An Inhibitory Effect of Furosemide on Sodium Reabsorption by the Proximal Tubule of the Rat Nephron

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ABSTRACT The evidence from previous micropuncture studies for an inhibitory effect of furosemide on proximal sodium reabsorption in the rat has been conflicting. Intrinsic reabsorptive capacity, estimated in free flow and shrinking drop experiments, has been reported to be depressed, whereas fractional reabsorption usually remains unchanged.

We have recently reported that, during conditions of elevated intraluminal hydrostatic pressure, unless care is taken to prevent retrograde flow of tubule fluid from more distal sites, the concentration of inulin in late proximal fluid is often factitiously elevated. Since furosemide raises intraluminal pressures, often markedly, the failure to detect a depression of fractional reabsorption might be the consequence of retrograde contamination during fluid collection.

Experiments were designed to compare the effect of furosemide on fractional sodium reabsorption by the proximal tubule when collections were obtained with distal oil blocks of conventional length as well as with unusually long blocks of oil of low and high viscosities.

When reflux is prevented, fractional sodium reabsorption is usually depressed by furosemide, whereas when conventional distal blocks are used, the calculated values for fractional reabsorption either remain unchanged or increase. Simultaneous measurements of nephron glomerular filtration rate indicate that the latter is the consequence of retrograde contamination.

INTRODUCTION

Both clearance and micropuncture techniques have provided information concerning the effects of drugs on relatively specific portions of the nephron and, in general, the results are complementary. On the basis of its effect on the urinary concentrating and diluting mechanism studied by means of clearance methods in dog and man, it has been suggested that furosemide inhibits sodium transport in the ascending limb of Henle's loop (1–4). The results from micropuncture studies in the dog (5) and rhesus monkey (6) add additional and somewhat more direct evidence to support this view.

There is disagreement, however, in the results obtained with these different methods regarding a possible action of furosemide in the proximal convoluted tubule. From data obtained in clearance studies during water diuresis in dog and man, it has been suggested that furosemide has an additional and perhaps important locus of action in the proximal portion of the nephron (1–4). This impression is based largely on the finding of an increase in delivery of sodium out of the proximal tubule, as measured by an absolute increase or a relatively smaller decline in free water clearance after furosemide than after other potent natriuretic agents. In addition it has been reported that furosemide in large doses occasionally results in the excretion of as much as two-thirds of the glomerular filtrate (2).

The results from studies utilizing micropuncture techniques have yielded conflicting conclusions. Rector, Sellman, Martinez-Maldonado, and Seldin observed a 40% inhibition of the intrinsic reabsorptive capacity of the proximal convoluted tubule in the rat after furosemide, as measured by the shrinking drop technique (7). Nevertheless, in agreement with the results in the dog (8) and monkey (6), they were unable to detect an inhibitory effect of the drug in the rat on fractional sodium reabsorption by the proximal tubule as measured in free flow studies. In two other reports, by Deetjen (9) and Malnic, Vieira, and Enokibara (10), small depressions were noted in fractional reabsorption, in the

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former, however, only when glomerular filtration rate (GFR) was reduced markedly, while in the latter, the magnitude of the changes were of doubtful statistical significance.

In a recent micropuncture study of the effect of elevated ureteral pressure on fractional sodium reabsorption by the proximal tubule in the rat (11), it was found that unless special care was taken to prevent retrograde flow of tubule fluid from more distal sites, the calculated value for fractional sodium reabsorption was elevated spuriously. This artifact results from the collection of fluid from more distal sites along the nephron where, as a result of continued water reabsorption, the inulin concentration is higher. An unusually long oil block placed distal to the site of puncture was found to be effective in preventing this retrograde flow.

With this potential technical hazard in mind, it seemed reasonable to reevaluate the effect of furosemide on fractional sodium reabsorption by the proximal tubule, since the high intraluminal pressures that develop after administration of this drug (12) might induce retrograde fluid flow and erroneously high tubule fluid–to-plasma inulin [$(TF/P)_{In}$] ratios. Thus an inhibitory effect on the absolute rate of sodium transport by the proximal tubule might be counteracted by a factitious elevation in the calculated value for fractional reabsorption, leading to the conclusion that the drug exerts no net effect on the proximal tubule. Accordingly micropuncture experiments were undertaken to measure the effect of furosemide on proximal sodium reabsorption, while unusually long distal oil blocks were used to prevent retrograde contamination. The results indicate that furosemide produces significant depression of fractional sodium reabsorption by the proximal tubule and that this is demonstrable when care is taken to prevent contamination from downstream sites. Furthermore, the results from these studies as well as from experiments utilizing the shrinking drop technique indicate that furosemide exerts a direct inhibitory effect on the absolute rate of sodium reabsorption by this portion of the nephron.

METHODS

Experiments were performed on male Sprague-Dawley rats weighing 205–370 g. They were allowed free access to a rat pellet diet except for 18–24 hr before study. Water was available ad lib. They were anesthetized with Inactin (100 mg/kg), placed on a heated micropuncture table, and prepared for study in a manner previously described (11). Indwelling polyethylene catheters were placed in the left jugular vein for infusion of inulin and furosemide, into the right jugular vein for injection of Lissamine green, and into the left femoral artery for periodic sampling of blood and measurement of arterial pressure. Replacement of urinary losses after furosemide administration was accomplished by infusion of isotonic saline through a catheter in the left femoral vein. To insure that the volume of fluid replaced was not in excess of the volume of urine excreted, the animals were allowed to incur a deficit of 0.5–1.5 ml at the onset of the diuresis. Isotonic saline then was administered at intervals of 1–2 min in amounts equal to the rate of urine formed per minute, as measured volumetrically in glass tubing calibrated in 0.01 ml divisions.

