The Relative Contributions of Reabsorptive Rate and Redistributed Nephron Filtration Rate to Changes in Proximal Tubular Fractional Reabsorption during Acute Saline Infusion and Aortic Constriction in the Rat

ETTORE BARTOLI and LAURENCE E. EARLEY

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

ABSTRACT The absolute rate of reabsorption by superficial rat proximal tubules was measured by the *in situ* microperfusion technique under conditions of hydropenia, infusion of saline, and infusion of saline plus aortic constriction sufficient to decrease whole kidney filtration rate below hydropenic levels. Fractional reabsorption was measured in adjacent filtering nephrons by collecting and recollecting tubular fluid from late proximal convolutions during each experimental condition. During hydropenia, the absolute rate of proximal tubular reabsorption averaged 3.56 ±0.60 nl/min per mm and late proximal tubular fractional reabsorption averaged 0.56 ±0.10. From these two measurements and measurements of tubule length to the site of micropuncture, a value for filtration rate was calculated for filtering nephrons. During hydropenia this value averaged 32.9 ±7.1 nl/min. Saline infusion increased sodium excretion to 5.5% of the filtered load as the absolute rate of proximal tubular reabsorption decreased 38% and fractional reabsorption decreased 45%. Calculated superficial nephron filtration rate increased 21% which on the average was identical with the simultaneously measured increase in whole kidney filtration rate. Similar results were obtained in a separate group of animals by the technique of total collection of late proximal tubular fluid. Aortic constriction during saline infusion decreased whole kidney and calculated nephron filtration rate to the same degree and to values lower than those during hydropenia. Fractional reabsorption increased but not to hydropenic values. The persistent natriuresis during aortic constriction was associated with a continued depression of the absolute rate of proximal tubular reabsorption which was sufficient to maintain an increased delivery of filtrate out of the proximal tubule despite the fall in nephron filtration rate. These results indicate that depressed fractional reabsorption in the proximal tubule during acute saline infusion is due predominantly to a decrease in absolute reabsorptive rate and to a lesser extent to an increase in superficial nephron filtration rate which is proportional to the increase in whole kidney filtration. Continued natriuresis when filtration rate is decreased during saline infusion can be accounted for entirely by the persistent large reduction in the absolute rate of proximal tubular reabsorption.

INTRODUCTION

The over-all rate of tubular reabsorption of sodium is depressed by saline infusion in the dog (1, 2) and rat (3, 4), and this decreased rate of reabsorption is extensive enough to result in increased sodium excretion even when total filtration rate is reduced experimentally (2, 5, 6). Micropuncture studies have demonstrated that saline infusion decreases the fraction of filtrate reabsorbed in the proximal tubule to a much greater extent than the simultaneous increase in the fraction of filtered sodium excreted (2, 4). Furthermore, the depression in fractional reabsorption by the proximal tubule resulting from infusion of saline persists during large experimental reductions in total filtration rate (2, 6). Therefore, it has been generally assumed that natriuresis during acute extracellular volume expansion, results for the most part, from depression of the absolute rate of reabsorption by the proximal tubule, and that this depressed rate of reabsorption is

Dr. Bartoli is a Fellow of the Italian National Research Council from Istituto di Patologia Medica, Torino, Italy.

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sufficiently great to produce continued natriuresis even when filtration rate is decreased experimentally. However, for several reasons, these interpretations of the role of proximal tubular reabsorption in the response to expansion of the extracellular volume require reexamination. Horster and Thurau (7) reported that during chronic salt loading in the rat, fractional reabsorption in superficial proximal tubules was not decreased, but an increased delivery of sodium to the distal nephron was present as a result of increased filtration rate by superficial nephrons. This increased rate of filtration in superficial nephrons occurred during salt loading despite little change in whole kidney filtration rate, and these investigators also found an associated decrease in filtration rate of juxtamedullary nephrons (7). These observations suggest that during chronic salt loading, the absolute rate of proximal tubular reabsorption is actually increased and natriuresis may be due to a disproportionate increase in filtration rate by superficial nephrons. Barger (8) and Carriere, Thornburn, O'Morchoe, and Barger (9) had proposed much earlier that a redistribution of glomerular filtrate away from superficial nephrons may play an important role in the sodium retention resulting from experimental heart failure or acute hemorrhage. Herrera-Acosta, Rector, and Seldin (10) have reported recently that in the rat, acute saline infusion also results in increases in superficial nephron filtration rate that are proportionately greater than the increase in whole kidney filtration rate, suggesting that a redistribution of filtration rate may be the major factor in the natriuresis accompanying acute volume expansion (10). Theoretically, if increases in nephron filtration rate were sufficiently large, tubular reabsorptive capacity could be exceeded and proximal tubular fractional reabsorption could be decreased without necessitating a decrease in the absolute rate of reabsorption. Several studies have reported that saline infusion results in a decreased rate of proximal tubular reabsorption as judged by prolongation of the reabsorptive half-time of isolated droplets in the proximal tubule (shrinking drop) (11–13). However, it is difficult to assess the quantitative contribution of depressed absolute reabsorption to the change in fractional reabsorption since the reabsorptive half-time will be influenced by small, perhaps immeasurable, differences in radius of the tubular lumen. In other studies, the contribution of a change in absolute reabsorptive rate to depressed proximal tubular fractional reabsorption during saline infusion has been assessed by microperfusion (14, 15) and by measurements of single nephron filtration rate determined by total collection of tubular fluid. This latter technique has yielded conflicting results regarding the relative roles of increased nephron filtration rate and decreased proximal tubular reabsorption in the depression of fractional reabsorption during saline infusion (10, 16–19).

The purpose of the present study was to reexamine the extent to which acute saline infusion in the rat alters the absolute rate of proximal tubular reabsorption, and the contribution of this change to the depression in proximal tubular fractional reabsorption and the increased excretion of sodium. From measurements of absolute and fractional reabsorption, single nephron filtration rate could be calculated and the changes compared with those occurring in whole kidney filtration rate, thereby allowing an assessment of the contribution of redistribution of nephron filtration rates. In addition, the effect of aortic constriction on the changes in proximal tubular reabsorption resulting from saline infusion, were examined in view of a recent report that aortic constriction may reverse the depression of proximal tubular reabsorption produced by extracellular volume expansion (13). The results indicate that the large decrease in the absolute rate of reabsorption by superficial proximal tubules during saline infusion, is the major factor contributing to the depression of fractional reabsorption. This depression of absolute reabsorptive rate, persisted during aortic constriction despite an associated increase in fractional reabsorption which was attributed to a fall in nephron filtration rate. Nevertheless, depressed proximal tubular reabsorption during aortic constriction was sufficient to account for the continued increase in sodium excretion. With each experimental maneuver calculated or directly measured, superficial nephron filtration rate changed in proportion to changes in whole kidney filtration rate, and there was no evidence that a redistribution of filtration rate accounted predominantly for changes in superficial proximal tubular fractional reabsorption, either during saline infusion or aortic constriction.

