The Relationship between Glomerular Filtration Rate and Sodium Reabsorption by the Proximal Tubule of the Rat Nephron

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ABSTRACT We have tested two of the hypotheses proposed to explain the adjustment in sodium reabsorption in the proximal tubule that follows a change in the rate of glomerular filtration (glomerulotubular balance). Using the recollection micropuncture technique, we were able to measure the immediate and late changes in reabsorptive rate after an acute alteration in filtration rate produced by aortic constriction and release of constriction. It was found that fractional reabsorption, as measured by the inulin tubule fluid to plasma (TF/P) ratio, increased after aortic constriction and decreased after release, but that in most instances, absolute reabsorptive rate changed in parallel to glomerular filtration rate. The change was similar whether the collections were made less than 1 or more than 5 min after the change in blood pressure. The rapid time course of this adjustment in reabsorptive rate is viewed as evidence against an intrarenal humoral feedback mechanism.

In the same experiments we measured the $(TF/P)_{In}$, transit time, and flow rate of fluid in single nephrons before and during aortic constriction or release of aortic constriction. The change in reabsorptive rate and the simultaneous change in calculated cross-sectional area of the tubule lumen were rarely proportional, i.e., $C/\pi r^2$ was not constant. In other experiments, these same measurements were made before and during periods of increased ureteral pressure. Despite large increments in calculated cross-sectional area, the absolute rate of reabsorption either remained relatively unchanged or fell in proportion to the change in filtration rate. It is concluded that under these conditions, reabsorptive rate is governed by some factor other than tubule geometry.

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INTRODUCTION

On the basis of indirect evidence obtained from clearance measurements, Homer Smith suggested that, despite wide variations in the rate of glomerular filtration (GFR), the proximal tubule reabsorbs a relatively constant fraction of the filtered sodium and water (1). Several studies in recent years, utilizing micropuncture techniques, have provided abundant evidence to support this hypothesis (2–6).

Three principal mechanisms have been proposed to explain this remarkable balance. One view holds that when the balance between filtration rate and reabsorption is upset, the resulting change in the

volume, composition, pressure, flow rate, or some as yet unknown factor at a more distal site triggers the release of a humoral substance whose ultimate action is to reset filtration rate or reabsorption, thereby establishing a new balance (7). Implicit in this type of servo-control mechanism is a finite period of delay between the initiating event and completion of the regulation via the feedback loop. A second view, initially advanced by Gertz holds that the rate of reabsorption varies in direct proportion to the square of the radius of the lumen (cross-sectional area of the lumen), and that glomerulotubular balance is the consequence of proportionate changes in the rate of glomerular filtration, tubule volume, and absolute reabsorptive rate (6, 8). A third proposal places the control of reabsorption at the peritubular and intercellular surfaces, and stresses the importance of changing local physical factors in this area in maintaining glomerulotubular balance (9).

The present studies were designed to evaluate the first two hypotheses. Initial experiments were concerned with the length of time required for an adjustment to occur in the rate of sodium reabsorption in the proximal tubule after an abrupt change in the rate of glomerular filtration. Whether filtration rate decreased (aortic constriction) or increased (release of aortic constriction), the adjustment occurred within 30 sec, an interval we believe to be too short for the operation of the proposed humoral feedback mechanism. In these same experiments, it was observed that the simultaneous changes in absolute reabsorptive rate and cross-sectional area in the same nephron were rarely proportional. To examine this relationship further, measurements of absolute reabsorptive rate and tubule cross-sectional area were made before and during periods of increased ureteral pressure. It was found that although cross-sectional area increased, often markedly, reabsorptive rate remained relatively constant, or fell. These results provide strong evidence against the proposal that tubule geometry is an important determinant of the absolute rate of sodium reabsorption in the proximal tubule.

METHODS

Studies were performed on male Sprague-Dawley rats, weighing 200–375 g. They were allowed free access to water and, except for 24 hr immediately before the study, were fed a rat pellet diet. They were anesthetized by intraperitoneal injection of Inactin (100 mg/kg), placed on a temperature regulated micropuncture table, and a tracheostomy was performd. Indwelling polyethylene catheters were inserted into the left jugular vein for infusion of inulin and fluids, into the right jugular vein for injection of lissamine green, and into the left femoral artery for periodic collection of blood and estimation of arterial pressure. The left kidney was exposed by a left subcostal incision and gently separated from the adrenal gland and perirenal fat. The renal capsule was left intact. The left ureter was cannulated near the renal pelvis with a 4–6-cm No. 10 polyethylene catheter. The kidney was suspended on a lucite holder, its surface illuminated by a quartz rod and bathed with mineral oil heated to 37°C.

Aortic constriction-release of constriction experiments. In 20 rats the GFR of the experimental kidney was altered acutely by changing left renal artery perfusion pressure. This required that the area of the abdominal aorta in the vicinity of the renal arteries be gently dissected free of surrounding structures, with care to preserve lymphatic vessels. A silk ligature was placed so as to encircle the aorta between the origins of the renal arteries. The ligature was threaded through a glass capillary tube so that tension exerted on the ligature produced compression of the aorta against the rigid end of the capillary tube. With this device, aortic constriction produced a prompt (2–3 sec delay) fall in renal artery perfusion pressure to a stable level, whereas release of the constriction resulted in an immediate elevation in perfusion pressure. These changes in pressure were monitored in the left femoral artery by means of a Statham strain gauge (Statham Instruments, Los Angeles, Calif.) connected to a Sanborn recorder (Sanborn Co., Cambridge, Mass.). Of these 20 rats, 7 received an isotonic saline infusion at the rate of 0.062 ml/min (non diuretic group); in 10 rats this solution was infused at the rate of 0.2 ml/min (saline diuretic group). Infusion of fluid was begun at the start of the surgical procedure and continued for the duration of the experiment. Inulin was added to the infusion to provide a plasma concentration of about 100 mg/100 ml. An equilibration period of at least 1 hr was allowed before the initiation of the experimental procedure.

In these studies the following protocol was employed. Late surface convolutions of three proximal tubules,[1] which for descriptive purposes are referred to as tubules 1, 2, and 3, were located by observing the passage of lissamine green which was injected rapidly (0.1 ml of a 2% solution) into the right jugular vein. The transit time, estimated with a stopwatch as the interval from the appearance of the dye in the peritubular capillaries to the arrival of the color wave at the proposed site of puncture

[1] In 54 randomly selected proximal tubules latex was injected and the casts microdissected. Puncture sites were localized between 35–65% of the total length of this segment; in 80% these sites were beyond 45% of the total length.

was recorded for each tubule. Using a sharpened micropipette (tip diameter 8–10 μ), a sample of fluid (1a) measuring about 75 nl was collected from the first tubule usually in a 2 min interval which was timed exactly, and the volume and inulin concentration in the collected fluid was determined. In some instances, the transit time measurement was repeated during the collection.[2] The rate of collection of fluid was adjusted to maintain the position of an oil block placed just distal to the site of puncture. Particular care was taken not to alter the diameter of the tubule. Then the aorta was constricted abruptly, and in most experiments a recollection from the same puncture site was begun and completed, usually within 40 sec and always within 1 min of the fall in blood pressure. This sample (1b) usually was less than 10 nl in volume, and only its inulin concentration was determined. The aorta remained clamped for periods varying from 5 to 25 min. The transit time to all three late convolutions was estimated during aortic constriction, either just before or during another timed collection from tubule 1 and the volume and inulin concentration of this sample (1c) was determined. With the aorta constricted, an initial timed collection (2a) was obtained from tubule 2. The aortic constriction was released, and in most experiments, a sample of tubule fluid (2b) measuring about 15 nl was recollected, usually within 30 sec and always within 1 min of the increase in blood pressure, and its inulin concentration determined. Between 5 and 25 min from the time the aorta was unclamped, the transit time for all three tubules again was measured, either just before or during the third collection (2c) from tubule 2. Tubule 3 was never punctured, but was used only for measurements of transit time. This same protocol was repeated one or two times in each animal.

The clearance of inulin by the experimental kidney was measured before, during, and after each period of reduced renal perfusion pressure. Urine collections were begun 3–5 min after each alteration in blood pressure. Blood samples, approximately 125 μl in volume, were drawn from the femoral artery at intervals of 15 min throughout the experiment.

