lower filtration rate and the other to lower the rate of proximal tubular reabsorption.

The present study provides no information on the mechanism whereby aortic constriction causes a decrease in the rate of proximal tubular reabsorption. However, it seems likely that this effect is a consequence of some hemodynamic change that controls tubular reabsorption. Earley and associates (16–18, 29, 30) and others (31) have presented evidence that tubular reabsorption may be depressed by increased peritubular capillary hydrostatic pressure, presumably as a consequence of diminished capillary removal of reabsorbate. Lewy and Windhager (19) proposed that increased capillary hydrostatic pressure could account, in part, for decreased proximal tubular reabsorption during constriction of the renal vein. This mechanism could not account for the present ob-

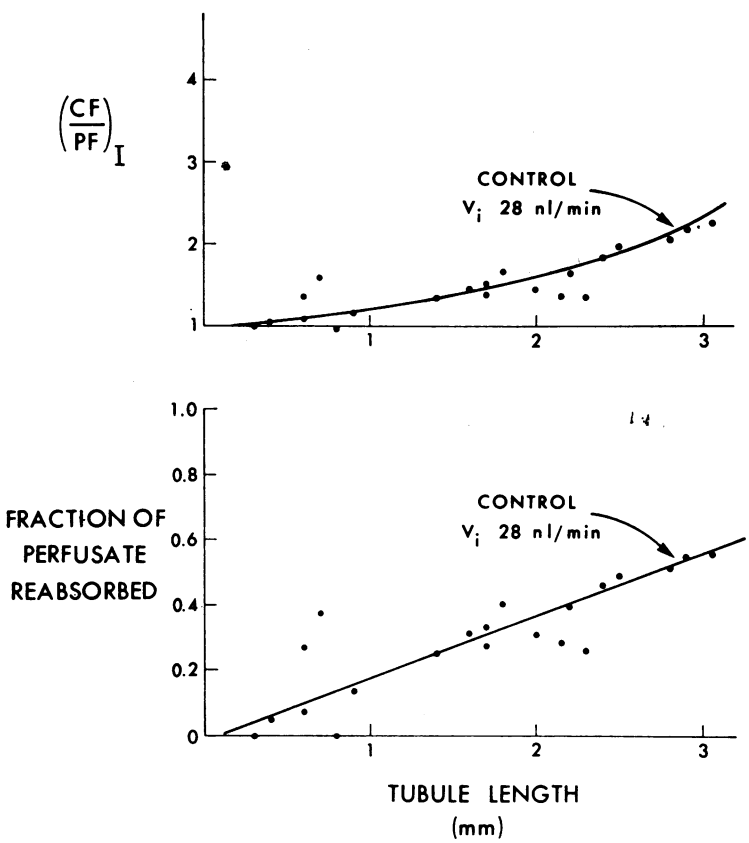

FIGURE 4 Comparison of proximal tubular reabsorption at reduced tubular perfusion rate during aortic constriction to reabsorption at the higher tubular perfusion rate in the absence of aortic constriction. Individual points are the same as shown in Fig. 3 for a tubular perfusion rate of 14 nl/min in the presence of aortic constriction, and the lines are those shown in Fig. 2 for reabsorption at a tubular perfusion rate of 28 nl/min in the absence of aortic constriction. Thus, the lower rate of tubular perfusion in the presence of aortic constriction resulted in increases in CF/PF and fractional reabsorption of perfusate similar to those observed at a perfusion rate of 28 nl/min in the absence of aortic constriction.

servation that aortic constriction decreases proximal tubular reabsorption since the expected reduction in capillary hydrostatic pressure should enhance capillary absorption. For that matter, adjustments in tubular reabsorption necessary in the phenomenon of glomerulotubular balance may not be mediated, under other experimental circumstances, primarily through changes in capillary hydrostatic pressure. This conclusion is based not only on the present study but also on consideration of other studies demonstrating glomerulotubular balance when filtration rate is decreased by arterial, ureteral, or renal venous constriction (7), maneuvers that should have grossly different effects on the gradient of hydrostatic pressure across the capillary wall. It seems possible that even though the absolute hydrostatic pressure within the kidney differs with these different maneuvers, little, if any, gradient of pressure between the various intrarenal compartments (tubule, interstitium, capillary) may be generated under either circumstance (19, 31–33). This is not to say that primary changes in capillary hydrostatic pressure do not affect the rate of proximal tubular reabsorption when filtration rate is relatively constant (15–18, 29–30).