METHODS

Experiments were performed on 38 male Sprague-Dawley rats weighing from 200 to 460 g. Anesthesia was induced by intraperitoneal injection of Inactin (Promonta, Hamburg, West Germany), 100 mg/kg body weight, and the animals were placed on a heated operating table that maintained body temperature at 37.5°C. An endotracheal tube was surgically placed, and polyethylene catheters were inserted into the right external jugular vein 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. As soon as the jugular catheter was inserted, a modified Ringer's solution (Na 142; K 3.5; Cl 125.5, and HCO_3 20 mEq/liter; containing inulin to achieve a plasma concentration of 50–100

mg/100 ml, and p-aminohippuric acid [PAH] [1] in an amount appropriate for clearance measurements) was infused at a rate of 50 μl/min per kg body weight.

Micropuncture techniques. Perfusion of superficial proximal tubules was performed by a technique similar to that described by Sonnenberg, Deetjen, and Hampel (20). The perfusion system was the same as previously described by Buentig and Earley (21). Convolutions of the same proximal tubule were identified by injecting an oil droplet through a 5 μ diameter pipette into one convolution and mapping its course through successive loops. The perfusion pipette was inserted into the most proximal convolution and a second pipette, 8–10 μ in diameter, was inserted even more proximally, through which castor oil was injected until the tubule was filled up to the perfusing pipette. In the process of removing the second pipette, a window was opened in the tubule to allow escape of filtrate proximal to the oil block. The second pipette was then inserted into one of the most distal convolutions, and a second oil block, 3–4 tubular diameters long, was placed distal to the pipette tip, after which collection of the perfused fluid was started. No attempt was made to keep the distal oil block in place when perfused fluid was flowing steadily into the collecting pipette without applying negative pressure. The solution used to perfuse proximal tubules contained Na 138, K 4.5, Cl 112.5, HCO_3 30, urea 5, and glucose 2.5 mM/liter, respectively, and was stained with lissamine green in a concentration of 0.05–0.1 g/100 ml. Iothalamate-^{125}I was added to the perfusion fluid as the marker for volume reabsorption (22, 23). 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 expelled quantitatively under oil into a 20 μl volume of water. This final dilution of the collected sample was transferred quantitatively into a capillary tube for measurement of radioactivity in a Packard automatic gamma counter (Packard Instrument Co, Downers Grove, Ill.) Triplicate samples of the perfusion solution were handled in precisely the same manner as the samples collected from perfused tubules. The samples were counted for 10–50 min yielding a total radioactivity 10–95 times the background. The error in the radioactive measurements was calculated from all the duplicate samples as $2(P_1 - P_2)/P_1 + P_2$, where P_1 is the duplicate with the higher reading. This calculated error was 1.7 ±1.4% (SD), n = 120. After completing collections from a single proximal tubule, the convolutions were filled with latex, and at the completion of an entire study, the kidney was macerated in 6 N HCl at 4°C, usually for 24–36 hr. 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 the perfused proximal tubular segments were varied intentionally and ranged between 0.2 and 5.0 mm. 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:

$$C(\text{nl/min per mm}) = V_t\,[1-(PF/CF)_I]/L,$$

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

Samples of tubular fluid were collected from late convolutions of adjacent filtering proximal tubules in 20 rats. The end proximal convolutions were identified with the same technique used for the perfused segments, and collection and recollections were made from the same convolutions under each experimental condition in each individual animal. In 13 rats, 163 samples were collected under the various experimental conditions utilizing the same technique used for collecting perfusion fluid in adjacent tubules (distal castor oil block), but no attempt was made to perform a total collection. In 7 rats, in which microperfusions were not performed, 72 total-timed free flow collections were made from the last accessible convolution of the proximal tubule. In these animals, a castor oil block was pushed upstream in the tubule and collections were accepted if its downstream movement was arrested without aspiration, or minimal intermittent aspiration, and it remained in place usually with slight to-and-fro movement. Collection time varied between 1 and 3.5 min. Recollections from the same tubule were not performed when the same last convolution was not recognizable or when the oil block from the previous collection was still in place. The total volume of collected fluid was measured in a constant bore capillary tube, and the samples were then transferred under oil into a volumetric pipette (18 nl) for measurement of inulin concentration.

Urine was collected from the left ureteral catheter directly into capillary micropipettes, and samples of arterial blood (0.1–0.2 ml) were withdrawn at the beginning and at the end of each experimental procedure in every individual animal. Pressure in the femoral artery was monitored throughout the experiment by a pressure transducer and a direct writing recorder (Hewlett-Packard, Avondale, Pa.).

In all experiments, the rate of microperfusion was 28 nl/min. The perfusion pump had been studied extensively in the past (21), and was calibrated frequently in vitro during the present study. In addition, nine total-timed collections were made in vivo during microperfusion, and the collected tubular fluid was tested for inulin as an index of contamination by glomerular filtrate or fluid from adjacent tubules. No inulin was detected, and the in vivo calculated perfusion rate was 27.1 ±4.3 nl/min. The absolute rate of tubular reabsorption measured in these recovery experiments was not statistically different from that measured in the bulk of studies when no attempt was made to collect all the perfused fluid reaching the collecting pipette. These findings suggest that there were no major errors either in the perfusion rate or from the contamination of collected perfusate with iothalamate-^{125}I-free fluid. 13 additional tubules in 6 rats were perfused at two different perfusion rates (28 and 56 nl/min) and samples were collected and recollected from the same site. The absolute rate of reabsorption calculated from the paired values decreased 5.6 ±12.2% when the perfusion rate was doubled. The change was not significant ($P > 0.05$) and this is in agreement with observations of others (14, 21, 24, 25), that the perfusion rate per se has little influence on the absolute rate of proximal tubular reabsorption. The rates 28 and 56 nl/min encompass most of the nephron filtration rates measured or calculated in the present study.