Three rats were studied in a similar manner at least 21 days after right nephrectomy. During these experiments the animals were infused with a solution containing 125 mM NaCl and 25 mM $NaHCO_3$ at the rate of 0.4 ml/min. The protocol followed in this group differed from the previous protocol in that only (a) and (c) collections were made and, therefore, only the late effects of reduced or elevated GFR were investigated. The transit time in this group was measured only during periods of tubule fluid collection.

In five rats infused with isotonic saline at the rate of 0.062 ml/min, the protocol followed was identical with the one initially described, except that tension was never exerted on the ligature and, therefore, aortic constriction was not produced. These rats served as the control group for these studies.

Increased ureteral pressure experiments. 10 rats were prepared for micropuncture as described above. Each received an isotonic saline infusion at the rate of 0.2 ml/min. A 1–4 min timed collection was obtained from a late convolution of each of 2–6 proximal tubules. The transit time was measured during each of these collections. The transit time also was measured in one or two other tubules (A, B) which were never punctured. Experimental kidney filtration rates were measured twice during this period. A length of polyethylene tubing was then attached to the end of the ureteral catheter and its free end raised to a height of 30–40 cm. Approximately 30 min later (about 15 min after the resumption of forward urine flow), the transit time was measured in all tubules. Samples of fluid were recollected from those tubules in which the transit time was prolonged and the transit time was repeated during each collection. During this period of elevated ureteral pressure, in an effort to prevent retrograde flow of tubule fluid into the micropipette, special care was taken to insert an oil block that was about three to four times as long as usual. The inulin concentration and exact volume of each tubule fluid sample were determined. Because of the extremely large dead space imposed by the long ureteral catheter during the period of elevated ureteral pressure, kidney GFR was not measured. After all recollections had been obtained, the attached portion of the ureteral catheter was removed and, after a brief washout period, the inulin clearance again was measured. In two rats which served as controls for these experiments, this exact protocol was followed except that the ureteral catheter was never raised.

Analytical. The volume of tubule fluid collected from individual nephrons 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, usually in duplicate, by the fluorophotometric method of Vurek and Pegram (10). Inulin in plasma and urine was measured by the anthrone method of Führ, Kaczmarczyk, and Krüttgen (11). In eight experiments the inulin concentration of a standard solution was measured by both micro and macro methods. The mean ratio (micro/macro) of the values obtained was 0.98 ± 0.045 SD.

Calculations. Glomerular filtration rate per nephron, (V_0), was calculated from the tubule fluid to plasma inulin ratio and the volume of tubule fluid (V_{TF}) collected per minute with the expression:

$$V_0 = (TF/P)_{In} \cdot V_{TF} \quad (1)$$

where V_0 and V_{TF} are in units of nl/min.

The absolute rate of tubule fluid reabsorption to the site of puncture was calculated with the expression:

$$\text{Reabsorptive rate} = V_0 - (V_{TF}) \quad (2)$$

where reabsorptive rate is given in nl/min.

[2] In 28 instances the transit time measured immediately before collection was compared with the value obtained during actual fluid collection. 14 such comparisons were made before aortic constriction and 14 during aortic constriction. The mean difference between the paired measurements in each period was less than 2%.

In the aortic clamp and release studies, the observed percentage change in the absolute reabsorptive rate

$$\frac{(\text{initial reabsorptive rate} - \text{final reabsorptive rate}) \times 100}{\text{initial reabsorptive rate}}$$

was compared with the change in the absolute reabsorptive rate predicted, assuming a change in reabsorptive rate exactly proportional to the change in V_0. The predicted reabsorptive rate was calculated with the expression:

Predicted reabsorptive rate =

$$\frac{\text{final } V_0}{\text{initial } V_0} \cdot \text{initial observed reabsorptive rate.} \quad (3)$$

% Glomerulotubular balance

$$= \frac{\text{observed change in reabsorptive rate}}{\text{predicted change in reabsorptive rate}} \times 100. \quad (4)$$

The relationship between the absolute reabsorptive rate and the tubule volume can be expressed as the ratio $Cd/\pi r^2 d$, the absolute reabsorptive rate per unit tubule volume (8). This ratio was calculated by the expression:

$$\frac{Cd}{\pi r^2 d} = \frac{\ln (TF/P)_{In}}{\text{transit time}} \quad (5)$$

where r is the tubule radius and d the length of the tubule to the site of puncture. Since the transit time was measured to the site of puncture, d in numerator and denominator are the same, $\frac{Cd}{\pi r^2 d}$ reduces to $C/\pi r^2$, which is in units of sec^{-1}.[3]

Fractional reabsorption of sodium and water was calculated from the plasma to tubule fluid inulin ratio according to the expression:

$$\text{Fractional reabsorption} = [1 - (P/TF)_{In}] \quad (6)$$

The relationship between tubule volume and nephron GFR, expressed as the ratio $\frac{\pi r^2 d}{V_0}$ was estimated from the value for fractional reabsorption, obtained from equation 6 and the value for $C/\pi r^2$ was obtained from equation 5. This relationship is described by the expression:

$$\frac{\pi r^2 d}{V_0} = \frac{[1 - (P/TF)_{In}]}{C/\pi r^2} \quad (7)$$

[3] For each of equations relating reabsorptive rate to tubule volume, it has been assumed that absolute reabsorptive rate and tubule radius are each uniform per unit length of proximal convoluted tubule. Experimental support for each assumption is available. Gertz noted that the absolute rate of fluid absorption by the proximal tubule, as measured by the split-oil droplet technique, is constant despite the position of the aqueous drop in early, middle, or late portions of the proximal tubule (12). With respect to the uniformity of the radius of the lumen of the proximal tubule, several groups have reported photographic and snap-frozen histological measurements of portions of this segment of the nephron selected at random to be remarkably similar and uniform (6, 9, 12–15).

where d represents the length of the tubule to the site of puncture. The units of this expression are nanoliter per nanoliter per second, which simplifies to seconds.

Since V_0 was determined independently in these studies, (equation 1), it was possible to estimate the volume of the tubule to the puncture site:

$$\pi r^2 d = \frac{(\pi r^2 d)}{V_0} \cdot V_0 \quad (8)$$

Since π and d were constant in any recollection pair, an estimate of the relative change in r^2 (or any other function of r) after each experimental manipulation was obtained:

$$\frac{(r^2) \text{ recollection}}{(r^2) \text{ initial}} = \frac{(\pi r^2 d) \text{ recollection}}{(\pi r^2 d) \text{ initial}} \quad (9)$$

RESULTS

Aortic constriction-release of constriction experiments. Glomerulotubular balance was studied in 7 nondiuretic rats (slow infusion) and in 10 rats during saline diuresis (moderate infusion). In a recent study of the mechanism of glomerulotubular balance by Rector, Brunner, and Seldin (6), experiments were performed in uninephrectomized rats receiving 0.4 ml/min of a saline-bicarbonate solution. In order to permit a comparison of the observations reported in the present study with their data, we performed aortic constriction-release of constriction experiments in three rats prepared in a similar manner (rapid infusion). Table I illustrates the protocol of a typical experiment in a nondiuretic rat in which the GFR was changed by aortic constriction or release of constriction, and the $(TF/P)_{In}$ ratio, volume flow of tubule fluid, and transit time measured.

In five control rats, the mean value for arterial pressure was 105 mm Hg and did not change significantly during the recollection period. In the 20 experimental animals arterial pressure during the unclamped period averaged 125 mm Hg and was lowered to about 75 mm Hg. With this degree of aortic constriction we noted a modest reduction in kidney volume, a decrease in tubule diameters, and dampening of the "renal pulse." Despite these changes it was almost always possible to begin the early (b) recollection within 20 sec of the change in blood pressure. Reversal of these changes occurred immediately upon release of constriction, and in nearly every instance, the blood pressure returned promptly to levels observed before clamping.