The effect of furosemide on fractional sodium reabsorption by the proximal tubule was studied in 12 rats. Special care was taken in the surgical preparation of each rat to avoid excessive losses of fluid; the small losses that were incurred were not replaced. Hypotonic sodium chloride solution (0.5%) containing inulin in concentrations calculated to provide plasma levels of 100 mg/100 ml was infused intravenously at the rate of 1.2 ml/hr beginning 45 min before micropuncture. The recollection micropuncture technique was used. Experiments were divided into two periods: an initial control period during hydropenia and a subsequent experimental period during the intravenous administration of furosemide (25 mg/kg prime and per hr). During the control period, quantitative, exactly timed collections of tubule fluid were obtained from late surface loops of proximal convoluted tubules (generally the penultimate surface loop so that the oil block placed distal to the site of puncture could be kept in view) previously identified after intravenous injection of 10% Lissamine green. The transit time, estimated with a stop watch as the interval from the appearance of Lissamine green in the peritubular capillaries to the arrival of the color wave at the site of puncture, was measured during each collection. From two to five tubules were punctured in each rat. Arterial blood pressure and urine flow rate were measured, and samples of blood and urine were obtained for estimation of inulin and electrolyte concentration.

The priming dose of furosemide was administered intravenously in a period of 1–2 min. Urine flow increased to a maximum value within 10 min, after which quantitative recollections from the same tubules were obtained. During each collection the transit time was measured again. All recollections were completed within 30–45 min from the onset of diuresis. The appropriate measurements of urine flow rate and concentration of inulin and electrolytes in blood and urine were repeated. It was possible therefore to evaluate [$(TF/P)_{In}$], individual nephron,[1] and experimental kidney GFR for both control and experimental periods.

In rat Nos. 1–7 the effect of furosemide on proximal sodium reabsorption was studied with polymer oil[2] as the material providing the distal block. In rat Nos. 8–12 castor oil was used in place of polymer oil. In order to assess the role of retrograde flow of tubule fluid during the furosemide-induced diuresis, recollections were obtained in 10 randomly chosen late proximal tubules of six rats in the polymer oil group with distal oil blocks of conventional length (2–3 tubule diameters) as contrasted with unusually long polymer oil blocks (allowed to extend downstream into at least one adjacent surface loop) used in all other recollections in these same animals. In rat Nos. 8–12 initial samples were obtained with a short polymer oil block, whereas during recollections, castor oil (a far more viscous oil than polymer oil) was substituted as the unusually long distal block.

In four rats, which served as the controls for this study, initial collections were obtained with polymer oil, while recollections were made with the long castor oil block. In

[1] Nephron GFR is equal to the product of $(TF/P)_{In}$ ratio and the volume of tubule fluid collected per minute.

[2] Polytrichloromonofluoroethane-Kel F, Minnesota Mining & Manufacturing Co., St. Paul, Minn.

TABLE I

Summary of Hemodynamic Data, Fractional Rates of Sodium and Water Excretion, and Plasma Electrolyte Composition in Control and Experimental Animals

	Arterial pressure		Experimental kidney GFR		Plasma Na		Plasma K		Na excretion		(V/GFR) ×100	
	mmHg		*ml/min*		*mEq/liter*		*mEq/liter*		*% of filtered*		*%*	
	H*	F*	H	F	H	F	H	F	H	F	H	F
Control (4)‡	121.2	111.7	1.06	1.10	145.0	146.9	4.6	4.4	0.03	0.07	0.30	0.34
	±4.3§	±1.7	±0.07	±0.07	±0.5	±0.3	±0.1	±0.1	±0.01	±0.03	±0.01	±0.02
Experimental (12)	116.7	116.3	0.96	0.80	145.5	147.6	4.3	3.6	0.10	19.5	0.34	20.2
	±2.7	±2.4	±0.05	±0.04	±0.6	±0.8	±0.4	±0.1	±0.02	±1.2	±0.02	±1.2

GFR, glomerular filtration rate; V, volume.

* H and F in this and subsequent Tables denote hydropenia and furosemide periods, respectively.

‡ Numbers in parentheses indicate number of animals.

§ Values represent mean of n animals ±1 SE.

these rats, the protocol as outlined above was followed exactly, except that furosemide was omitted from the prime and sustaining infusions.

The concentration of inulin in tubule fluid was measured by the method of Vurek and Pegram (13). Inulin concentrations in plasma and urine were determined by the method of Führ, Kaczmarczyk and Krüttgen (14). Sodium and potassium concentrations in plasma and urine were determined by flame photometry with lithium as an internal standard.