Experimental maneuvers. In 13 rats, the experimental protocol was as follows. Each animal was studied during hydropenia, following infusion of saline and again after

[1] *Abbreviations used in this paper:* GFR, whole kidney filtration rate; PAH, p-aminohippuric acid; SNGFR, single nephron filtration rate.

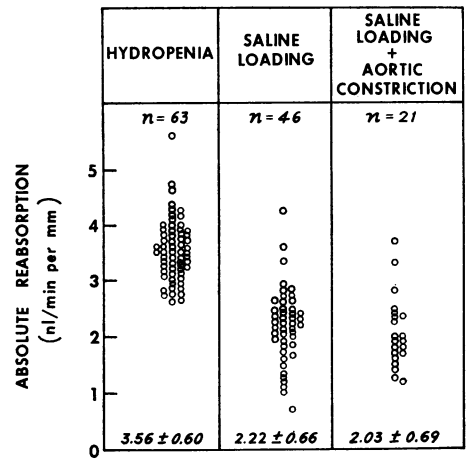

FIGURE 1 *Absolute rate of proximal tubular reabsorption determined by microperfusion before and after infusion of saline and during aortic constriction in the presence of infusion of saline.* Each point represents the result from a single proximal tubular perfusion. 34 measurements were made in the 13 animals represented in Table I under control (hydropenic) conditions and 26 measurements were made in these same 13 animals during saline infusion. In 12 of the same animals 20 measurements were made during aortic constriction in the presence of infusion of saline. Data are shown from 18 additional animals not represented in Tables I and II. Means and standard deviations are reported for each experimental situation.

constricting the aorta during infusion of saline. Infusion of saline is defined as the infusion of a modified Ringer's solution (Na 142, K 4, Cl 118, and HCO_3 28 mEq/liter, respectively) until a volume equal to 10% of the animal's weight was infused over a period of 50–60 min. Afterwards, the Ringer solution was infused at 200–400 μl/min throughout the remainder of the study. Following the initial infusion, experimental collections were begun. The aorta was constricted during volume expansion to reduce femoral artery (and renal artery) pressure from an average of 125 to an average of 80 mm Hg. 30–45 min after constricting the aorta, experimental collections were begun again. Under each experimental situation in every animal, one to six microperfusions of proximal tubular segments were performed and two to six end-proximal tubular free-flow samples were collected from adjacent tubules. Two to six timed urine collections for clearances of inulin and PAH were made during each experimental maneuver. In one rat the aorta was not constricted, and in one rat, urine samples during aortic constriction were lost. In 18 additional animals, only microperfusion studies were performed. 11 of these animals were studied during both hydropenia and volume expansion but the aorta was constricted in only 1. In 5 of the 18 animals, microperfusions were performed only during hydropenia, and in two microperfusions were performed only during volume expansion. The results obtained in these animals were not different statistically from those obtained in the other 13 animals and were included, therefore, in the over-all values shown in Fig. 1. In seven rats, only free-flow total collections were performed during hydropenia and following volume expansion. In three of these animals, the aorta was constricted before volume expansion, but the data during aortic constriction are not included in this report.

In the 13 animals in which the absolute rate of reabsorption was determined by microperfusion and fractional reabsorption was determined in adjacent end-proximal tubules, single nephron filtration rate (SNGFR) was calculated as:

$$\text{SNGFR (nl/min)} = C \cdot L/1\text{-}(PF/TF)_{In}$$

where C = absolute reabsorptive rate in nl/min per mm determined by microperfusion, L = length of proximal tubular segment (in millimeters) from glomerulus to site of end-proximal tubular free-flow collection, and $1\text{-}(PF/TF)_{In}$ = fractional reabsorption of the filtered volume to the point of free flow collection. PF and TF are the concentrations of inulin in plasma and tubular fluid respectively. L was determined by microdissection of latex casts of 59 proximal tubules and averaged 5.2 ±0.4 mm (SD). In the seven animals in which total collections of late proximal tubular fluid were made, nephron filtration rate was calculated as:

$$\text{SNGFR} = V(TF/P)_{In}$$

where V = the rate (nl/min) of collection of tubular fluid.

Determinations of Na, K, inulin, and PAH in plasma and urine were performed by the techniques previously described for this laboratory (26). Inulin in duplicate samples of tubular fluid was determined by a modification of the microtechnique of Vurek and Pegram (27). The error of this determination was calculated from 143 duplicate determinations and was 4.4 ±3.6% (SD). The significance of differences between means of measured variables was calculated using the variance analysis.

RESULTS

Absolute reabsorptive rate. The absolute rate of proximal tubular reabsorption was measured by microperfusion of 133 tubular segments in 31 rats and the length of the perfused segment ranged from 0.2 to 5.0 mm. The absolute rate of reabsorption determined by microperfusion was independent of the length of perfused tubule. Between tubular lengths ranging from 0.2 to 5.0 mm, the linear correlation coefficients of the fraction of perfusate absorbed per segment length were 0.96, 0.88, and 0.89 during hydropenia, infusion of saline, and constriction of the aorta, respectively. The intercept of zero reabsorption for the three experimental conditions was between 0.10 and 0.25 mm tubular length. During hydropenia, the rate of reabsorption in 63 tubules in 13 rats averaged 3.56 ±0.60 (SD) nl/min per mm. After saline loading, 46 different proximal tubules were perfused in the same animals and the rate of reabsorption averaged 2.22 ±0.66 nl/min per mm. This decrease of 38% in the absolute rate of proximal tubular reabsorption after saline loading was highly significant ($P < 0.01$). During saline diuresis, the aorta was constricted in 12 of the animals to decrease renal perfusion pressure from an average of 125 mm Hg to an average of 80 mm Hg, and 21 different proximal tubular segments were perfused. During saline loading in the

TABLE I

Effects of Saline Infusion and Aortic Constriction on Absolute and Fractional Volume Reabsorption by the Rat Proximal Tubule and on Whole Kidney and Calculated Nephron Filtration Rates