A large body of evidence exists consistent with the view that a direct relationship obtains between plasma protein concentration and the rate of tubular reabsorption (16–18, 30, 34, 35). This association has been attributed also to a direct causal relationship between capillary removal of reabsorbate and the rate of proximal tubular reabsorption. In the present studies, as well as other studies employing renal venous or ureteral constriction, decreased proximal tubular reabsorption could have been a consequence of decreased oncotic (protein) absorptive force due to a reduction in renal blood flow. Although the initial concentration of protein in postglomerular capillary plasma relates directly to the filtration fraction, the total peritubular oncotic force will be a function of both the concentration of protein in plasma and the rate of plasma flow through peritubular capillaries. Therefore, the simultaneous reduction of renal plasma flow when filtration rate is reduced by aortic, ureteral, or renal venous constriction could decrease proximal tubular reabsorption as a consequence of limited capacity for removal of reabsorbate by the reduced peritubular plasma flow. This suggestion may seem at variance with observations that increased renal plasma flow during induced renal vasodilatation is accompanied by decreased tubular reabsorption of sodium (15, 18, 29, 30). However, during induced vasodilatation filtration fraction (29, 30) and presumably the initial concentration of protein in postglomerular plasma are decreased, and therefore, the total peritubular oncotic

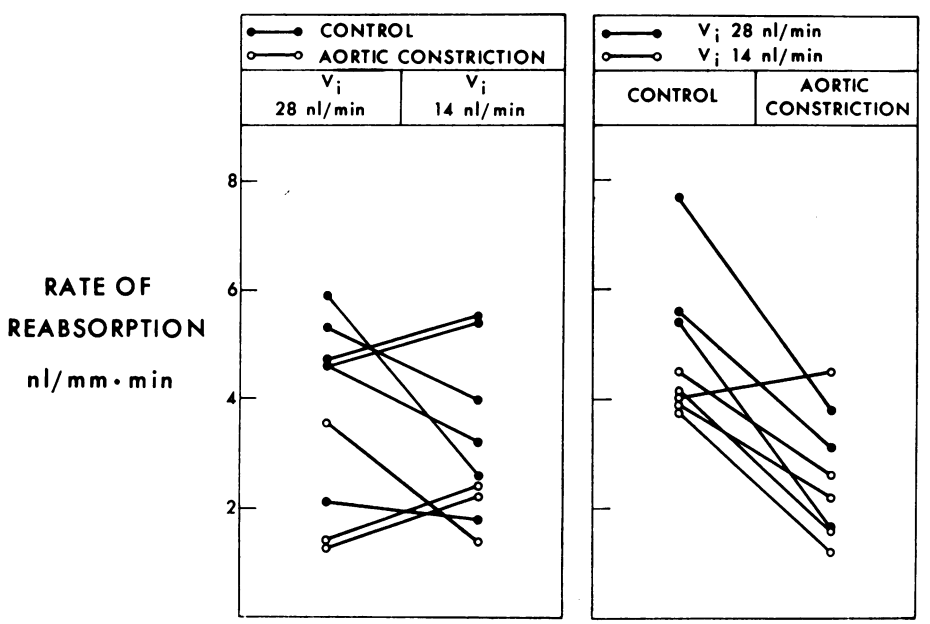

FIGURE 5 Independent effects of different perfusion rates and aortic constriction on the absolute rate of proximal tubular reabsorption in individual animals. Each point is the mean of three or more proximal tubular perfusions. As shown in the panel on the left decreasing the perfusion rate alone had variable effects on the rate of proximal tubular reabsorption, whether in the presence of or absence of aortic constriction. In all but one animal aortic constriction resulted in a large decrease in the rate of reabsorption at both perfusion rates (right hand panel).