The effect of furosemide on the absolute rate of sodium reabsorption was studied in three rats by the shrinking drop technique. Measurements of lengths of aqueous drops between oil columns were made from photographs taken at intervals of 4 sec. Photomicrographs were made with a Nikon motor-driven camera (Nikon, Inc., Garden City, N. Y.) mounted on a Leitz Ortholux microscope (E. Leitz, Inc., New York). Illumination was provided by an Ascor electronic flash (American Speedlight Corp., Middle Village, N. Y.). Double-barreled pipettes were used to deliver 150 mM NaCl solution and castor oil stained with Sudan black. Determinations, usually in duplicate, were made in three to five tubules before and four to six tubules after furosemide administration. Black and white negatives (Plus-X) were projected and measurements were made at × 820. The width of the oil columns also was measured in each of the photographs to provide an estimate of the radius of the tubule lumen.

RESULTS

The average results of 12 experiments showing the effects of furosemide on renal hemodynamics and several measures of nephron function are summarized in Tables I and II, together with the average values from control animals. Within minutes of administration there was an increase in urine flow, from an average of 3.0 μl/min during control hydropenia to 161 μl/min shortly after the drug. The excreted fraction of filtered sodium

TABLE II

Summary of Average Values of Several Parameters of Individual Nephron Function in Control and Experimental Animals

	$(TF/P)_{In}$			Proximal Na reabsorption			Nephron GFR		Transit time	
	H	F	F/H*	H	F	%Δ*	H	F	H	F
				% of filtered			*nl/min*		*sec*	
Control (4)	2.30	2.22	0.98	55.4	54.2	−1.3	37.2	36.0	12.4	12.4
	±0.12	±0.10	±0.04	±2.3	±2.4	±3.4	±2.2	±3.0	±0.4	±0.6
Experimental (12)	2.67	2.19	0.84	61.6	53.0	−13.4	36.9	36.4	12.1	15.9
	±0.08	±0.07	±0.04	±1.2	±1.4	±3.0	±2.7	±2.5	±0.3	±0.4
Polymer oil (7)	2.51	2.27	0.91	59.1	54.5	−7.9	40.0	37.0	12.0	15.9
	±0.08	±0.10	±0.05	±1.4	±2.1	±3.9	±3.9	±2.7	±0.5	±0.6
Castor oil (5)	2.90	2.07	0.73	65.1	50.9	−21.0	32.4	35.4	12.2	15.8
	±0.09	±0.03	±0.03	±1.0	±0.6	±1.7	±2.7	±5.0	±0.4	±0.7

$(TF/P)_{In}$, tubular fluid-to-plasma inulin ratio.

* Values represent the mean of these ratios.

increased, on the average, from 0.10% (range = 0.03–0.24%) before furosemide, to 19.5% (range = 12.6–27.8%) after the drug. Similarly, the percentage of filtered volume excreted (V/GFR × 100) rose from 0.30% (range = 0.25–0.42%) to 20.2% (range = 13.5–28.6%) (Table I). No significant change in fractional excretion rates were noted in control animals. The concentration of sodium in plasma did not change after furosemide (Table I). Although the average plasma potassium concentration fell after furosemide, this difference, relative to control values, was not significant.[3] Experimental kidney GFR fell, on the average, by 15% (from 0.96 to 0.80 ml/min) after furosemide administration. The values for nephron GFR observed in control and experimental rats were similar (Table II) and agree closely with values reported by others for comparably hydrated animals (16, 17). It should be noted that in these antidiuretic animals the variability in this measurement was considerable. In 12 tubules (from four control animals) although the mean change in nephron GFR between initial and recollection periods was only − 0.7%, the standard deviation was ±16.0%.

Fig. 1 and Table II show the results of recollection $(TF/P)_{In}$ ratios obtained from 12 tubules in four control rats. During the hydropenic conditions of this study the recollection $(TF/P)_{In}$ ratio did not differ significantly from unity. Table III shows the $(TF/P)_{In}$ concentration ratios of 10 tubules (from six rats studied before and during furosemide diuresis) in which a polymer oil block of conventional length was used (i.e., 2–3 tubule diameters). Despite replacement of urinary losses to maintain extracellular fluid (ECF) volume, the recollection $(TF/P)_{In}$ ratio increased in eight, often mark-

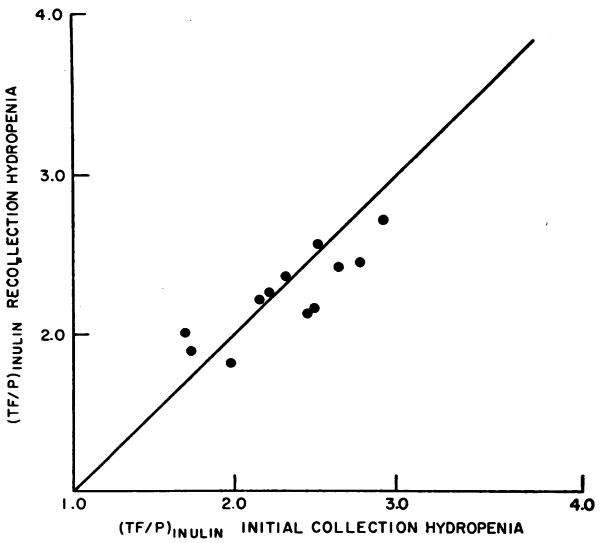

FIGURE 1 A comparison of tubule fluid-to-plasma inulin $(TF/P)_{In}$ concentration ratios obtained during initial and recollection periods in four control hydropenic rats. The initial collection was made with a polymer oil distal block of conventional length and the recollection with an unusually long castor oil block.