Experiment	BP	Wt	C	TF/P_{in}	$1 - P/TF_{in}$	SNGFR	GFR	$\frac{SNGFR}{GFR} \times 10^6$
	mm Hg	*g*	*nl/min per mm*			*nl/min*	*µl/min*	
1 Hydropenia	145	470	4.97 (2)	2.13 (5)	0.48 (5)	51.9	1874 (2)	27.6
Saline infusion	150		2.67 (2)	1.27 (5)	0.19 (5)	68.8	1638 (2)	42.0
Aortic constriction	100		3.32 (1)	1.67 (4)	0.33 (4)	49.7	1570 (2)	31.7
2 Hydropenia	100	460	3.39 (2)	2.97 (5)	0.66 (5)	25.8	1432 (2)	18.0
Saline infusion	117		2.41 (1)	1.64 (5)	0.37 (5)	32.1	1890 (2)	17.0
Aortic constriction	90		2.36 (1)	1.97 (5)	0.44 (5)	26.8	1647 (2)	16.2
3 Hydropenia	115	400	3.74 (2)	2.52 (5)	0.57 (5)	32.9	1121 (3)	29.3
Saline infusion	120		2.03 (4)	1.43 (5)	0.28 (5)	36.6	1330 (3)	27.5
Aortic constriction	80		1.77 (4)	1.84 (5)	0.41 (5)	21.6	852 (3)	25.3
4 Hydropenia	145	230	3.41 (3)	2.37 (4)	0.56 (4)	30.3	1410 (3)	21.5
Saline infusion	140		1.96 (2)	1.77 (5)	0.39 (5)	25.2	1720 (2)	14.6
Aortic constriction	70		1.96 (2)	2.43 (4)	0.42 (4)	23.5	1033 (2)	22.7
5 Hydropenia	115	260	3.04 (4)	1.98 (2)	0.49 (2)	30.8	1344 (2)	22.9
Saline infusion	130		1.54 (2)	1.50 (5)	0.29 (5)	26.4	1172 (4)	22.5
Aortic constriction	70		1.03 (1)	1.19 (4)	0.15 (4)	33.4	643 (2)	52.0
6 Hydropenia	120	330	3.22 (1)	2.66 (3)	0.62 (3)	26.0	1237 (3)	20.9
Saline infusion	130		2.61 (1)	1.49 (4)	0.33 (4)	39.7	2106 (3)	18.8
Aortic constriction	65		1.55 (3)	1.98 (4)	0.47 (4)	16.5	1309 (4)	12.6
7 Hydropenia	133	215	2.75 (1)	2.47 (5)	0.59 (5)	23.4	943 (3)	24.8
Saline infusion	132		1.78 (2)	1.43 (3)	0.29 (3)	30.5	1150 (3)	26.5
Aortic constriction	75		1.80 (1)	2.02 (4)	0.47 (4)	19.2	—	—
8 Hydropenia	115	230	3.89 (3)	2.64 (4)	0.59 (4)	33.0	857 (2)	38.5
Saline infusion	130		3.23 (3)	1.32 (3)	0.23 (3)	70.2	1410 (3)	49.8
Aortic constriction	80		3.7 (1)	2.04 (2)	0.44 (2)	42.0	1072 (2)	39.2
9 Hydropenia	125	220	3.76 (3)	2.16 (5)	0.51 (5)	36.6	1177 (3)	31.1
Saline infusion	130		2.10 (1)	1.46 (5)	0.28 (5)	37.9	1423 (3)	26.6
Aortic constriction	93		2.80 (1)	2.01 (4)	0.49 (4)	28.7	699 (3)	41.0
10 Hydropenia	125	255	4.03 (3)	2.32 (6)	0.56 (6)	36.0	1186 (3)	30.3
Saline infusion	123		2.54 (4)	1.48 (7)	0.29 (7)	44.3	1534 (4)	28.8
Aortic constriction	90		1.55 (2)	2.13 (6)	0.50 (6)	15.5	1310 (3)	11.8
11 Hydropenia	113	265	4.10 (3)	2.32 (3)	0.56 (3)	36.7	1137 (3)	32.3
Saline infusion	110		2.35 (1)	1.67 (2)	0.37 (2)	31.4	1191 (3)	26.4
Aortic constriction	70		1.94 (1)	2.45 (3)	0.57 (3)	17.0	702 (3)	24.2
12 Hydropenia	105	265	3.23 (5)	2.25 (5)	0.52 (5)	31.1	874 (3)	35.5
Saline Infusion	103		2.29 (3)	1.67 (5)	0.39 (5)	29.4	920 (4)	31.9
13 Hydropenia	100	320	3.49 (2)	2.09 (4)	0.52 (4)	33.6	1384 (3)	24.3
Saline infusion	110		2.73 (1)	1.44 (4)	0.29 (4)	46.9	2210 (3)	21.2
Aortic constriction	75		2.66 (1)	1.53 (5)	0.33 (5)	39.8	2110 (3)	18.9
Means*								
Hydropenia (13)	120	302	3.62	2.38	0.56	32.9	1229	27.5
	±15	±89	±0.57	±0.27	±0.05	±7.1	±274	±6.1
Saline infusion (13)	125		2.33	1.51	0.31	40.0	1515	27.2
	±13		±0.45	±0.15	±0.07	±14.2	±387	±9.8
Aortic constriction (11)	80		2.20	1.94	0.42	27.8	1177	27.3
	±11		±0.78	±0.36	±0.11	±11.2	±464	±12.3

* Values are averages of individual means from respective experimental condition in each animal, ±SD.

Abbreviations are as follows: BP, aortic pressure below point of constriction; Wt., weight of animal; C, absolute rate of proximal tubular reabsorption measured by microperfusion; $1 - (P/TF)_{in}$, fraction of filtrate reabsorbed at site of collecting late proximal tubular fluid; $(TF/P)_{in}$, ratio of tubular fluid inulin concentration to plasma inulin concentration; SNGFR, superficial nephron glomerular filtration rate; GFR, whole kidney glomerular filtration rate.

Numbers in parentheses indicate number of individual measurements made and used to calculate mean value shown.