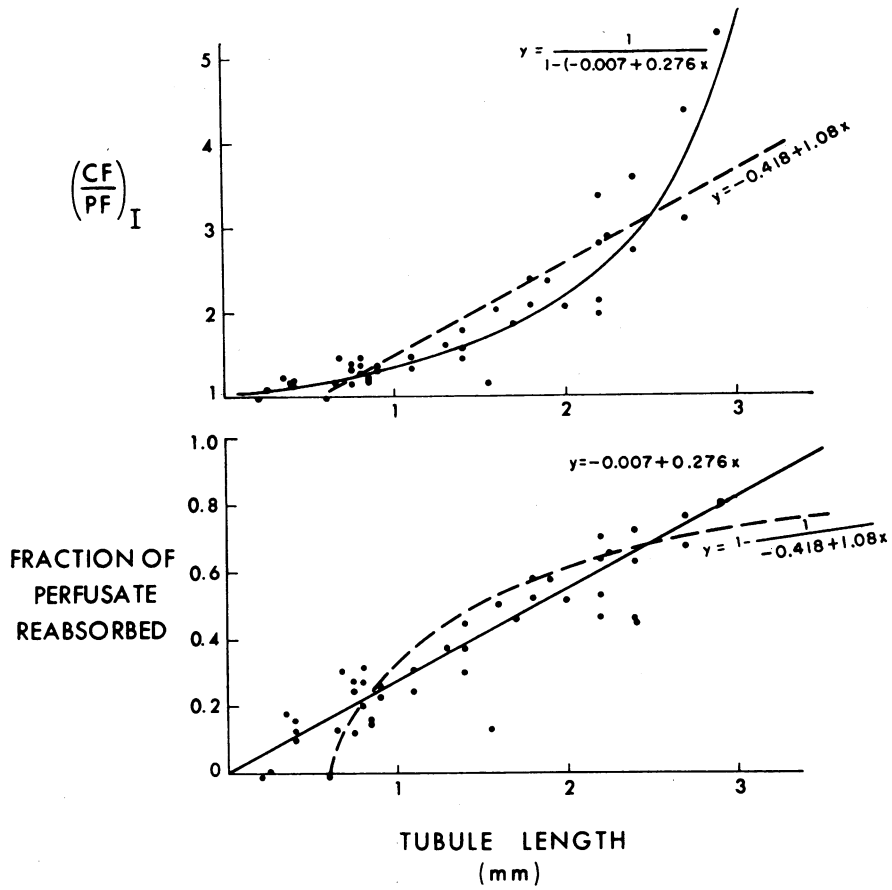

FIGURE 6 Relationship between reabsorption and the length of proximal tubule perfused. The points are the same as those shown in Fig. 2. The broken lines represent the least squares fit of $(CF/PF)_I$ against tubule length (upper panel) and the mathematical conversion of this regression to fraction of perfusate reabsorbed (lower panel). As seen, the intercept of these curves is displaced from zero tubule length, with many experimental observations falling away from the curves. The solid lines are the least squares regression of fractional reabsorption of perfused fluid against tubule length (lower panel) and the mathematical conversion of this curve to CF/PF against tubule length (upper panel). As seen this latter regression intercepts close to zero tubule length and the derived curve lies close to the experimental points in the upper panel.

force may not be increased. Furthermore, increases in transmitted hydrostatic pressure may play the predominant role in decreasing tubular reabsorption during renal vasodilatation (18).

The present studies do not eliminate the possibility that a decrease in filtration rate by the nephrons not under study activates some indirect pathway that influenced reabsorption by the perfused tubules. If the tubules under study are bathed by the circulation of adjacent proximal tubules, then it is conceivable that the decrease in filtration rate of adjacent nephrons causes the release, or suppression, or some intrarenal humoral factor which leads to a change in reabsorption by the nonfiltering proximal tubule under study. There is no way to evaluate this possibility from the present data, or for that matter, from any other studies of glomerulotubular balance.

The observation in the present study that the absolute rate of reabsorption by the proximal tubule appears to be constant along the length accessible for micropuncture affords additional information on the mechanism of glomerulotubular balance. The observation in this study and by others (4, 19, 20) that the rate of tubular reabsorption is largely independent of a change in the load per se does not answer the question as to whether the rate of reabsorption decreases progressively along the length of the proximal tubule. Even though the rate of nephron filtration per se may not set the over-all rate of proximal tubular reabsorption, a proportionality could still exist between the rate of reabsorption and

volume of intratubular fluid reaching a given site along the proximal tubule. Such a relationship could result conceivably from decreasing tubular diameter (5) with distance along the proximal tubule, or from an effect of reabsorbate to progressively decrease the concentration of protein in peritubular plasma. Analysis of the relationship between absolute reabsorption and the length of proximal tubule perfused indicated that the rate of reabsorption was constant and not influenced either by an effect of the decreasing intratubular volume of perfusate or the volume of perfusate absorbed by the peritubular circulation.

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