The mean GFR of the experimental kidney dur-

TABLE I

A Typical Experiment Showing the Effect of Acute Alterations in Blood Pressure on Glomerular Filtration Rate, (TF/P) Inulin, and Transit Time

Time	Tubule fluid collection number	Femoral arterial pressure	Time of collection from outset of change in blood pressure	Duration of collection	Tubule fluid volume	$(TF/P)_{In}$	Nephron GFR	Kidney GFR	Transit time, Tubule number 1	2	3*	4	5	6*
min		*mm Hg*		*sec*	*nl/min*		*nl/min*	*ml/min*	*sec*					
−219 to −104	Infusion of 0.9% NaCl at rate of 0.062 ml/min													
−104	Inulin added to infusion													
0	1 a	130	—	120	31.9	1.36	43.4	0.96	6.0	7.5	9.0			
7			Aorta constricted											
7	1 b	80	20 sec	20	—	2.23	—	—	—	—	—			
15	1 c	80	8 min	120	8.1	2.62	21.2	0.36	8.0	7.0	12.0			
20	2 a	90	—	120	4.6	3.30	15.2	—	—	—	—			
26			Constriction released											
26	2 b	140	20 sec	20	—	2.17	—	—	—	—	—			
33	2 c	140	7 min	120	lost	2.39	—	1.25	7.5	8.0	9.0			
73	4 a	135	—	120	47.8	1.24	59.3	1.70				6.0	9.5	13.0
78			Aorta constricted											
78	4 b	80	20 sec	20	—	lost	—	—				—	—	—
94	4 c	90	16 min	120	19.6	1.46	28.6	1.34				6.0	10.0	12.5
97	5 a	90	—	120	20.4	1.50	30.6	—				—	—	—
102			Constriction released											
102	5 b	140	20 sec	20	—	1.31	—	—				—	—	—
107	5 c	135	5 min	60	44.4	1.25	55.5	1.41				6.0	9.0	12.0

* Tubules 3 and 6 are unpunctured tubules on the surface of the kidney.

ing the absence of aortic clamping was 1.07 ml/min ± 0.03 SE for the slow infusion group, 1.25 ml/min ± 0.04 SE for the moderate infusion group, and 1.79 ml/min ± 0.07 SE for the rapid infusion (uninephrectomized) group. The kidney GFR during constriction of the aorta fell 30% on the average for all experimental rats. In control rats, the mean values for kidney GFR in the initial collection and recollection periods were 0.97 ml/min ± 0.10 SE and 0.91 ml/min ± 0.09 SE, respectively. The fraction of filtered water excreted was proportional to the rate of fluid administration, averaging less than 1% in the slow, 2.4% in the moderate, and 4.5% in the rapid infusion group.

Table II summarizes the mean values for nephron GFR, transit time, and $(TF/P)_{In}$ concentration ratios obtained in the three experimental groups. It was possible to obtain both (a) and (c) collections from 85 tubules, and in each, to measure the minute volume, inulin concentration, and transit time. Since the experimental design called for a change in nephron GFR, the data are considered from only those tubules in which the filtration rate during the clamp period was at least 30% lower than the filtration rate in the same nephron during the unclamped period. Of these 85 recollection pairs, nephron GFR during aortic contriction or release changed less than 30% in 18, and these were excluded. Of the remaining 67 recollection pairs, 37 were associated with aortic constriction and 30 with release of constriction. The mean nephron GFR during the recollection period in control animals was very nearly the same as the mean value during the initial collection (Table II). These mean values do not differ significantly from the mean value obtained during the unclamped period in the slow infusion experimental group and are similar to values reported by others (5, 9, 16). The mean nephron GFR in the absence of clamping in the moderate infusion group was about 20% higher, presumably reflecting the effect of extracellular fluid (ECF) volume expansion. A higher filtration rate also was seen in the whole kidney, a finding previously noted by others (17, 18, 13). In the rapid infusion group, the mean nephron GFR was 74 nl/min, a value about 70% higher than that found in rats receiv-

TABLE II

Mean Values for Nephron GFR, Transit Time, and (TF/P) Inulin in Aortic Constriction-Release of Constriction Experiments

Condition	Nephron GFR			Transit time			$(TF/P)_{In}$				
	Initial	Recoll.		Initial	Recoll.		Initial	Early recoll.		Late recoll.	
	(a)	(c)	c/a*	(a)	(c)	c/a*	(a)	(b)	b/a*	(c)	c/a*
		nl/min			*sec*						
Control [5]‡§	37.6	37.9	1.08	9.4	10.1	1.08	1.82	1.87	1.03	1.77	0.99
	±2.98	±2.74	±0.09	±0.79	±1.06	±0.08	±0.09	±0.10	±0.03	±0.09	±0.02
	(14)‖	(12)	(12)	(14)	(14)	(14)	(14)	(13)	(13)	(12)	(12)
Experimental											
Slow infusion [7]											
Constriction	44.9	22.6	0.52	6.8	8.2	1.22	1.51	1.84	1.22	1.92	1.29
	±4.37	±2.19	±0.04	±0.52	±0.46	±0.05	±0.07	±0.14	±0.15	±0.14	±0.10
	(8)	(8)	(8)	(8)	(8)	(8)	(8)	(5)	(5)	(8)	(8)
Release	20.7	34.8	1.66	9.3	9.2	0.97	1.74	1.50	0.96	1.54	0.88
	±3.81	±7.07	±0.07	±1.02	±1.44	±0.05	±0.13	±0.18	±0.14	±0.17	±0.05
	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(3)	(3)	(5)	(5)
Moderate infusion [10]											
Constriction	54.1	26.4	0.50	8.1	9.5	1.22	1.48	1.91	1.26	1.94	1.31
	±2.94	±2.42	±0.03	±0.55	±0.63	±0.07	±0.05	±0.14	±0.08	±0.14	±0.09
	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(11)	(11)	(20)	(20)
Release	26.3	47.9	1.93	9.3	7.8	0.89	1.81	1.55	0.86	1.50	0.84
	±2.20	±3.60	±0.13	±0.95	±0.58	±0.05	±0.08	±0.03	±0.03	±0.04	±0.02
	(18)	(18)	(18)	(18)	(18)	(18)	(18)	(10)	(10)	(18)	(18)
Rapid infusion [3]											
Constriction	74.0	36.5	0.50	7.8	8.7	1.15	1.46	—	—	1.81	1.24
	±5.24	±3.37	±0.04	±0.72	±0.56	±0.05	±0.04	—	—	±0.08	±0.07
	(9)	(9)	(9)	(9)	(9)	(9)	(9)			(9)	(9)
Release	32.2	72.8	2.36	9.9	9.6	0.96	1.69	—	—	1.48	0.88
	±3.76	±8.68	±0.20	±0.78	±0.73	±0.03	±0.08	—	—	±0.04	±0.03
	(7)	(7)	(7)	(7)	(7)	(7)	(7)			(7)	(7)

* These values represent means of ratios, rather than ratios of mean values.
‡ Numbers in brackets refer to the number of animals.
§ Values are expressed as mean ±1 *SE*.
‖ Numbers in parentheses refer to the number of observations.

ing fluid at the slowest infusion rate. This presumably reflects the combined influences of additional ECF volume expansion and compensatory renal hypertrophy.[4] During aortic constriction the filtration rate of individual nephrons in each experimental group fell on the average by 50% (Table II).

Upon release of the aortic constriction, nephron filtration rates tended to return to the level measured before constriction (Table II). Although the simultaneous changes in nephron and kidney filtration rates usually were similar, often the two changed disproportionately. As noted earlier, in these studies individual nephron reabsorption always was evaluated in relation to its own GFR.

[4] The weight of the remaining kidney in each rat was about 35% greater at the time of puncture than the right kidney at the time of its removal.

The mean $(TF/P)_{In}$ ratios observed at the end of the accessible portion of the proximal tubule in the different states of hydration[5] (Table II) are comparable to values reported by others (9, 17–19). In the control animals, there were no significant changes in the $(TF/P)_{In}$ ratios in either early or late recollections. In the slow and moderate infusion groups, a mean increase of 30% was

[5] As shown in Table II, the mean inulin TF/P ratio in control rats was higher than that observed in the experimental group receiving saline at the same infusion rate (slow infusion group). This most likely reflects differences in the exact location of the puncture sites in each group, since in 10 of 13 control tubules subsequently microdissected, puncture sites were beyond 50% of the total length of the proximal tubule, whereas in the experimental group only 3 of 8 were beyond 50%. Similarly, the mean transit time in the control group also was slightly longer.

found in 28 late recollection $(TF/P)_{In}$ ratios obtained 5–25 min after the onset of aortic constriction. In 20, the change was at least + 10%. There was no significant difference between the two groups in this respect. It is apparent from Table II that the late recollection $(TF/P)_{In}$ ratios in the uninephrectomy (rapid infusion) group increased to a similar extent. A comparable mean change (+ 25%) also was observed in the 16 early clamp (b) recollections obtained within 1 min of the fall in blood pressure (Table II). In 23 late recollections obtained 5–25 min after release of constriction in the slow and moderate infusion groups, the mean change in $(TF/P)_{In}$ was − 15%. A decline of at least 10% occurred in 18 and in none was there an increase. There were no significant differences between the two groups. The change observed after release of constriction in the uninephrectomized group was essentially the same. Furthermore a similar decrease in fractional sodium reabsorption occurred within 1 min of the increase in blood pressure (Table II). Table III lists all $(TF/P)_{In}$ ratios for those tubules in which initial (a), early (b), and late (c) recollections were obtained.