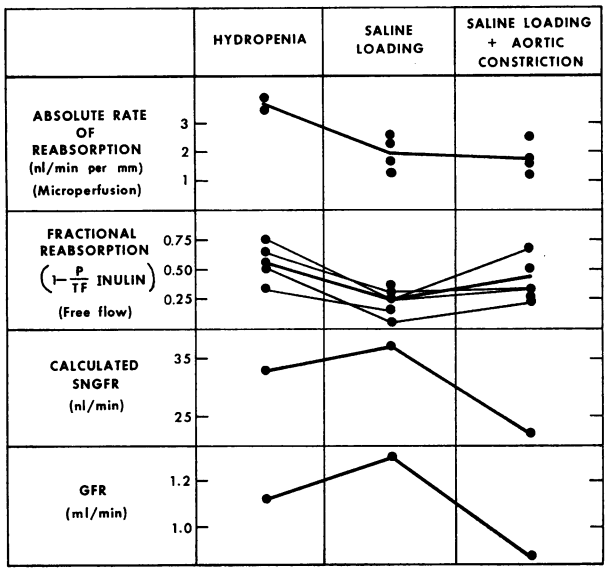

FIGURE 2 *Effects of saline infusion and subsequent aortic constriction on nephron filtration and proximal tubular reabsorption in a single rat.* Individual measurements of proximal tubular reabsorption (microperfusion) and fractional reabsorption (collection and recollection) are shown. Nephron filtration rate (SNGFR) was calculated from the mean values for absolute and fractional reabsorption. Whole kidney filtration rate (GFR) is the mean of two to three consecutive clearance periods during each phase of the experiment.

presence of aortic constriction, proximal tubular reabsorptive rate averaged 2.03 ±0.69 nl/min per mm which was significantly lower than that during hydropenia ($P < 0.01$), but was not different from that during saline loading ($P > 0.05$) in the absence of aortic constriction. The mean values for each maneuver are presented in Table I, and all individual measurements from all animals are shown in Fig. 1.

Fractional reabsorption. In 13 of the animals, 163 samples of tubular fluid were collected from the most distal surface convolution of 76 proximal tubules. Fractional reabsorption at the site of collection was calculated from the ratio of tubular fluid to plasma inulin concentration of each sample. During hydropenia, fractional reabsorption measured in 56 end-proximal tubules averaged 0.56 ±0.10. During saline loading, tubular fluid was recollected from the same puncture site in 51 of these tubules and from an additional 6 late convolutions. In these 57 proximal tubules, fractional reabsorption during saline loading averaged 0.31 ±0.15; a decrease of 45% from the mean value during hydropenia which was a highly significant change ($P < 0.01$). During aortic constriction in the presence of saline loading in 12 animals, tubular fluid was recollected for the second time from the same puncture site in 34 proximal tubules and from an additional 16 late convolutions. Fractional reabsorption in these 50 late proximal tubules averaged 0.42 ±0.18 which was significantly higher ($P < 0.05$) than that observed during saline infusion in the absence of aortic constriction, and significantly lower ($P < 0.05$) than that observed during hydropenia. Mean values for each maneuver are presented in Table I, and individual measurements in one animal are shown in Fig. 2.

Calculated nephron filtration rates. In 59 microdissections, measurements of the distance from glomerulus to the site of collection of proximal tubular fluid (for measuring fractional reabsorption) averaged 5.2 ±0.4 mm. These measurements are in agreement with those reported by other authors (28, 29). The method for identifying late proximal tubular convolutions to be punctured was always the same, and, therefore, a value of 5 mm was assumed to be the distance from the glomerulus to puncture site for all tubules. From the absolute rate of reabsorption and fractional reabsorption at 5 mm, superficial nephron filtration rate was calculated (see Methods). Since the absolute reabsorptive rate and fractional reabsorption were determined in different nephrons, calculations of nephron filtration rate were made from the mean values of the two measurements for each animal under each experimental condition. During hydropenia, calculated superficial nephron filtration rate in 13 animals averaged 32.9 ±7.1 nl/min. This value increased 21% to 40.0 ±14.2 nl/min during saline infusion which was significantly different from the value during hydropenia ($P < 0.05$). During aortic constriction in the presence of saline infusion,

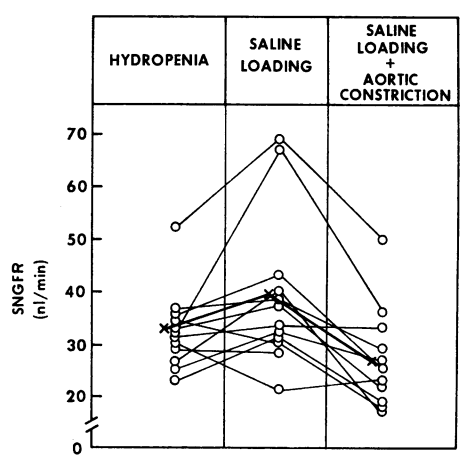

FIGURE 3 *Effects of saline infusion and subsequent aortic constriction on calculated nephron filtration rate.* Points are the values from individual animals calculated from the means of multiple measurements of absolute and fractional reabsorption during each phase of the experiment. The heavy line and crosses are the means of the individual values shown.

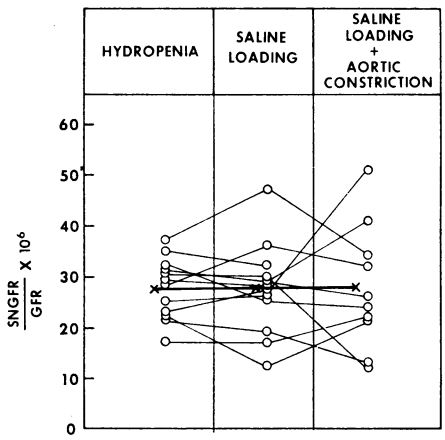

FIGURE 4 *Proportionality between calculated nephron filtration rate (SNGFR) and whole kidney filtration rate (GFR) during saline infusion and subsequent aortic constriction.* In general, saline infusion resulted in proportionately similar changes in nephron and whole kidney filtration rates. During aortic constriction the two measurements exhibited more divergence. However, overall there was no change in the mean ratio of filtration rates during either experimental maneuver as shown by the heavy line and crosses.

nephron filtration rate was calculated for 12 of the animals and averaged 27.8 ±11.2 nl/min. This value was significantly lower than that observed during saline infusion ($P < 0.05$). These values are summarized in Table I, and measurements in individual animals are shown in Figs. 2 and 3.

Filtration rate by the whole kidney was measured during each experimental maneuver in all 13 animals (Table I). During hydropenia, filtration rate by the kidney averaged 1229 ±274 μl/min and increased significantly ($P < 0.05$) to 1515 ±387 μl/min during saline infusion. When the aorta was constricted during saline infusion, kidney filtration rate decreased to an average of 1177 ±464 μl/min which was significantly lower than that observed during saline infusion without aortic constriction ($P < 0.01$).