[3] Recently, it has been suggested that proximal sodium reabsorption is depressed during acute metabolic acidosis and markedly reduced plasma bicarbonate concentration (15). In the present experiments bicarbonate concentration in plasma and arterial pH were measured 1 hr after the initiation of a furosemide diuresis. In those animals in which proximal sodium reabsorption was depressed significantly, plasma bicarbonate and arterial pH ranged from 19.7 to 22.6 mmoles/liter and from 7.35 to 7.39 mmoles/liter, respectively, values which were not different from those found in two control rats.

TABLE III

Effect of Furosemide on $(TF/P)_{In}$ Ratios, Nephron GFR, and Tubule Fluid Flow Rates in 10 Tubules When using Polymer Oil Blocks of Conventional Length

Rat No.	Tubule No.	$(TF/P)_{In}$			Nephron GFR			Collected fluid volume	
		H	F	%Δ	H	F	%Δ	H	F
					nl/min			*nl/min*	
2	3	2.19	2.25	+ 3	39.0	34.2	− 12	17.8	15.2
3	4	2.23	3.53	+ 58	58.9	78.4	+ 33	26.4	22.2
4	2	2.26	2.00	− 12	13.1	37.6	+187	5.8	18.8
5	2	2.80	3.58	+ 28	36.4	150.7	+314	13.0	42.1
6	1	2.56	3.04	+ 19	36.1	57.4	+ 59	14.1	18.9
	2	2.57	5.53	+115	32.1	264.7	+724	12.5	47.9
7	1	2.17	4.28	+ 97	37.5	89.9	+140	17.3	21.0
	2	2.33	3.61	+ 55	57 1	72.3	+ 27	19.7	75.8
	3	1.97	3.01	+ 53	49.5	218.3	+341	25.1	72.5
	4	2.25	3.38	+ 51	45.7	175.8	+285	20.3	52.0

edly, resulting in an average increase in the calculated value for fractional sodium reabsorption in all 10 tubules of 18.5%. The calculated values for nephron GFR also increased markedly (average 242%) in the eight instances in which fractional reabsorption increased and was due not only to higher values for $(TF/P)_{In}$ ratios but also, in the majority of instances, to a marked increase in the volume of fluid collected per minute (Table III). We wish to emphasize that in each of these ten collections it was never apparent to the worker performing the micropuncture that reflux of distal fluid was taking place. Nevertheless, under these conditions of high intraluminal pressure, despite the presence of a seemingly adequate oil block, retrograde contamination occurred with disturbing frequency.[4] An attempt was made therefore to reduce the likelihood of reflux by inserting considerably longer oil blocks, at least of a length sufficient to fill one downstream surface loop. As shown in Tables II and IV and Fig. 2 A, the presence of the added length of polymer oil in the recollections after furosemide now was associated in 14 of 22 tubules with a reduction in fractional sodium reabsorption of as much as 35%, the difference between the values for all recollection ratios and unity being statistically significant ($P = 0.05$). However in two of the seven rats in this group (Nos. 3 and 6), depression of fractional reabsorption did not occur in any of the five tubules studied. Consequently, when the data are evaluated on the basis of animals the average change in fractional reabsorption for all seven rats (−7.9%) is not significant statistically ($0.1 > P > 0.05$). Note in Table IV that

[4] It should be recognized that the extent of elevation in the $(TF/P)_{In}$ ratio as a result of retrograde contamination will be less when intraluminal pressures are raised after administration of a diuretic than when pressures are raised in similarly hydropenic animals by elevating ureteral pressure. In the former instance, the final urine-to-plasma inulin $(U/P)_{In}$ ratios are usually less than 10, whereas in the latter, this ratio often exceeds 300. Thus for an elevation in the $(TF/P)_{In}$ ratio to be detected after a diuretic, the volume of fluid entering the pipette from downstream sites will have to be larger.

TABLE IV

Effect of Furosemide on $(TF/P)_{In}$ Ratios, Nephron GFR, and Transit Time in 22 Tubules When using Unusually Long Polymer Oil Blocks

Rat No.	Tubule No.	$(TF/P)_{In}$			Nephron GFR		Transit time	
		H	F	F/H	H	F	H	F
					nl/min		*sec*	
1	1	2.54	2.34	0.92	51.3	49.4	11.5	14.0
	2	2.34	2.04	0.87	46.1	27.7	10.4	11.0
	3	3.04	2.50	0.82	44.4	37.2	11.5	16.0
	4	2.63	2.71	1.03	41.5	35.1	11.0	16.0
2	1	2.81	2.34	0.83	34.8	40.0	11.5	16.0
	2	3.05	2.66	0.87	22.3	37.0	16.0	16.5
	4	2.14	1.82	0.85	28.0	38.9	11.0	12.5
3	1	2.24	2.22	0.99	42.3	47.1	11.0	14.5
	2	2.38	2.33	0.98	65.2	45.0	13.3	17.0
	3	2.55	2.80	1.10	45.9	37.8	13.5	15.0
4	1	3.38	2.24	0.66	29.1	27.8	11.3	11.3
	3	1.73	1.58	0.91	17.3	28.3	12.5	19.0
	4	2.32	2.00	0.86	29.2	36.4	10.3	13.0
5	1	2.76	1.72	0.62	37.0	35.9	15.0	17.0
	3	2.56	1.88	0.73	38.9	48.3	13.5	19.0
	4	2.57	1.90	0.74	35.2	29.4	10.0	16.0
	5	3.35	2.50	0.75	31.2	48.0	13.0	16.5
6	3	2.20	2.52	1.14	42.0	30.0	11.3	15.0
	4	2.64	2.87	1.09	50.1	18.4	15.0	20.0
7*	1	2.17	2.65	1.22	37.5	30.5	9.5	20.0
	2	2.33	2.28	0.98	57.1	56.2	10.5	19.0
	3	1.97	1.45	0.74	49.5	47.1	9.8	14.0

* Tubules Nos. 1, 2, and 3 in rat No. 7 were repunctured initially with conventional length oil blocks (see Table III) and again with unusually long distal blocks.