TABLE III

Tubule Fluid to Plasma Inulin Concentration Ratios Obtained before (a), Immediately after (b), and 5–25 Min after (c) Acute Alterations in Blood Pressure

Experiment No.	Aortic constriction			Release constriction		
	Initial (a)	Early (b)	Late (c)	Initial (a)	Early (b)	Late (c)
3	1.84	1.40	1.79			
	1.57	1.79	1.98			
12	1.41	2.06	1.89			
13	1.36	2.23	2.62	1.50	1.31	1.25
14	1.58	1.74	2.03	1.96	1.51	1.43
				1.36	1.19	1.21
15	1.47	1.47	1.75	2.03	1.57	1.69
	1.58	1.81	1.78	2.07	1.43	1.54
				1.80	1.79	1.65
16	1.29	1.52	1.37	1.81	1.44	1.46
17	1.89	1.95	2.63			
18	1.30	1.44	2.51	1.34	1.30	1.16
19	1.54	2.85	2.64			
20	2.05	2.61	2.96	1.97	1.75	1.69
21	1.39	1.97	1.58	1.77	1.58	1.40
	1.28	1.83	1.65	1.46	1.47	1.31
22	1.57	1.62	1.68	1.95	1.55	1.73
	1.35	1.90	3.64	2.06	1.58	1.41

In agreement with two recent studies in the rat (8, 20), constancy of fractional sodium reabsorption was not observed in the present study utilizing the recollection micropuncture technique. By comparing the inulin TF/P ratios in separate tubules (rather than by recollection from the same tubules) before and after variations in the filtered load of sodium, others have concluded that fractional reabsorption remains relatively constant (5). The experimental design of the present study also allowed us to examine the effect of aortic constriction on separate tubules as well, by comparing the inulin TF/P ratios of 1a collections for tubules before constriction with the values for this ratio in 2a collections from other tubules during the period of aortic constriction. As shown in Table II, fractional reabsorption was found not to be constant, the changes in the inulin TF/P ratios being similar to those observed in the comparison of recollection pairs. However there was no difference between the first (mean 1.53 ± 0.04 SE) and last (mean 1.48 ± 0.03 SE) (a) collections made in each animal while the aorta was not clamped.

In 57 of the 67 recollections, reabsorptive rate and nephron GFR changed in the same direction. The situation in which the change in the absolute reabsorptive rate was exactly proportional to the change in GFR in the same nephron has been designated as 100% or ideal glomerulotubular balance. Fig. 1 indicates the relationship between the observed changes in reabsorption and those predicted for ideal glomerulotubular balance. The observed change in the rate of proximal sodium reabsorption produced by aortic constriction was nearly always less than the value predicted from the change in filtration rate for that same nephron. Most of the points, therefore, fall above the line of identity (Fig. 1, lower left). After release of the constriction, the observed change in the rate of sodium reabsorption was again nearly always less than predicted. Consequently most of these points fall below the identity line (Fig. 1, upper right). During aortic constriction as well as after release of constriction, the observed change in reabsorption was on the average more than half of predicted. The distribution of the points about the identity line did not vary over a wide range of changes in GFR and was similar whether the late

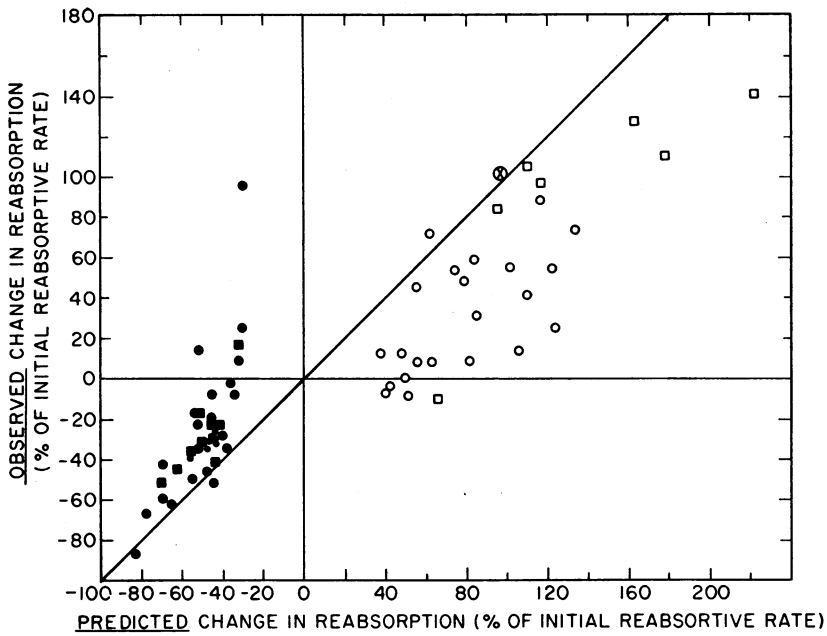

FIGURE 1 A comparison of the observed change in reabsorption after aortic constriction (closed symbols) and release of constriction (open symbols) with the change predicted for ideal glomerulotubular balance. Equations 2 and 3 were used to calculate observed and predicted reabsorptive rates, respectively. Circles denote values obtained from rats infused at slow and moderate rates; squares represent values obtained from uninephrectomized rats receiving fluid at the rapid rate. The symbol ⊗ represents an instance in which the observed reabsorptive rate was 303% and that predicted 279% of the initial value; to simplify the scale used, the value on each coordinate was divided by 3.0.

recollection was made at 5 or 25 min after the change in blood pressure.

In order to evaluate the relationship between the absolute reabsorptive rate and tubule volume (equation 5), we measured the transit time of lissamine green to the site of puncture before and after alterations in GFR. As shown in Table II the mean values for transit time during initial and recollection periods in control rats were very nearly the same and agree with values reported by others (6, 9, 18, 13, 20). The shorter transit time in the nondiuretic experimental group undoubtedly reflects the somewhat earlier collection sites in this group. The mean transit time for all experimental groups increased by approximately 20% during aortic constriction. Although not shown, measurements of transit time in tubules that were never punctured also were made in each rat. The changes in these in almost every instance were similar to the simultaneous changes noted in the tubules that were punctured.

The relationship between the absolute reabsorptive rate and tubule volume, expressed as the ratio $C/\pi r^2$ was determined in each nephron before and after the change in filtration rate (equation 5). As shown in Table IV, the mean values for $C/\pi r^2$ during initial and late recollections in control animals did not differ from one another or from the value in the unclamped period for the slow infusion experimental group. Lower mean values for $C/\pi r^2$ during the unclamped period were associated with higher rates of saline infusion, a finding previously noted by others (17, 18). For all experimental groups aortic constriction was associated with a mean increase in $C/\pi r^2$ of about 40%, whereas after release of constriction this ratio decreased by an average of 20%. The change in $C/\pi r^2$

$$\frac{(C/\pi r^2 \text{ recollection})}{C/\pi r^2 \text{ initial}},$$

after a change in GFR was extremely variable, as shown in Fig. 2. This variability was apparent to

TABLE IV

*The Relationship between Reabsorptive Rate and Tubule Volume, and between Tubule Volume and Nephron GFR in Aortic Constriction-Release of Constriction Experiments**

Condition	$C/\pi r^2$ sec^{-1}			$\pi r^2 d/V_0$ sec‡		
	Initial	Recoll.		Initial	Recoll.	
	(a)	(c)	(c/a)	(a)	(c)	(c/a)
Control [5]	0.062	0.060	0.99	7.11	7.33	1.05
(12)	±0.004	±0.004	±0.08	±0.59	±0.68	±0.08
Experimental						
Slow infusion [7]						
Constriction	0.062	0.080	1.34	5.48	6.10	1.12
(8)	±0.008	±0.011	±0.15	±0.43	±0.46	±0.04
Release	0.060	0.049	0.77	7.10	7.64	1.06
(5)	±0.010	±0.014	±0.13	±0.82	±1.34	±0.08
Moderate infusion [10]						
Constriction	0.049	0.064	1.38	6.61	7.13	1.12
(20)	±0.005	±0.005	±0.17	±0.45	±0.39	±0.06
Release	0.065	0.051	0.79	7.00	6.44	0.97
(18)	±0.005	±0.004	±0.10	±0.63	±0.44	±0.06
Rapid infusion [3]						
Constriction	0.050	0.070	1.46	6.44	6.49	1.04
(9)	±0.005	±0.003	±0.15	±0.61	±0.38	±0.04
Release	0.053	0.041	0.80	7.75	8.12	1.05
(7)	±0.005	±0.003	±0.06	±0.59	±0.39	±0.02

* Notations are the same as in Table II.

