JCI The Journal of Clinical Investigation

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J Clin Invest. 1978;62(2):311-320. https://doi.org/10.1172/JCI109131.

Research Article

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ABSTRACT The present studies were designed to further investigate the possibility of heterogeneity of nephron function during Ringer loading in the rat, and to determine the specific nephron segment responsible for this finding. As in previous studies from this laboratory with smaller rats (50–125 g), net addition of sodium between late distal tubule and papillary base (6.9 vs. 10.4% of the filtered load, respectively, $P < 0.005$) was found in more mature rats (170–230 g). In contrast, there was net reabsorption of sodium between these two segments in nonvolume-expanded animals, 1.70 vs. 0.45% of the filtered sodium load, $P < 0.005$. Because nephron heterogeneity of sodium transport during extracellular volume expansion is the most likely explanation for these findings, further studies were performed to determine the specific juxtamedullary nephron segment responsible for the net addition pattern between late distal tubule and papillary base in Ringer-loaded animals. First, a comparison was made of sodium delivery to the late proximal tubule of superficial nephrons vs. the delivery rate to the bend of Henle's loop of juxtamedullary nephrons in both hydropenia and Ringer loading. Fractional sodium delivery was quite comparable between the superficial and juxtamedullary nephrons in both hydropenia and Ringer loading although the absolute level was much greater in both groups of nephrons in the Ringer studies. Chlorothiazide (15 mg/kg loading and 15 mg/kg per h) given during Ringer loading markedly increased late distal sodium delivery, 19% of the filtered load, but did not prevent net addition of sodium at the papillary base. In contrast, furosemide (5 mg/kg loading and 5/mg/kg per h) given during Ringer loading completely reversed the segmental pattern, 35.5 and 28.8% at late distal tubule and papillary base, respectively, $P < 0.005$. These studies demonstrate that the net addition of sodium between late distal tubule and papillary base during Ringer loading is not limited to immature rats and that the segmental pattern does not occur in nonvolume-expanded animals. Further, the reversal of the net addition pattern with furosemide, but not chlorothiazide, and the comparable proximal nephron delivery rates in Ringer loading suggest that the loop of Henle of juxtamedullary nephrons reabsorbs less sodium than the same portion of superficial nephrons in this setting. A model is proposed to explain this finding.

Received for publication 3 June 1977 and in revised form 22 March 1978.

INTRODUCTION

In a recent study from this laboratory, evidence was presented which suggested that there was a difference in sodium transport between superficial and more inner cortical nephrons in the rat during Ringer loading (1). Although there was net reabsorption of sodium between the late distal tubule of superficial nephrons and the final urine in this setting, direct micropuncture of the most proximal portion of the papillary collecting duct consistently revealed a greater fractional delivery of sodium to this segment than to the late distal tubule. Because net sodium reabsorption occurred along the papillary collecting duct, it was suggested from these data that there was greater inhibition of sodium transport in more inner cortical nephrons during Ringer loading.

The present studies were designed to further investigate the possibility of heterogeneity of nephron function during Ringer loading and to determine the specific nephron segment responsible for this finding. The results further strengthen the view that sodium transport in inner cortical nephrons is inhibited to a greater extent during extracellular volume expansion and suggest that this is primarily because of altered sodium transport in the thin ascending limb of the loop of Henle of these nephrons.

METHODS

Studies were performed on male and female Munich-Wistar rats weighing 170–230 g. This specific breed of rats was utilized because they are endowed with an exceptionally large and accessible papilla (2 mm or more in most instances). The rats were anesthetized with Inactin (100 mg/kg; Promonta, Hamburg, West Germany) and placed on a thermoregulated heating board. Two polyethylene catheters were inserted in one jugular vein for infusion and administration of Lissamine green, and one catheter was placed in the carotid artery for blood withdrawal and monitoring of blood pressure. A tracheostomy was performed and a PE-50 catheter was inserted in the bladder. A small left subcostal incision was made and the left kidney was gently separated from the adrenal gland and contiguous perirenal fat and placed in a plexiglass cup. The surface was illuminated with a fiberoptic light source and the kidney was bathed with mineral oil at 37°C. If the rat had a proximal tubular transit time greater than 15 s, undue retention of the dye in the distal tubule, or a mean arterial pressure less than 100 mm Hg, it was discarded.