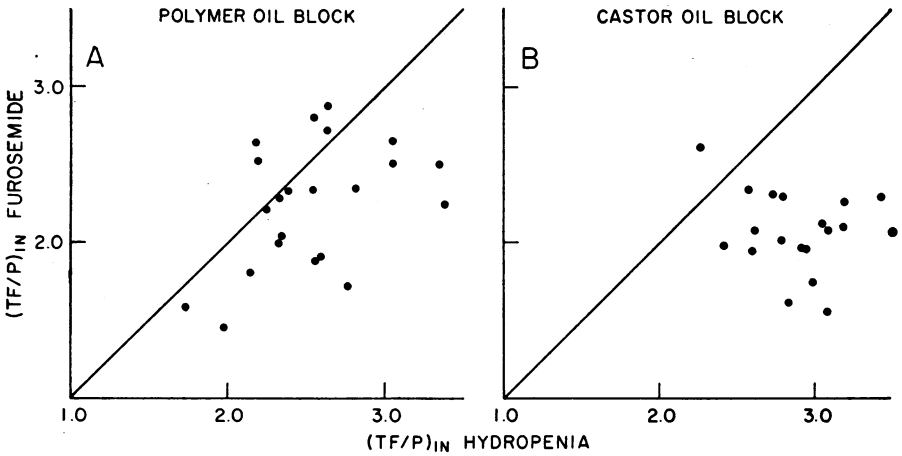

FIGURE 2 A The effect of furosemide on $(TF/P)_{In}$ concentration ratios in 22 tubules studied during control hydropenia and furosemide diuresis. During the initial collection a polymer oil block of conventional length was used while in the recollection a longer polymer oil block was used. B The effect of furosemide on $(TF/P)_{In}$ concentration ratios in 19 tubules studies during control hydropenia and furosemide diuresis in which the initial collection was obtained with a conventional length polymer oil distal block and the recollection with an unusually long castor oil block.

a depression of the $(TF/P)_{In}$ ratio was observed in tubules that had reduced, unchanged, or increased nephron GFR. It is difficult to know whether these calculated values do, in fact, represent changes in nephron GFR, or merely reflect the inherent variability of the analytical methods as well as the not inconsiderable difficulties in obtaining quantitative fluid collections, particularly during periods of high intraluminal pressure. In recent studies in the rat, it has been observed that fractional reabsorption increases after a reduction in nephron GFR (produced by aortic constriction) (11, 18, 20), and decreases after an elevation in nephron GFR (release of aortic constriction) (11). If it is assumed that nephron GFR changed in the present study to the extent calculated, the data were examined to determine whether the changes in fractional reabsorption after furosemide could be the consequence of these changes in nephron GFR. Fig. 3 presents the relationship between changes in $(TF/P)_{In}$ ratios and changes in nephron GFR when changes in the latter were produced by furosemide (as indicated by the data points) or by aortic constriction and release (shaded band). The shaded band is derived from data previously reported (11), consisting of 58 tubules in which recollection $(TF/P)_{In}$ ratios and nephron GFR were measured simultaneously. The boundaries of the shaded zone encompass 95% of the measurements; none of the three data points excluded fall below this shaded area. After furosemide, in 14 of 22 recollections made with polymer oil, values for nephron GFR were either unchanged or lower. In seven of these, fractional reabsorption was depressed, and in all but one the change in fractional reabsorption was independent of the influence of adjustments mediated by glomerulotubular balance, i.e., the points fall below the shaded zone. In seven tubules, fractional reabsorption was not depressed. Nephron GFR increased in 8 of 22 tubules, and in 6 of these, a decline in fractional reabsorption was observed. However, in only one instance was fractional reabsorption depressed to an extent greater than that seen when GFR is raised by mechanical means (i.e., release of aortic constriction).

In five other rats (Nos. 8–12) we examined the effect of furosemide on proximal tubules blocked with an exaggerated length of castor oil in an attempt to exclude with greater certainty any possibility of reflux of distal fluid. The results are shown in Tables II, V, and Fig. 2 B. Fractional reabsorption declined in 18 of 19 tubules. The depression of reabsorption in these five rats averaged 21%, a value significantly different from that in the control group ($P < 0.005$). The degree of inhibition in the castor oil group was similar whether nephron GFR fell or increased. The relationship between recollection $(TF/P)_{In}$ ratios and changes in nephron GFR induced by furosemide is shown in Fig. 3. Nephron GFR fell in eight tubules, and in seven fractional reabsorption was depressed. In 11 tubules nephron GFR increased and fractional reabsorption fell. In four the change in fractional reabsorption was similar to that which follows mechanical elevation of GFR. However, in seven tubules the depression in fractional reabsorption was well below values that can be attributed to the glomerulotubular balance mechanism alone.