‡ Note that in this term V_0 is in units of nl/sec.

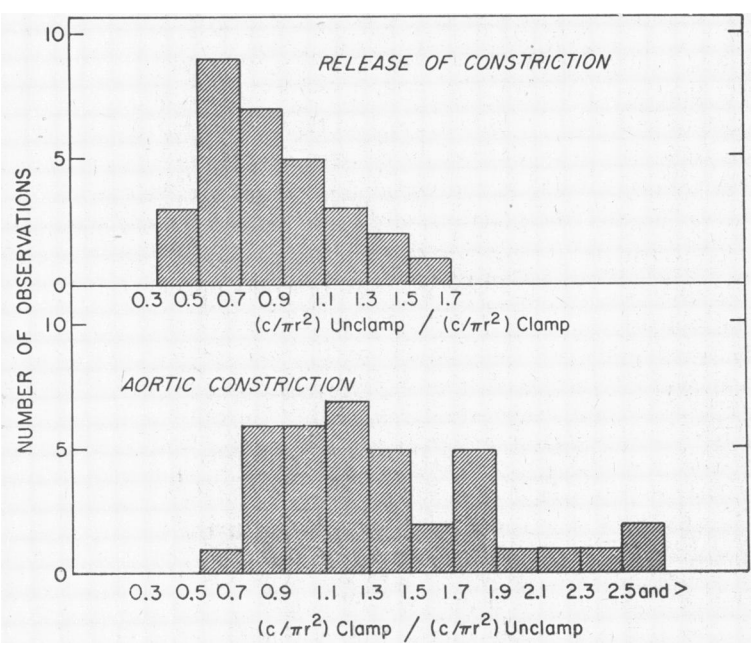

FIGURE 2 Frequency histogram showing the effects of aortic constriction (lower) and release of constriction (upper) on the ratio of reabsorptive rate to tubule volume ($C/\pi r^2$). For each experimental maneuver the value for $C/\pi r^2$ (calculated by means of equation 5) of the final collection is divided by the value obtained for that same tubule for the initial collection.

the same extent whether the observed change in absolute reabsorption was greater or less than half of predicted.

In control as well as in experimental animals the relationship between tubule volume and individual nephron filtration rate $\frac{(\pi r^2 d)}{V_0}$ remained, on the average, relatively constant despite spontaneous variations in filtration rate or those induced by saline loading or aortic constriction and release (Table IV).

Increased ureteral pressure experiments. Increased ureteral pressure, produced in 10 rats by elevation of the ureteral catheter to a height of about 33 cm (24 mm Hg) resulted in a noticeable increase in kidney volume and dilatation of individual tubules.

The effect of increased ureteral pressure on nephron GFR, transit time, and $(TF/P)_{In}$ ratios in a typical experiment is shown in Table V. Despite a previous puncture, after elevation of ureteral pressure the transit time nearly always was prolonged. If, before recollection, extravasation of tubule fluid from the initial puncture site was detected, the tubule was no longer studied and the initial sample discarded. Recollection pairs that were apparently technically satisfactory were obtained from 48 tubules before and during increased ureteral pressure and in each, minute flow rate and inulin concentration were measured. In 10 of these recollection pairs from 8 of the 10 animals the calculated nephron filtration rates during the period of elevated ureteral pressure were more than 50% higher (range 69–540%) than the corresponding initial value. These higher values, which we believe to be spurious, were caused by a sharp rise in inulin TF/P ratios [6] (range 56–980%) and a relatively constant or increased minute volume. It is thought that during these 10 recollections, the distal oil block was inadequate.

[6] In one of these experiments, inulin ^{14}C and nonradioactive inulin were administered simultaneously. Recollection pairs from five tubules were obtained and in one of these, after the elevation in ureteral pressure, the TF/P_{In} ratio, minute flow rate, and calculated filtration rate increased by more than 50%. Inulin TF/P ratios measured by fluorescence and isotopic counting were similar.

TABLE V

A Typical Experiment Showing the Effect of Increased Ureteral Pressure on Nephron GFR, (TF/P) Inulin and Transit Time

Time	Tubule fluid collection number	Kidney GFR	Transit time		Tubule fluid	$(TF/P)_{In}$	Nephron GFR
			Before puncture	During puncture			
min		*ml/min*	*sec*		*nl/min*		*nl/min*
−169 to −109	Infusion of 0.9% NaCl at rate of 0.20 ml/min						
−109	Inulin added to infusion						
0	Sl a	0.90	—	8.5	37.6	1.44	54.1
+1	Control tubule A		7.0				
+3	S2 a		—	7.5	30.6	1.39	42.5
+7	S3 a	1.00	—	8.0	33.8	1.51	51.0
+16	Control tubule B		7.5				
+33	Left ureteral catheter raised to height of 33 cm						
+50	Control tubule A		11.5	—			
+51	S1 b		15.5	—			
+54	S1 b		—	16.0	35.6	1.42	50.5
+57	S2 b		13.5	—			
+59	S2 b		—	15.5	31.5	1.43	45.0
+63	S3 b		19.0	—			
+65	S3 b		—	19.5	32.1	1.56	50.1
+71	Control tubule B		18.5	—			
+85	Catheter lowered						
+95 to +100		0.89					

TABLE VI

*Nephron GFR, transit Time, and $(TF/P)_{In}$ in Increased Ureteral Pressure Experiments**

Experiment No.	Nephron GFR		Transit time		$(TF/P)_{In}$	
	Initial period	Recollection period	Initial period	Recollection period	Initial period	Recollection period
	nl/min		*sec*			
36	58.6	43.4	11.5	16.0	1.48	1.48
	73.4	66.6	10.5	19.0	1.62	2.05
	85.0	74.9	9.0	31.0	1.67	1.81
	66.3	59.2	8.5	16.0	1.62	1.71
	75.4	53.0	11.0	26.5	1.98	2.03
	56.9	78.3	9.0	25.0	1.68	2.62
37	44.0	46.1	11.0	14.0	1.78	1.51
	33.5	37.9	9.0	15.5	2.04	2.12
	67.7	73.7	9.0	15.5	1.81	2.03
	61.0	34.2	8.5	17.5	1.60	2.11
38	55.4	52.1	13.0	21.0	1.97	1.99
	48.6	41.0	9.0	11.0	1.60	1.50
	55.4	33.1	11.5	30.5	1.63	1.48
	43.6	35.3	13.0	25.0	1.53	1.56
	42.8	39.1	9.5	15.5	1.69	1.73
41	49.9	39.1	9.0	16.0	1.63	1.73
	39.9	40.4	7.5	17.5	1.88	2.15
42	54.1	50.5	8.5	16.0	1.44	1.42
	42.5	45.0	7.5	15.5	1.39	1.43
	51.0	50.1	8.0	19.5	1.51	1.56
43	50.1	36.7	6.5	12.5	1.25	1.43
	37.1	8.3	7.0	19.0	1.34	1.39
44	51.7	41.9	9.0	13.5	1.53	1.47
	56.1	50.3	6.5	12.0	1.27	1.27
	67.0	84.9	8.0	19.0	1.74	2.49
	66.4	64.4	12.5	22.5	1.72	1.86
45	52.6	45.7	7.5	11.5	1.38	1.42
	32.8	31.8	9.0	15.0	1.12	1.03
	58.8	38.2	7.5	16.5	1.43	1.51
	27.9	33.3	8.0	24.0	1.61	1.78
46	39.0	29.6	9.0	23.0	1.94	1.86
	51.0	36.3	8.0	21.5	1.58	1.39
	49.2	59.1	12.5	16.5	1.79	1.97
	50.4	43.0	8.5	20.5	1.21	1.33
50	72.4	37.2	6.0	18.5	1.42	1.28
	58.2	41.6	6.5	10.5	1.59	1.36
	50.0	39.9	8.0	10.5	1.72	1.28
	43.6	26.9	8.0	22.5	1.44	1.67
Mean (38)	53.1 ±2.04	45.3 ±2.65	9.0 ±0.30	18.2 ±0.84	1.60 ±0.03	1.71 ±0.04
Controls [2] Mean (11)	49.5 +3.95	47.0 ±2.53	8.1 ±0.42	7.9 ±0.62	1.52 ±0.05	1.48 ±0.05

* Notations are the same as in Table II.

permitting fluid from more distal sites to enter the pipette. Consequently the data from these collections were discarded.