The animals were then given a solution containing 10% inulin dissolved in Ringer solution at a rate of 20 μl/min. While the inulin was equilibrating, late proximal and (or) late distal segments were localized with two or three injections of Lissamine green (10 μl of a 10% solution).

1 h after the inulin infusion had been started, the papilla was exposed and bathed in 37°C mineral oil as previously described (1). The tubules were then punctured with sharpened pipettes varying from 7 to 10 μM for proximal samples, 5 to 7 μM for loop, vasa recta, and distal tubule collections, and 10 to 13 μM for samples obtained from either the base or tip of the collecting duct. The late proximal, late distal, and collecting duct samples were obtained by methods previously described (1, 2).

A puncture of the collecting duct was performed as far proximally in the papilla as possible (this will hereafter be called the base sample). In each instance, the specific papillary tip punctured with each base sample was chosen because the oil column injected at the base was seen to exit at that particular duct. Samples were obtained from Henle's loop as close to the hairpin turn as possible, and quantitative fluid collections were made at the intratubular flow rate so that nephron glomerular filtration rate (GFR)[1] could be calculated. In additional studies, samples were obtained from the loop of Henle and adjacent ascending vasa recta, by the techniques described by Johnston et al. (3). Ascending vasa recta samples were immediately centrifuged and plasma was separated for subsequent analysis. As specified by the particular protocol, two to four late proximals, two or three late distals, one to four loop samples, one to four ascending vasa recta, or two to four papillary base and tip collections were obtained in a given study. Seven groups of studies were performed.

Group I, nondiuretic studies (n = 6)

In our previous study (1), it was not possible to accurately collect late distal tubular fluid during hydropenia because of the small size of the rats utilized and the consequential low tubular flow rate. In the present study, much larger rats were utilized and control experiments were performed to determine the fractional sodium delivery to the late distal tubule and papillary base in a nonvolume-expanded state. In four of the six studies, 10 μl/min of 5% mannitol was given in addition to the 20 μl/min maintenance infusion to further optimize tubular fluid collection. After initial preparation of the animal as described above, late distal and papillary collecting duct (base and tip) samples were collected.

Group II, Ringer loading studies (n = 8)

After initial preparation, 10%-body-weight Ringer solution was given over 40 min. The infusion was then reduced to a rate slightly above urinary losses. 20 min after the infusion rate had been reduced, late distal tubule and papillary collecting duct punctures were obtained.

Group III, comparison of superficial and juxtamedullary nephron sodium transport

(a) Hydropenia (n = 7). After initial preparation, late proximal and Henle's loop samples were obtained during continued hydropenia (infusion of only the maintenance infusion at 20 μl/min).

(b) Ringer loading (n = 6). These animals were expanded with Ringer solution in the manner described above, and late proximal and Henle's loop punctures were obtained in these particular studies.

Group IV, Ringer loading plus chlorothiazide studies (=7)

At the time of completion of the 10%-vol load, chlorothiazide (Merck, Sharp and Dohme, West Point, Pa.), 15 mg/kg loading and 15 mg/kg per h maintenance was begun. The Ringer infusion was continued at a rate slightly above urinary losses. 20 min after the initiation of diuretic administration, late distal tubule and collecting duct collections were obtained.

Five additional studies were performed in hydropenic animals given the same dose of chlorothiazide. 20 min after the diuretic was initially administered, and as volume replacement was being maintained, late distal tubular and collecting duct punctures were obtained.

Group V, Ringer loading plus furosemide studies (n = 8)

The same protocol was utilized as in the previous group except that furosemide (HoechstSoussel Co., Somerville, N. J.), 5 mg/kg loading and 5 mg/kg per h maintenance was given at the conclusion of the 10%-body-weight expansion. This does was chosen because it has been shown to have no effect on sodium delivery out of the superficial proximal tubule or to the bend of Henle's loop of juxtamedullary nephron (JMN) (4). The Ringer infusion was again kept at a level slightly above the urinary losses. 20 min after furosemide administration had begun, late distal tubule and collecting duct collections were obtained.

Group VI, comparison of superficial nephron (SN) and JMN sodium transport during Ringer loading and furosemide administration (n = 5)

Animals were prepared in a manner identical to those in the previous group. Samples were obtained from the late

[1] *Abbreviations used in this paper*: GFR, glomerular filtration rate; JMN, juxtamedullary nephron; SN, superficial nephron; $(TF/P)_{In}$, tubular fluid to plasma inulin ratio; $(TF/P)_{Na}$, tubular fluid to plasma sodium ratio; $(TF/P)_{Na/In}$, tubular fluid to plasma sodium to inulin ratio.

proximal tubule of SN and the loop of Henle of JMN, with the same protocol as in Group V.