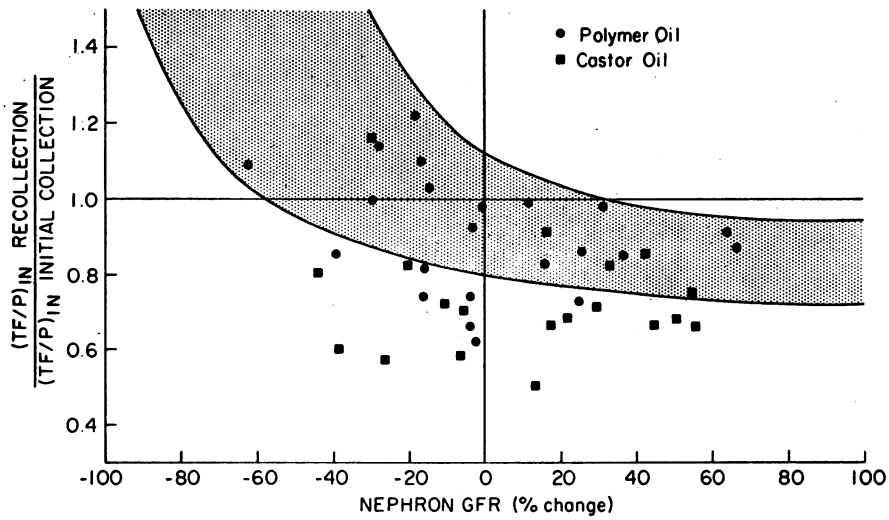

FIGURE 3 A comparison of the relationships between $(TF/P)_{In}$ recollection ratios and percentage changes in nephron glomerular filtration rate (GFR) for changes in nephron GFR produced either by furosemide (data points) or by means of aortic constriction and release (shaded band). In the furosemide experiments, the initial collections and recollections were obtained before and during the diuretic period, respectively. The shaded band is obtained from data, previously reported (11) in which $(TF/P)_{In}$ ratios were obtained before and during aortic constriction and before and after release of constriction.

TABLE V

Effect of Furosemide on TF/P_{In} Ratios, Nephron GFR, and Transit Time in 19 Tubules When using Unusually Long Castor Oil Blocks

Rat No.	Tubule No.	$(TF/P)_{In}$			Nephron GFR		Transit time	
		H	F	F/H	H	F	H	F
					nl/min		*sec*	
8	1	3.08	1.55	0.50	33.0	37.6	11.0	16.0
	2	2.73	2.31	0.85	24.3	34.6	10.8	14.5
	3	3.09	2.09	0.68	29.6	44.3	14.0	20.5
	4	2.78	2.01	0.72	35.3	31.1	8.3	17.0
9	1	2.95	1.95	0.66	38.4	49.5	11.5	14.6
	2	3.05	2.13	0.70	50.9	47.7	12.0	13.0
	3	3.18	2.10	0.66	37.5	54.0	12.8	14.6
10	1	3.19	2.26	0.71	21.4	27.6	14.7	16.2
	2	2.83	1.61	0.57	20.1	14.7	12.8	15.8
	3	2.26	2.62	1.16	34.1	23.8	10.0	18.0
	4	2.61	2.08	0.80	39.6	22.1	15.5	16.6
11	1	2.91	1.97	0.68	40.7	49.4	13.0	15.5
	2	2.80	2.29	0.82	27.2	21.5	12.5	16.5
	3	2.60	1.94	0.75	32.0	49.2	10.0	12.5
	4	2.41	1.98	0.82	32.8	43.4	12.0	12.5
12	1	3.44	2.29	0.66	29.6	34.6	15.0	18.4
	2	3.50	2.09	0.60	22.4	13.6	13.0	21.0
	3	2.57	2.34	0.91	26.7	30.9	11.3	13.2
	4	2.99	1.74	0.58	30.5	28.4	12.0	16.5

TABLE VI

Effects of Furosemide on Reabsorptive Half-Time and Intrinsic Reabsorptive Capacity ($C/\pi r^2$) as Studied by the Shrinking Drop Technique

Experiment No.	Transit time		Reabsorptive half-time		$C/\pi r^2$		Per cent change
	H	F	H	F	H	F	
	sec		*sec*		sec^{-1}		
1	14.9	23.5	12.1	21.0	0.061	0.043	−29.5
2	14.0	22.0	10.6	28.0	0.069	0.030	−56.5
3	14.8	20.0	14.0	25.0	0.055	0.032	−41.8
Mean	14.6	21.8	12.2	24.7	0.062	0.035	−42.6

In two experimental rats (Nos. 3 and 6, Table IV) in which fractional reabsorption was not inhibited, the excreted fractions of filtered sodium (15.6 and 19.8%, respectively) and water (17.0 and 20.2%) were not much lower than values observed in rats (e.g. rat No. 8, Table V, 22.4 and 22.7%) which showed the greatest decline in proximal sodium reabsorption.

The average values for transit time to the late proximal tubule (which represents about 50% of the total length of this segment of the nephron) were similar in control and pretreatment experimental rats (Table II). After furosemide, the transit time measured during fluid collection was increased in nearly every tubule (Tables IV and V).