The degree of proportionality between the changes in calculated nephron filtration rate and measured changes in whole kidney filtration rate was assessed as the ratio (Single Nephron Filtration Rate/Total GFR) × 10^6. This proportionality index (Table I) averaged 27.5 ±6.1 during hydropenia and was essentially unchanged at 27.2 ±9.8 ($P > 0.05$) during saline infusion. When the aorta was constricted during saline infusion, there was some degree of variability in the relationship between nephron filtration rate and the whole kidney filtration (Fig. 4), but the mean value of the ratio was 27.3 ±12.3 which was not significantly different from that during hydropenia or saline infusion without aortic constriction ($P > 0.05$ in both). Thus, overall, calculated, superficial nephron filtration rate and whole kidney filtration rate increased proportionately during saline infusion and decreased proportionately when the aorta was constricted (Figs. 2 and 4).

Nephron filtration rate determined by total collection. In a separate group of seven rats, the effect of saline infusion on nephron filtration rate and the calculated absolute rate of proximal tubular reabsorption was determined by the technique of total collection of proximal tubular fluid. In 33 measurements during hydropenia, superficial nephron filtration rate averaged 32.0 ±9.5 nl/min and whole kidney filtration rate ranged from 703 to 1814 μl/min. The average proportionality index, (SNGFR/TGFR) × 10^6, in these animals was 25.8 ±5.2 during hydropenia. Saline infusion increased filtration rate in these same nephrons, an average of 31%. The proportionality index was essentially unchanged at 23.2 ±4.8 ($P > 0.05$). During saline infusion the absolute rate of proximal tubular reabsorption decreased an average of 49.7% ($P < 0.01$). These data are summarized for all animals in Table II. Thus, the effects of saline infusion on superficial nephron filtration rate and the absolute rate of proximal tubular reabsorption determined by the technique of total collection of tubular fluid were qualitatively and quantitatively similar to those calculated or measured in the larger group of animals by the microperfusion technique. In these seven animals the infusion of saline was associated with a fall in mean fractional reabsorption from 0.55 to 0.23 (Table II).

Relation between sodium excretion and segmental tubular reabsorption. During hydropenia, sodium excretion averaged 471 ±509 nEq/min which represented an average over-all tubular reabsorption of 99.7% of the filtered load of sodium. During saline infusion the excretion of sodium increased to an average of 10,604 ±6,701 nEq/min, representing an average excretion of 5.5% of the filtered load of sodium. From the calculated nephron filtration rate and absolute and fractional reabsorption, the delivery of filtrate (and sodium) out of the proximal tubule was estimated. A proximal tubular length of 7 mm was used for this calculation. During hydropenia, an average of 22.2 ±7.1% of the filtered sodium was delivered from the proximal tubule, and since sodium excretion was negligible, virtually all of this was reabsorbed by the distal nephron. In absolute amount, this calculated distal reabsorption averaged 37.0 ±18.3 μEq/min. During saline infusion, delivery of sodium from the proximal tubule increased to 114.5 ±36.4 μEq/min and the absolute rate of distal reabsorption increased to 106.1 ±33.8 μEq/min. Thus, during saline infusion, excreted sodium represented only 10.1% of the increased delivery from the proximal tubule. When the aorta was constricted during saline infusion, delivery of sodium from the proximal tubule

TABLE II

Proximal Tubular Function Determined by Total Collection

Experiment	BP	C	ΔC	TF/P_{in}	$1 - P/TF_{in}$	SNGFR
	mm Hg	*nl/min*	%			*nl/min*
Hydropenia	111 ±12	17.6 ±5.3 (n = 33)		2.33 ±0.23 (n = 33)	0.55 ±0.04 (n = 33)	32.0 ±9.5 (n = 33)
Saline infusion	109 ±16	8.8 ±4.0 (n = 38)	−49.7 ±19.0 (n = 7)	1.34 ±0.13 (n = 38)	0.23 ±0.06 (n = 38)	38.4 ±11.6 (n = 38)

Abbreviations are the same as in Tables I and III. C in this table refers to nanoliters per minute of tubular fluid reabsorbed from the glomerulus to the site of collection. ΔC is the per cent change in absolute rate of reabsorption from hydropenia to saline loading. Numbers in parentheses indicate the total number of measurements in seven rats. Values are means ±SD of mean values in each animal.

decreased to 67.5 ±37.5 μEq/min, due on the average entirely to the fall in calculated nephron filtration rate. This latter value was still greater than the delivery from the proximal tubule during hydropenia despite the fall in nephron filtration rate and increased proximal tubular fractional reabsorption. The calculated absolute rate of sodium reabsorption by the distal nephron decreased during aortic constriction to 63.9 ±33.7 μEq/min, but this was still greater than that during hydropenia. Even though aortic constriction during saline infusion decreased whole kidney and calculated nephron filtration rates below hydropenic values, the excretion of sodium remained increased. This persistent increase in sodium excretion during aortic constriction related to a maintained increased delivery of filtrate from the proximal tubule entirely due to depressed absolute reabsorption and a failure of distal tubular reabsorption to continue at the high rate present during saline infusion before aortic constriction. The absolute rate of distal tubular reabsorption was least during hydropenia and greatest during saline infusion, with the rate during aortic constriction falling between the two. These data relating to filtered, reabsorbed, and excreted sodium are summarized for all experiments in Table III.

DISCUSSION

The results of the present studies indicate that acute saline loading in the rat depresses the absolute rate of proximal tubular reabsorption approximately 38%. Reabsorptive rates in the proximal tubule were determined by perfusing single tubular segments *in situ*, and, therefore, the observed changes could not be influenced by filtration rate of the nephron under study. Free flow recollections from the most distal accessible segment of adjacent proximal tubules indicated that fractional reabsorption was simultaneously depressed an average of 45% during saline infusion, in agreement with the observations of others (2, 5, 10, 16). If the percentage decrease in absolute reabsorptive rate observed in the perfused tubules occurred in the adjacent tubules, in which fractional reabsorption was measured, then the change in absolute reabsorptive rate would account for 77% of the observed decrease in fractional reabsorption. Therefore, the remaining decrease in fractional reabsorption must have been due to an increase in filtration rate by the nephrons under study. This would indicate that saline infusion resulted, on the average, in a 21% increase in filtration rate by superficial cortical nephrons. The simultaneously measured whole kidney filtration rate increased an average of 23% during saline infusion. Therefore, the present studies suggest that saline infusion depresses proximal tubular fractional reabsorption by a combination of a decrease in the absolute reabsorptive rate and an increase in nephron filtration rate which is proportionately the same as the increase in whole kidney filtration rate. In other words, the present results do not support the view that the fall in proximal tubular fractional reabsorption during saline infusion is due primarily to an increase in superficial nephron filtration rate out of proportion to the increase in whole kidney filtration rate. This is in contrast to the reported effects of chronic salt loading (7, 30, 31) to depress proximal tubular fractional reabsorption largely as a result of increased nephron filtration rate. This difference could relate to differences in the effects of acute and chronic expansion of the extracellular volume. Our present observations during infusion of saline of proportionately similar increases in whole kidney filtration rate and superficial nephron filtration rate (whether calculated from absolute and fractional reabsorption or measured directly), are in agreement with some (4, 16, 18, 19), but not all (10), studies employing only direct measurements of nephron filtration rate.