Table VI shows the data for nephron GFR, transit time, and TF/P inulin ratios from these 38 tubules. In 26 tubules, increased ureteral pressure was associated with a fall in nephron GFR (range − 6 to − 78%); in five nephron GFR was unchanged (± 5%) and in seven GFR increased (range + 6 to + 38%). For all tubules, the mean change was − 15%.[7] Increased ureteral pressure prolonged transit time in all tubules punctured (mean change = + 106% ± 9.2 SE) which was very similar to the mean change (+ 88% ± 19.2 SE) observed in 14 other tubules in these same rats which were never punctured.

A comparison of the $(TF/P)_{In}$ ratios before and during increased ureteral pressure is shown in Fig. 3 and Table VI. In most instances the recollection $(TF/P)_{In}$ ratio was very similar to the initial ratio, and this was true whether there was no change in transit time (controls), a moderate increase (< 100%), or a large increase (> 100%). The dashed line in Fig. 3 indicates the recollection $(TF/P)_{In}$ ratio to be expected for a doubling of the transit time and a constant value for $C/\pi r^2$ i.e., a change in reabsorption which is directly proportional to the change in tubule volume. The theoretical curve as drawn represents minimal changes in $(TF/P)_{In}$ in that it assumes no change in GFR; if GFR falls, the curve rises more steeply.

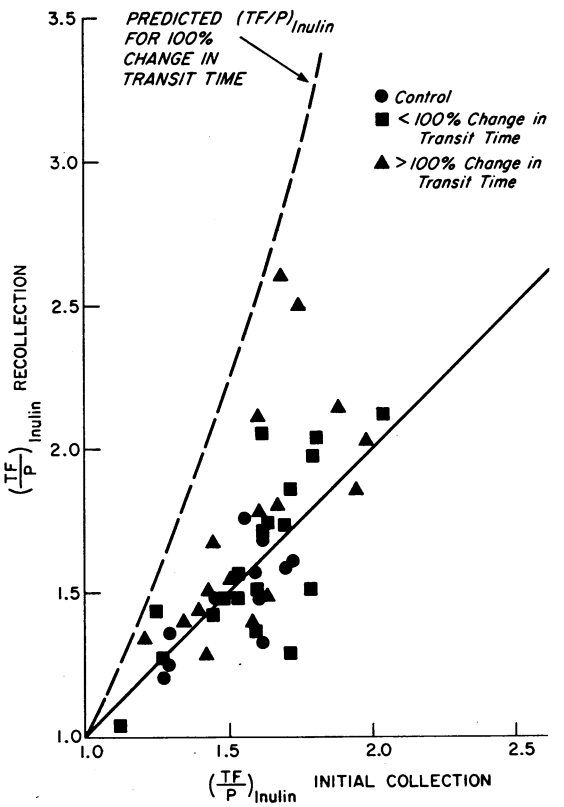

FIGURE 3 Effect of elevated ureteral pressure on proximal inulin TF/P ratios from 10 experimental and 2 control rats. The data are grouped according to the per cent change in transit time measured during the recollection relative to the initial collection from the same tubule. The curve indicates the inulin TF/P ratio that would be expected for a 100% increase in transit time and a constant value for $C/\pi r^2$ i.e., a change in reabsorption which is directly proportional to the change in tubule volume.

[7] Although kidney filtration rates were not measured as part of this study (because of the very large catheter dead space) inulin clearance measurements subsequently were made in four additional rats before and during similar elevations in ureteral pressure. In these experiments in which adequate time was allowed for washout of the dead space (30–45 min), the GFR was always reduced and the change varied from − 15 to − 40%.

TABLE VII

*The Relationships among Reabsorptive Rate, Tubule Volume, and Nephron GFR in Increased Ureteral Pressure Experiments**

	$C/\pi r^2$ sec^{-1}			$\pi r^2 d/V_0$ sec				
	Initial	Recollection		Initial	Recollection		(Radius)² recollection / (radius)² initial	Reabsorptive rate recollection / reabsorptive rate initial
	(a)	(b)	(b/a)	(a)	(b)	(b/a)		
Control [2] (11)	0.051 ±0.004	0.050 ±0.005	0.98 ±0.069	6.62 ±0.31	6.65 ±0.41	1.00 ±0.038	0.99 ±0.077	0.94 ±0.089
Experimental [10] (38)	0.051 ±0.002	0.028 ±0.002	0.55 ±0.028	7.21 ±0.21	14.43 ±0.66	2.03 ±0.091	1.72 ±0.095	0.92 ±0.061

* Notations are the same as in Table II.

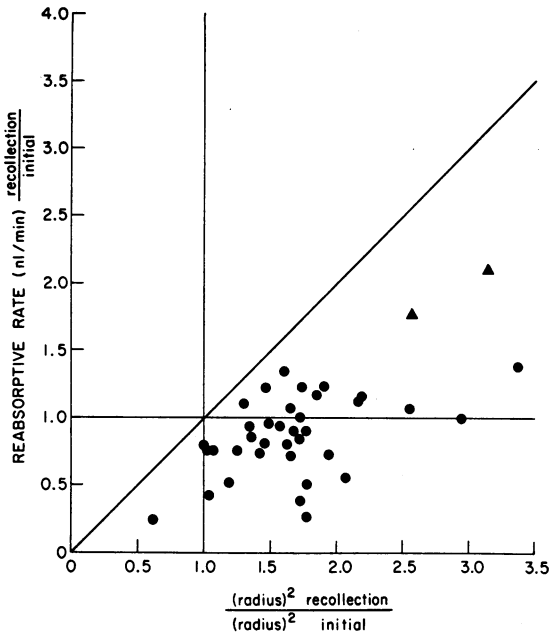

FIGURE 4 Effect of changes in the square of the radius of the proximal tubule produced by elevation of ureteral pressure on absolute sodium reabsorption in these same tubules. The 45° line corresponds to a change in reabsorptive rate exactly proportional to the change in r^2. The change in reabsorptive rate was measured in each tubule whereas the change in r^2 was calculated from measurements of inulin concentration, transit time, and nephron filtration rate (see text and equations 8 and 9). The two values shown as triangles represent instances in which nephron GFR appeared to increase by 27 and 38 per cent, respectively.

The relationship between the ln $(TF/P)_{In}$ and transit time, expressed as the mean values for the ratio $C/\pi r^2$ is summarized in Table VII for control and experimental tubules. When ureteral pressure was increased, in every instance this ratio fell (range 13–85%). As can be seen in Fig. 4, the fall in this ratio was the consequence of a relatively constant reabsorptive rate (Cd) at a time when the radius increased. In addition, since V_0 in these experiments also changed little, (Table VII) $\pi r^2 d/V_0$ increased. In control animals the mean changes in nephron GFR, transit time, $(TF/P)_{In}$, and calculated values of $C/\pi r^2$ and $\pi r^2 d/V_0$ were small (Tables VI and VII).

DISCUSSION

It has been observed by others that fractional sodium reabsorption in the proximal tubule, as measured by the inulin TF/P ratio, remains relatively constant after a reduction in the rate of glomerular filtration (4–6). This has been interpreted to indicate that a very precise quantitative relationship exists between reabsorption and filtered load. In the present study, after acute reductions or elevations in GFR, although fractional reabsorption was not constant, in nearly every instance there was a change in the absolute rate of reabsorption, in the same direction, and usually more than half that required to maintain perfect glomerulotubular balance. These findings are similar to those recently reported by Landwehr, Schnermann, Klose, and Giebisch (20). The extent to which reabsorption in the proximal tubule is modified when glomerular filtration rate is changed is highly relevant to interpretation of the effect of such changes on sodium excretion. However this will not be considered further since the issue with which this investigation is mainly concerned is the mechanism by which the adjustment is effected.