The effect of furosemide on proximal sodium reabsorption was studied in three rats by the shrinking drop technique. The reabsorptive half-time and transit time were determined in consecutive 1 hr periods before and after the administration of the drug, and the results are summarized in Table VI. To assess the effect of the drug on absolute reabsorptive rate (C), the value for intrinsic reabsorptive capacity ($C/\pi r^2$) was calculated from the reabsorptive half-time.[5] This index of the volume of fluid reabsorbed per unit time per unit tubule volume decreased in each experiment (average = − 43%) (Table VI). To obtain a value for C, an estimate of tubule radius is required. It is difficult to be certain of exact luminal boundaries as estimated from photographs (11), but if the oil margins are used both before and after furosemide, the measured estimates of radius change little (average = + 6%). Since $C/\pi r^2$ is reduced markedly, and the values for radii are relatively unchanged, the values for absolute reabsorptive rate also fall significantly.

DISCUSSION

Despite the relative ease with which micropuncture methods can be applied to studies of the proximal convoluted tubule, at present there is little agreement concerning the effects of potent natriuretic drugs on sodium transport by this segment. The information obtained in the present study accounts in part for some of the differences. One important consequence of the inhibition of sodium and water reabsorption by the distal nephron after furosemide is the marked increase in intratubular hydrostatic pressure. In the present study, after furosemide, when fluid was collected from proximal convoluted tubules using conventional methods to prevent reflux of downstream fluid, unexpectedly high values for nephron GFR were found. Since furosemide is not likely to result in a large increase in nephron GFR (whole kidney GFR usually falls), it is believed that these recollections were contaminated with downstream fluid. This retrograde flow was not apparent at the time of fluid collection. To obviate this difficulty, recollections during furosemide diuresis were carried out with unusually long columns of polymer or castor oil to insure more effective distal blockade. This single maneuver allowed us to recognize a depression in the recollection $(TF/P)_{In}$ ratio after furosemide in 32 of 41 tubules and net depression in fractional sodium reabsorption in 10 of 12 animals.

Mechanical alterations in GFR produced either by aortic constriction or release of constriction have been shown to increase and decrease, respectively, fractional sodium reabsorption by the proximal tubule (11). In the present study, furosemide was associated in most instances with widely varied alterations in nephron GFR. It therefore was necessary to compare the changes in fractional sodium reabsorption after furosemide with those obtained when GFR was altered by means of aortic constriction and release. As shown in Fig. 3, although fractional reabsorption often fell after furosemide, when this change in reabsorption was accompanied by elevations in nephron GFR, it was not possible to attribute the depression to an action of the drug (i.e., comparable elevations in nephron GFR that follow an abrupt increase in renal artery perfusion pressure result in similar reductions in fractional reabsorption). In numerous other instances, despite reductions in nephron GFR,

[5] $C/\pi r^2 = 0.693/t_{\frac{1}{2}}$.

reabsorption fell after furosemide. Whereas in the former group the change in fractional reabsorption could be explained entirely on adjustments mediated by the mechanism(s) which set glomerulotubular balance, in the latter fractional reabsorption was changed in the direction opposite to that resulting from the glomerulotubular balance mechanism. This fall in fractional reabsorption, despite a fall in nephron GFR, is taken as evidence for a direct action of furosemide on the proximal tubule. In addition to demonstrating an inhibitory effect of furosemide on fractional reabsorption the results from tubules in which nephron GFR remained relatively unchanged indicate that absolute reabsorption also was depressed.

Although depression of fractional reabsorption was found more often in the castor oil group than in the group studied with equally long columns of polymer oil, it is not possible to conclude that castor oil provides a more effective distal block. In each group increases as well as decreases in nephron GFR were observed. The uniform failure to demonstrate an action of the drug on the proximal tubule in two rats (Nos. 3 and 6) studied with polymer oil is in part responsible for the quantitative differences between groups. It is possible that if greater numbers of animals were studied these differences would disappear. However, the primary purpose of the present study was not to evaluate the effectiveness of the different oils under these conditions, but rather to determine, when the technical difficulties are minimized, whether furosemide has an action on sodium transport in the proximal tubule of the rat.

On the basis of experimental and, to a large extent, inferential evidence, it has been widely held until recently that the cross-sectional area of the lumen of the proximal tubule is the principal determinant of the rate of sodium reabsorption by this segment (19–22). More recent studies in the rat (11, 23) and rabbit (24) have provided information that strongly opposes this interpretation. In these studies despite marked increases in calculated and measured values for tubule radius produced by elevation of ureteral pressure, renal venous occlusion and dilatation of isolated perfused proximal tubules, respectively, far less than proportionate increases, and often reductions in absolute reabsorptive rate were noted. Although these findings are in direct disagreement with the results predicted on the basis of the geometry hypothesis, an additional line of evidence remains in support of this hypothesis. In a recent study in the rat by Rector and coworkers (7), furosemide, in a dose similar to that used in the present study, was observed to lower the intrinsic reabsorptive capacity of the proximale tubule for sodium by 40% but was found to exert no effect on fractional sodium reabsorption. The following will help to illustrate the nature of this disparity. Fractional reabsorption, generally referred to by the expression $[1 - (P/TF)_{In}]$ may be rewritten:

$$\text{fractional reabsorption} = \frac{Cd}{V_0} \tag{1}$$

where C is the absolute reabsorptive rate, V_0 is the nephron GFR, and d is the distance from glomerulus to the site of puncture. When considered in the context of geometry hypothesis, the expression for fractional reabsorption may be rewritten as follows:

$$\text{fractional reabsorption} = \frac{Cd}{\pi r^2 d} \times \frac{\pi r^2 d}{V_0} \tag{2}$$

where r is the radius of the tubule, and the term $\pi r^2 d$ represents tubule volume to the point of puncture. Rector and associates have viewed fractional reabsorption, as shown by equation (2), as being determined by two independent processes, one that governs intrinsic reabsorptive capacity, given by the term $Cd/\pi r^2 d$, or more simply, $C/\pi r^2$, and the other, the process that governs the relationship between tubule volume ($\pi r^2 d$) and nephron GFR (V_0). Hence, fractional reabsorption will be depressed only when the product of these terms is reduced. After furosemide, Rector and associates (7) observed that $C/\pi r^2$ was reduced, on the average, by 39% when determined from the relationship between the $(TF/P)_{In}$ ratio and transit time[6] and by 40% when estimated by the shrinking drop technique.[7] However, the calculated value for $(\pi r^2 d/V_0)$[8] uniformly was observed to increase, reflecting the relatively larger increase in tubule volume than V_0. This calculated increase in tubule volume was due principally to a prolongation in transit time. The absence of an effect of the drug on fractional reabsorption in their study was thought to be the consequence of roughly equal and opposite changes in $C/\pi r^2$ and $\pi r^2 d/V_0$, and the dilatation of individual tubules was believed to counterbalance the inhibitory action of furosemide on absolute reabsorptive rate. In the present study, fractional reabsorption often fell after furosemide, despite an average increase in the calculated value for $\pi r^2 d/V_0$ of 46%. In agreement with the results of Rector et al., $C/\pi r^2$ fell, on the average, by 39% in free flow experiments and by 43% in experiments utilizing the shrinking drop technique. Thus, in the present study, despite an increase in tubule volume, we found the absolute and fractional rates of sodium reabsorption to be reduced after furosemide, and the extent of inhibition was similar for free flow and shrink-

[6] Calculated from the expression: $C/\pi r^2 = \frac{\ln (TF/P)_{In}}{\text{transit time}}$.

[7] Calculated from the expression: $C/\pi r^2 = \frac{0.693}{t_{1/2}}$.

[8] Calculated from the expression: $\frac{\pi r^2 d}{V_0} = \frac{[1 - (P/TF)_{In}]}{C\pi r^2}$.

mg drop techniques. Added to the results of our earlier studies (11), the present findings provide further evidence to suggest that the rate of reabsorption of sodium and water by the proximal tubule is governed by factors other than tubule geometry.

Although furosemide increased fractional delivery of sodium out of the proximal convoluted tubule from 35% of filtered to 50% in several of these hydropenic rats, there appeared to be little or no additional increase in fractional sodium excretion beyond that observed in other equally antidiuretic rats in which proximal reabsorption was not depressed. This suggests that furosemide, at least in the dosage studied, does not completely abolish sodium transport in the more distal portions of the nephron, since 50–65% of the glomerular filtrate emerged from the proximal tubule, whereas only one-third to one-half of that was excreted.

The magnitude of the depression in fractional reabsorption after furosemide (15–25%) is considerably less than that seen after the infusion of large quantities of isotonic saline solutions (which depress fractional reabsorption by 40–60%). It has become apparent from our own[9] as well as from the studies of others (25) that fractional reabsorption by the proximal tubule is associated intimately with the ECF volume compartment and that graded changes in the latter result in what appears to be relatively proportional changes in fractional reabsorption. Thus, mild to moderate expansion of ECF volume causes depression of fractional reabsorption by about 15–30% respectively, whereas massive volume expansion reduces fractional reabsorption by about 60%. This property of the proximal tubule to adjust fractional reabsorption over a relatively wide range implies that reabsorptive capacity is influenced by a number of factors, including, for example, the hemodynamic factors that create the balance of colloid osmotic and hydrostatic pressures at the peritubular surface, and the as yet little understood factor(s) that act on tubule epithelial cells to influence sodium transport directly. It is possible that furosemide, which produces a modest depression of fractional reabsorption, has as its principal action in the proximal tubule a direct inhibitory effect on an active sodium transport step, whereas after the infusion of large quantities of isotonic saline, the resulting greater depression of fractional reabsorption reflects the combined influences of the latter maneuver on renal hemodynamic and peritubular factors, as well as directly on the sodium transport mechanism.

Micropuncture studies in the dog and rhesus monkey have failed to demonstrate an inhibitory effect of furosemide on fractional reabsorption by the proximal tubule (6, 8). Since measurements of nephron GFR were not made in these studies, retrograde flow of tubule fluid may have occurred and was unrecognized, leading to $(TF/P)_{In}$ ratios that were elevated falsely. It should be emphasized, however, that in these same micropuncture studies, the fraction of filtered water reaching the early distal tubule (hence emerging from the water permeable proximal portion of the nephron) was not greater after furosemide than that observed before administration of the drug. Consequently, although the evidence from the present study supports an action of furosemide on the proximal tubule of the rat, there remains at present little direct evidence for a similar effect in this segment in other species.

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