of Tubular Fluid before and after Saline Infusion

GFR	$\frac{SNGFR}{GFR} \times 10^6$	V	C_{PAH}	F_{Na}	$U_{Na}V$	U_KV	T_{Na}/F_{Na}
μl/min		*μl/min*	*μl/min*	*μEq/min*	*nEq/min*	*nEq/min*	%
1,263	25.8	4.4	4,256	170.6	200	1,226	99.9
±391	±5.2	±2.3	±1,447	±52.8	±163	±862	±0.1
(n = 21)	(n = 7)	(n = 21)	(n = 21)	(n = 21)	(n = 21)	(n = 21)	(n = 21)
1,657	23.2	51.2	6,432	223.7	7,873	3,037	96.6
±494	±4.8	±48.1	±2,140	±66.7	±7,800	±415	±2.9
(n = 29)	(n = 7)	(n = 29)	(n = 29)	(n = 29)	(n = 29)	(n = 29)	(n = 29)

Reported values for individual superficial nephron filtration rate in the rat vary widely. Techniques involving the total collection of tubular fluid have yielded values ranging from 12 (32) to 45 (38) nl/min, and in individual studies, the range of values may be quite variable. It is not surprising that the total collection technique may yield a wide range of values for nephron filtration rate since the technique involves occlusion of the proximal tubules. To the extent that this may alter intratubular hydrostatic pressure, one of the major variables determining filtration rate is changed by the procedure. Schnermann, Horster, and Levine (33) have measured intratubular hydrostatic pressure before occlusion of the proximal tubule and then maintained this pressure constant while making total collections of tubular fluid. They report a value for individual superficial nephron filtration rate measured in this manner close to the value in hydropenic animals measured by total collection of tubular fluid in the present study and close to that calculated from absolute and fractional reabsorption. Gertz and associates (32, 34) reported a much lower value (8–17 nl/min) for superficial nephron filtration rate. The latter authors used the technique of total collection of tubular fluid while controlling pressure in the collection circuit at the previously determined intratubular pressure. However, it would seem that in order for tubular fluid to flow into the collecting pipette against this controlled pressure, intratubular pressure would be greater than the previously determined free flow value and this change should lower filtration rate. Nevertheless, it should be pointed out that this lower value for superficial nephron filtration rate is close to the value Gertz, Braun-Schubert, and Brandis calculated from measurements of transit time, tubular radius, and free-flow TF/P inulin ratio (34). However, small errors in these measurements, especially that of tubular radius, will introduce a large error in the calculation of absolute reabsorptive rate necessary for calculating filtration rate. Using information available in the literature, individual nephron filtration rate

TABLE III

Effects of Saline Infusion and Aortic Constriction on Proximal and Distal Tubular Sodium Reabsorption

Experiment	V	C_{PAH}	F_{Na}	$U_{Na}V$	U_KV	T_{Na}/F_{Na}	T_PNa	T_PNa/F_{Na}	Distal delivery	Distal reabsorption	Distal reabsorption / delivery
	μl/min	*ml/min*	*μEq/min*	*nEq/min*	*nEq/min*	%	*μEq/min*	%	*μEq/min*	*μEq/min*	%
Hydropenia	5.9	3.8	165.9	471	1,144	99.7	128.4	77.8	37.5	37.0	98.5
(n = 35)	±3.5	±1.4	±36.9	±509	±536	±0.4	±27.0	±7.1	±18.3	±18.3	±1.6
Saline infusion	54.7	5.8	204.5	10,604	1,422	94.5	90.0	43.0	114.5	106.1	89.1
(n = 39)	±36.7	±2.5	±52.2	±6,701	767	±3.7	±28.2	±8.5	±36.4	±33.8	±8.0
Aortic constriction	14.9	4.9	158.9	3,344	1,404	98.3	91.4	58.6	67.5	63.9	95.3
(n = 31)	±20.9	±1.9	±62.7	±5,596	±1,116	±2.0	±35.5	±15.0	±37.5	±33.7	±4.6

Abbreviations are as follows: V, rate of urine flow; C_{PAH}, clearance of *p*-aminohippurate; F_{Na}, rate of filtration of sodium (GFR × plasma sodium × 0.95); $U_{Na}V$, rate of excretion of sodium; U_KV, rate of excretion of potassium; T_{Na}/F_{Na}, over-all fraction of filtered sodium reabsorbed; T_PNa, absolute amount of sodium reabsorbed by 7 mm length of proximal tubule; T_PNa/F_{Na}, fraction of filtered sodium reabsorbed at 7 mm length of proximal tubule.
Numbers in parentheses indicate total number of measurements. Values are means ±SD of mean values in the 13 animals shown in Table I.

calculated from measurements of reabsorptive rate determined by the "shrinking drop" technique, end-proximal tubular fractional reabsorption, and segment length to the site of collection of tubular fluid, yield values ranging from 22 (35) to 38 nl/min (36).

The present observation of decreased absolute reabsorption in the proximal tubule during saline infusion is in keeping with inferences that may be derived from previous studies (1–5), with studies in which single nephron filtration rates were measured by the technique of total collection of tubular fluid, (4, 6, 10, 16) with studies in which reabsorptive rates were estimated by the "shrinking drop" technique (11–13) [2] and with studies utilizing *in situ* microperfusion (14, 15). However, most of these earlier studies have not provided sufficient quantitative data that would permit direct comparisons between observed or calculated nephron filtration rates and simultaneous whole kidney filtration rates during saline infusion or aortic constriction.