One proposed mechanism for this adjustment involves a humorally mediated feedback control of proximal sodium reabsorption (7). A system of this type, in its simplest form might be viewed as involving three components: a sensor, which in this case might perceive a physical or chemical change in the fluid arriving at some site in the distal nephron (for example, the macula densa); a site for hormone synthesis, storage, and release; and finally a pathway via which the humoral factor would reach the proximal tubule. It is very likely that a finite period of time would be required from disruption of the steady state to completion of this sequence of events. The finding that the adjustment in reabsorption takes place within 30 sec of the change in GFR, therefore, makes this proposed mechanism seem unlikely. Moreover, an important prediction of this hypothesis, whether the hormone is one which inhibits or stimulates sodium reabsorption, is that the time required for onset of its effect probably would not be identical with the time required for its dissipation. Consequently, the finding that there was an immediate change in reabsorption whether the GFR was lowered or raised, while not entirely conclusive, can be considered to be evidence against this hypothesis.

As a consequence of the experimental design,

fractional reabsorption in the proximal tubule was measured when tubule volume ($\pi r^2 d$) and nephron GFR (V_0) were changed in the same and opposite directions. When the changes were parallel (aortic constriction and release) the ratio $(\pi r^2 d)/V_0$ remained relatively constant whereas when the changes were divergent, (increased ureteral pressure) $(\pi r^2 d)/V_0$ doubled. Because they found that fractional reabsorption remained relatively constant during aortic constriction and appeared to increase during periods of increased ureteral pressure, Rector and coworkers concluded that this relationship between tubule volume and GFR is one important determinant of fractional reabsorption (6). In contrast, our data do not support this interpretation. In the initial experiments in which, on the average $(\pi r^2 d)/V_0$ remained relatively constant, fractional sodium reabsorption increased (aortic constriction) or decreased (release of aortic constriction), whereas in the increased ureteral pressure experiments, this ratio doubled while fractional reabsorption remained relatively unchanged.

In order to evaluate the proposed relationship (6, 8) between absolute reabsorptive rate and tubule volume, we calculated the ratio $(C/\pi r^2)$ (equation 5), where C is the reabsorptive rate and πr^2 the tubule volume, each per unit tubule length. It also was possible to obtain a more direct estimate of $\pi r^2 d$ (equations 8 and 9) and thereby compare the change in tubule volume directly with the observed change in absolute reabsorption. In agreement with others (6, 8, 20) reabsorptive rate (C) and tubule volume (πr^2) per unit length often changed in the same direction when the GFR was changed by means of an aortic clamp. However, in contrast to others, the change in C was rarely proportional to the change in tubule volume; consequently $C/\pi r^2$ varied widely (Fig. 2). On the basis of these initial findings, we suspected that absolute reabsorption was not directly related to tubule volume. The results from the increased ureteral pressure experiments, shown in Fig. 4, confirm this suspicion, in that tubule volume increased while absolute reabsorptive rate remained relatively constant or declined.

It is apparent that the strongest evidence to support or refute the geometry hypothesis in this study and in those reported by Rector and associates (6) is derived from those experiments in which ureteral pressure was increased. For methodological and theoretical reasons, we believe the present findings to be the correct ones. Both studies have in common the finding of prolonged transit time after increased ureteral pressure and values for TF/P inulin of which some increased and some remained relatively unchanged. Rector and associates (6) found that during periods of increased ureteral pressure, if the distal oil block was not adequate, retrograde contamination resulted in very high inulin TF/P ratios (range 10–100). However it was not possible for them to evaluate the contribution of retrograde flow to the TF/P inulin ratios in the range of 2–5. In the current study, the GFR of individual nephrons was measured and it was found that in those few tubules in which the TF/P inulin increased (range 2.2–15), calculated nephron GFR was also increased. Since increased ureteral pressure is not likely to produce elevations in GFR of surface nephrons while depressing whole kidney GFR, we conclude that in these 10 instances the TF/P_{In} was elevated spuriously, presumably by contamination with fluid from more distal sites. Another difference between the studies is that in the present experiments the recollection micropuncture method was used; consequently, each tubule served as its own control. This served to eliminate the scatter in the data resulting from variability among tubules and among animals in response to the experimental maneuver.

In the initial studies by Rector and coworkers (6), the seemingly proportional relationship between reabsorptive rate and tubule volume during the period of increased ureteral pressure was demonstrated in antidiuretic rats. More recently, these same workers using the recollection micropuncture technique and measuring nephron filtration rates found results during antidiuresis similar to our own, namely an increase in tubule volume without a concomitant change in reabsorption.[8] During saline loading again they found increased inulin (TF/P) ratios during the period of elevated ureteral pressure; however, in each of these recollections, nephron filtration rate was increased. In the present study, also conducted during saline loading, most inulin (TF/P) ratios were unchanged, and most nephron and all kidney filtra-

[8] Rector, F. C., Jr. Personal communication.

tion rates fell slightly after ureteral pressure was elevated. Since there is nothing in the experimental design that could lead to falsely low inulin (TF/P) ratios and one would predict, both a priori and from the change in whole kidney GFR, that the GFR in surface nephrons should fall, we conclude that the unchanged inulin (TF/P) values and the lower nephron filtration rates are the correct ones.

The most obvious and concise interpretation of these data is that proximal sodium reabsorption is not dependent upon tubule volume or any other function of tubule geometry. However, this is not the only possible interpretation. For example, in these experiments raising the ureteral pressure conceivably could have changed reabsorptive capacity (C) in some fashion independent of its hypothetical relation to πr^2. The superimposed increase in πr^2 might then have been such as to have increased the absolute rate of reabsorption exactly enough to result in constancy of fractional reabsorption. Not only does this combination of events seem unlikely a priori, but there is experimental evidence that seems definitely to exclude it. If elevated ureteral pressure were to alter C, one would predict that this maneuver would prolong the reabsorptive $t_{\frac{1}{2}}$ of the shrinking drop as measured by the technique of Gertz (12), since in this procedure r^2 is large and more or less independent of ureteral pressure. However, in two studies it has been found that elevation of ureteral pressure to levels as high as 60 mm Hg resulted in no significant change in $t_{\frac{1}{2}}$ (14, 21). Although as previously mentioned we have reservations about high $(TF/P)_{In}$ values obtained during elevated ureteral pressure, this maneuver should not cause falsely low $t_{\frac{1}{2}}$ values, and we assume that these latter measurements are correct. Consequently the most reasonable conclusion to be drawn from the experiments done during ureteral pressure elevation is that reabsorptive rate is not dependent upon tubule volume.

Another line of evidence, apparently contrary to the above conclusion, derives from the remarkable agreement between the $t_{\frac{1}{2}}$ and the transit time to the site in the tubule at which $(TF/P)_{In} = 2$ (50% reabsorbed). The relationship between reabsorption in free-flow experiments and in the split drop technique (12) is given by the expression:

$$\frac{\ln (2.0)}{t_{\frac{1}{2}}} = \frac{C}{\pi r^2} = \frac{\ln (TF/P)_{In}}{\text{transit time}}$$

Since the radius at the split drop appears invariably to be larger, and often twice the radius of the tubule during free-flow collections (9, 13, 14), the finding of nearly identical fractional reabsorption under the two circumstances suggests that absolute reabsorptive rate per unit length of tubule (C) is proportional to the square of the radius. This agreement between $t_{\frac{1}{2}}$ and transit time (to the point in the tubule where $(TF/P)_{In} = 2$) has been shown both during hydropenia (8, 9, 14) and saline diuresis (17, 18). Giebisch, Klose, Malnic, Sullivan, and Windhager have reported less exact agreement between the reabsorptive rate predicted from $t_{\frac{1}{2}}$ measurements and that actually measured from the change in inulin concentration in different spilt drops (22). However, the comparison of values for $C/\pi r^2$ calculated from these two different kinds of measurements reveals quite good agreement in the majority of instances. These findings are difficult to reconcile with the data presented in the present paper.

This dilemma of course would be resolved if the radius of the aqueous column in the split drop were in actual fact not greater than the radius of the tubule under free-flow condition. All reported measurements of radii of split drops have been of the oil columns on either side of the aqueous drop, rather than of the aqueous drop itself. There is no reason why the radii at the two different points necessarily must be the same.[9] Another source of uncertainty is in the measurement of radius during free-flow collections. Using our own measurements of (TF/P) inulin, transit time, volume flow, and tubule length, we calculated a radius approximately 50% greater than that usually measured from photographs of rat proximal tubules under comparable conditions (13, 14). On the other hand using the measurements of (TF/P) inulin,

[9] Although it is possible to color the saline droplet and thus to arrive at a better estimate of its diameter relative to that of the castor oil two problems remain: (*a*) the diameter of the saline droplet can easily be varied by the pressure applied to the mineral oil; (*b*) the measurement of the diameter of the colored droplet in photographs of the kidney surface is subject to the same uncertainties that effect these measurements in free flow.

transit time, volume flow, and radius (from photographs) by Lewy and Windhager (9), one calculates that many of their collections would have to have been made several millimeters beyond what has been found to be the end of the surface convolutions of the proximal tubule in the rat. Using data kindly supplied to us by Windhager, we were able to calculate $\pi r^2 d$ for each tubule (equations 5, 7 and 8), and by using their value for r^2 (obtained from photographs), we calculated d, the distance to the puncture site from the glomerulus. From their data d varied from 4.5 to 14.4 mm in control animals (with 15 of 23 values being beyond 7.0 mm) and from 2.6 to 62.0 mm in animals with renal venous occlusion (8 of 15 beyond 7.0 mm). The average measured length of the proximal tubule was found by us to be 8.6 mm $\pm$ 1.7 SD, a value similar to that reported by Sperber (23). Since it is rarely possible to puncture beyond 65% of this segment in this species, data which so frequently yield calculated values for d of 7 mm or more suggest an error in one or more of the measurements necessary to make these calculations. Although we have no good evidence upon which to base an objection to any specific measurement it is difficult to see how the relatively precise measurements of inulin concentration, transit time, and volume flow would be in error to an extent sufficient to explain these discrepancies. Moreover we feel that the measurements of the radii from photographs of the tubules during free flow contain a large degree of uncertainty. This uncertainty stems both from the difficulty in deciding from the photograph what the true boundaries of the lumen are, and also from the fact that a considerable portion of the proximal tubule (from which the collection is made) is below the surface and cannot be measured at all. We conclude that from the available information it is not possible to be certain about a value for the radius either during free-flow collections or in the shrinking drop, and before more definitive measurements are made the agreement between the $t_{\frac{1}{2}}$ and the transit time (to a TF/P of 2) cannot be interpreted as evidence for the geometry hypothesis.

A recent study by Wiederholt, Hierholzer, Windhager, and Giebisch (15), utilizing the technique of continuous microperfusion, investigated the relationships among flow rate, tubule diameter, and reabsorptive rate, both in normal hydropenia and during elevated ureteral pressure. Although they conclude that tubule volume governs reabsorptive rate, inspection of the data obtained from individual tubules studied at different perfusion speeds (hence when the radius was varied) often failed to show proportional changes in absolute reabsorptive rate. Considering the frequency with which disproportionate changes between tubule volume and reabsorptive rate occurred, their conclusion of a causal relationship between tubule volume and reabsorptive rate seems less convincing.

If one accepts then, that neither tubule geometry nor an intrarenal hormone mediate the balance between glomerular filtration rate and tubular reabsorption, one should consider other possible mechanisms. Lewy and Windhager have suggested that reabsorption may be regulated in large part by extratubular events, particularly the rate at which the peritubular capillary circulation removes the reabsorbate (9). According to their view the control of proximal sodium and water reabsorption is the resultant of the dynamic interplay between oncotic and hydrostatic pressure operating at the peritubular level. Although this is an attractive hypothesis, their evidence thus far cannot be viewed as conclusive and it is clear that further work will be required to elucidate the mechanism of this intriguing adjustment in proximal sodium reabsorption.

REFERENCES

1. Smith, H. W. 1951. The Kidney: Structure and Function in Health and Disease. Oxford University Press, New York.
2. Lassiter, W. E., M. Mylle, and C. W. Gottschalk. 1964. Net transtubular movement of water and urea in saline diuresis. *Am. J. Physiol.* **206**: 669.
3. Giebisch, G., R. M. Klose, and E. E. Windhager. 1964. Micropuncture study of hypertonic sodium chloride loading in the rat. *Am. J. Physiol.* **206**: 687.
4. Dirks, J. H., W. J. Cirksena, and R. W. Berliner. 1965. The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. *J. Clin. Invest.* **44**: 1160.
5. Glabman, S., H. S. Aynedjian, and N. Bank. 1965. Micropuncture study of the effect of acute reductions in glomerular filtration rate on sodium and water reabsorption by the proximal tubules of the rat. *J. Clin. Invest.* **44**: 1410.
6. Rector, F. C., Jr., F. P. Brunner, and D. W. Seldin. 1966. Mechanism of glomerular balance. I. Effect

of aortic constriction and elevated ureteropelvic pressure on glomerular filtration rate, fractional reabsorption, transit time, and tubular size in the proximal tubule of the rat. *J. Clin. Invest.* **45**: 590.
7. Leyssac, P. P. 1967. Intrarenal function of angiotensin. *Federation Proc.* **26**: 55.
8. Gertz, K. H., J. A. Mangos, G. Braun, and H. D. Pagel. 1965. On the glomerular tubular balance in the rat kidney. *Arch. Ges. Physiol.* **285**: 360.
9. Lewy, J. E., and E. E. Windhager. 1968. Peritubular control of proximal tubular fluid reabsorption in the rat kidney. *Am. J. Physiol.* **214**: 943.
10. Vurek, G. G., and S. E. Pegram. 1966. Fluorometric method for the determination of nanogram quantities of inulin. *Anal. Biochem.* **16**: 409.
11. Führ, J., J. Kaczmarczyk, and C. D. Krüttgen. 1955. Eine einfache colorimetrische Methode zur Inulinbestimmung fur Nieren-clearance-untersuchungen bein Stoffwechselgesunden und Diabetikern. *Klin. Wochschr.* **33**: 729.
12. Gertz, K. H. 1963. Transtubuläre natriumchloridflüsse und permeabilität fur nichtelektrolyte im proximalen und distalen konvolut der rattenniere. *Arch. Ges. Physiol.* **276**: 336.
13. Hayslett, J. P., M. Kashgarian, and F. H. Epstein. 1967. Changes in proximal and distal tubular reabsorption produced by rapid expansion of extracellular fluid. *J. Clin. Invest.* **46**: 1254.
14. Brunner, F. P., F. C. Rector, Jr., and D. W. Seldin. 1966. Mechanism of glomerulotubular balance. II. Regulation of proximal tubular reabsorption by tubular volume, as studied by stopped-flow microperfusion. *J. Clin. Invest.* **45**: 603.
15. Wiederholt, M., K. Hierholzer, E. E. Windhager, and G. Giebisch. 1967. Microperfusion study of fluid reabsorption in proximal tubules of rat kidneys. *Am. J. Physiol.* **213**: 809.
16. Flanigan, W. J., and D. E. Oken. 1965. Renal micropuncture study of the development of anuria in the rat with Mercury-induced acute renal failure. *J. Clin. Invest.* **44**: 449.
17. Landwehr, D. M., R. M. Klose, and G. Giebisch. 1967. Renal tubular sodium and water reabsorption in the isotonic sodium chloride-loaded rat. *Am. J. Physiol.* **212**: 1327.
18. Rector, F. C., Jr., J. C. Sellman, M. Martinez-Maldonado, and D. W. Seldin. 1967. The mechanism of suppression of proximal tubular reabsorption by saline infusions. *J. Clin. Invest.* **46**: 47.
19. Cortney, M. A., M. Mylle, W. E. Lassiter, and C. W. Gottschalk. 1965. Renal tubular transport of water, solute, and PAH in rats loaded with isotonic saline. *Am. J. Physiol.* **209**: 1199.
20. Landwehr, D., J. Schnermann, R. M. Klose, and G. Giebisch. 1967. The effect of acute reduction in glomerular filtration rate on renal tubular sodium and water reabsorption. *Federation Proc.* **26**: 547. (Abstr.)
21. Steinhausen, M. 1967. Studies on the microcirculation of renal tubular flow under the special aspect of the glomerulo-tubular balance. *Bibliotheca Anat.* **9**: 135.
22. Giebisch, G., R. M. Klose, G. Malnic, W. J. Sullivan, and E. E. Windhager. 1964. Sodium movement across single perfused proximal tubules of rat kidneys. *J. Gen. Physiol.* **47**: 1175.
23. Sperber, I. 1944. Studies on the mammalian kidney. *Zool. Bidrag. Uppsala.* **22**: 249.