Measurements of the absolute rate of proximal tubular reabsorption by the technique of microperfusion are subject to several sources of error, and these have been discussed in detail elsewhere (21). The technique does have some distinct advantages when used to determine absolute reabsorptive rates. It is not dependent on a knowledge of tubular radius, it is not subject to an influence of variations in delivery of fluid for reabsorption, and it does not require measuring another variable such as the volume of tubular fluid reaching the site of micropuncture. In the present study, proximal tubular reabsorptive rate in hydropenic rats averaged 3.56 nl/min per mm. This value is somewhat higher than that reported by Morgan and Berliner (14) who also used the microperfusion technique to study proximal tubular reabsorption in the rat. However, the value reported by the latter authors is too low to be consistent with most estimates of individual nephron filtration rate and measured end-proximal tubular fractional reabsorption. If the individual nephron filtration rate in the rat is approximately 30 nl/min (33) and the length of proximal tubule to the site of "end-proximal tubular" punctures is 5 mm (28, 29), then the frequently observed end-proximal tubular TF/P inulin ratios of 2.0–2.5 would require a rate of reabsorption of 3.0–3.6 nl/mm per min, close to the rate observed in the present study, and close to the rates calculated from the microperfusion data of Sonnenberg and Solomon (15).

When the aorta was constricted during saline infusion to reduce whole kidney filtration rate below the presaline infusion value, the absolute rate of proximal tubular reabsorption was unchanged from the decreased value observed after infusing saline, despite the reduced arterial pressure and whole kidney filtration rate. However, end-proximal tubular fractional reabsorption increased during aortic constriction, as has been reported in other recent studies (6, 47–49). If the absolute rate of reabsorption in all proximal tubules remained decreased as measured by microperfusion, then the increase in fractional reabsorption in adjacent proximal tubules must have been due to a fall in nephron filtration rate. From the two measurements (absolute rate of reabsorption and fractional reabsorption), it was calculated that the increase in fractional reabsorption during aortic constriction must have been due almost entirely to a fall in nephron filtration rate, and this fall was on the average proportional to the fall in whole kidney filtration rate.

The present observation that the depressed rate of proximal tubular reabsorption during saline infusion persisted during a reduction in renal perfusion pressure and whole kidney filtration rate differs from the observations of Bank, Koch, Aynedjian, and Aras (13). These latter authors reported that the depressed rate of proximal tubular reabsorption during saline infusion as measured by the "shrinking drop" technique was reversed by aortic constriction even though an average of 3.8% of filtered sodium was being excreted (13). The reasons for this difference are not apparent but the results of the present study are in agreement with a number of studies which have demonstrated a failure of aortic constriction to reverse completely the effect of saline infusion on both sodium excretion (1, 2) and fractional reabsorption by the proximal tubule (2, 5, 6, 48).

During aortic constriction in the present study, whole kidney filtration was decreased to rates below those present before saline infusion, yet increased sodium excretion persisted as has been demonstrated in numerous other studies (1, 2, 11, 13, 51). Delivery of filtrate beyond the late proximal tubule, calculated from absolute and fractional reabsorption, remained sufficiently increased during aortic constriction to account for the continued natriuresis despite increases in proximal tubular fractional reabsorption. Therefore, it was unneces-

[2] With the "shrinking drop" technique the basic measurement is the reabsorptive half-time of an isolated droplet of saline in the proximal tubule. In addition to a number of difficulties involved in reproducibly determining reabsorptive half-time by this technique (37), conversion of the measurement to an absolute reabsorptive rate (nl/min per mm) requires an accurate measurement of the tubular radius (nl/min per mm $= 0.694\ \pi r^2/t_{\frac{1}{2}}$). Even though values for reabsorptive half-times are in close agreement in a number of studies, estimates of tubular radii are not (11–13, 35–46). Consequently, the "shrinking drop" technique yields a wide range of calculated absolute reabsorptive rates. Nevertheless, reabsorptive half-time determined by this technique is prolonged by saline infusion, and this prolongation is not accounted for completely by changes in luminal volume calculated from estimates of tubular radii (11–13).

sary to postulate that distal tubular reabsorption was decreased below the level present before saline infusion. It is possible, however, that saline infusion limited distal tubular reabsorptive capacity permitting the maintenance of increased sodium excretion when delivery of filtrate from the proximal nephron was decreased during aortic constriction. Howards, Davis, Knox, Wright, and Berliner (50) have reported that sodium excretion may be increased minimally during infusion of hyperoncotic albumin in the dog, despite decreased proximal tubular fractional reabsorption similar to that observed during saline infusion and extensive natriuresis. Although, these latter authors obtained no data on the absolute rate of proximal tubular reabsorption, the implication of their observation is that distal tubular reabsorptive capacity is lower during infusion of saline than during infusion of hyperoncotic albumin (50). Our results suggest that persistent natriuresis during saline infusion and aortic constriction does not require a decrease in the absolute rate of distal tubular reabsorption.

Buentig and Earley, (21) using the microperfusion technique, reported that constriction of the aorta to reduce whole kidney filtration rate 50% resulted in approximately a 50% decrease in proximal tubular reabsorptive rate. This differs from the present observation that aortic constriction had no effect on reabsorptive rate. However, the former studies were performed in nonsaline-loaded animals with high reabsorptive rates (21). In the present study, proximal tubular reabsorption was depressed by saline infusion before constricting the aorta, and this may have limited any further decrease during aortic constriction. Implicit in this conclusion is that there may be some lower limit for proximal reabsorptive rate which disrupts glomerulotubular balance when filtration rate is reduced in the presence of a depressed rate of proximal tubular reabsorption. The observation that proximal tubular fractional reabsorption increased in filtering nephrons during aortic constriction in the present study is consistent with this conclusion. Also, the observation in this study and in a previous report from this laboratory (21) that a reduction in renal perfusion pressure is not accompanied by an increase rate of reabsorption by the proximal tubule may seem at variance with numerous other studies that indicate an inverse relationship between renal perfusion pressure and over-all tubular reabsorption of sodium (13, 41, 51, 52). However, some of these demonstrations have involved manipulations of perfusion pressure through a range that produced small changes in filtration rate. When the change in perfusion pressure is large enough to effect gross changes in filtration rate, such as was the case in this and our previous study (21), other factors such as blood flow and postglomerular oncotic absorptive capacity may predominate over hydrostatic pressure as determinants of capillary absorption and proximal tubular reabsorption (31, 51–56).

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