Group VII, determination of loop of Henle to vasa recta sodium concentration gradients

In these studies, the gradient between the thin loop of Henle and ascending vasa recta was examined in three settings.

(a) Hydropenia (n = 5). The protocol in these studies was the same as that used in group IIIA. However, samples were obtained from Henle's loop and adjacent ascending vasa recta.

(b) Ringer loading (n = 6). These animals were expanded with 10%-body-weight Ringer loading as described, and loop of Henle and ascending vasa recta samples were obtained.

(c) Ringer loading plus furosemide (n = 5). Again, ascending-vasa-recta and loop-of-Henle punctures were performed. This group of animals was prepared in the same manner as that described for groups V and VI.

In all studies, the experimental collections were begun at approximately the same time after initial preparation of the animal and were completed in a similar period of time. Blood was obtained at the beginning and end of all collection periods. Clearance determinations were obtained from the right kidney at the same time as the micropuncture samples were being collected.

Plasma and urine inulin concentrations were determined by the anthrone method (5) whereas the concentration of inulin in tubular fluid was measured by the method of Vurek and Pegram (6). Sodium concentration in tubular fluid and vasa recta was measured with an Aminco helium-glow photometer (American Instrument Co., Travenol Laboratories, Inc., Silver Spring, Md.) and in urine and plasma with an Instrumentation Laboratory flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass.).

Calculations. (*a*) A fraction of filtered load of sodium delivered to a given nephron segment = $(TF/P)_{Na/In} \times 100$, where $(TF/P)_{Na/In}$ is the tubular fluid to plasma sodium to inulin ratio; (*b*) a fraction of filtered load of sodium absorbed along papillary CD = $(TF/P)_{Na/In}$ base − $(TF/P)_{Na/In}$ tip × 100; (*c*) the nephron filtration rate $(V_0) = V_F \times (TF/P)_{In}$, where V_F is the tubular flow rate in nl/min and $(TF/P)_{In}$ is the tubular fluid to plasma inulin ratio.

The data were analyzed by standard statistical methods (paired or unpaired *t* test) and all results are presented as the mean±SEM.

RESULTS

Nondiuretic studies. These studies are summarized in Table I and Fig. 1. GFR and fractional sodium excretion from the contralateral kidney were 0.82 ml/min and 0.06%, respectively. There was no difference in the results obtained with or without a small infusion of isotonic mannitol, and the studies have been combined. As can be noted from Table I, fractional sodium delivery to the end of the distal tubule was 1.7% of the filtered load. There was net reabsorption of sodium from late distal tubule to papillary base, the mean difference being 1.25% of the filtered load, $P < 0.005$ (Fig. 1). As was previously demonstrated in hydropenia, there was also net reabsorption of sodium from base to tip, 0.45 to 0.08% of the filtered load ($P < 0.01$).

Ringer loading studies. In these studies, mean GFR and fractional sodium excretion from the contralateral kidney were 1.03 ml/min and 4.9%, respectively. Fractional sodium delivery to the late distal tubule averaged 6.9%, a value significantly higher than in hydropenia ($P < 0.001$). Further, as we had demonstrated in our previous study in smaller rats, there was net addition between late distal tubule and papillary base in seven of the eight studies with the mean value of the latter parameter being 10.7%, $P < 0.005$, in comparison with late distal delivery (Table I and Fig. 2). This difference varied from 3.1 to 6.9% of the filtered load. Furthermore, as has been previously described (1, 7), there was substantial net sodium reabsorption along the papillary collecting duct, the difference being 5.4% of the filtered sodium load, $P < 0.01$.

Comparison of SN and JMN sodium transport. These studies were designed to determine whether the net addition of sodium between late distal tubule and papillary base during Ringer loading (Fig. 2) was because of a greater inhibition of sodium transport in the

TABLE I
Summary of Micropuncture Results

Model	Late distal tubule			Papillary base			Papillary tip		
	$(TF/P)_{Na}$*	$(TF/P)_{In}$‡	$(TF/P)_{Na/In}$§ × 100	$(TF/P)_{Na}$	$(TF/P)_{In}$	$(TF/P)_{Na/In}$ × 100	$(TF/P)_{Na}$	$(TF/P)_{In}$	$(TF/P)_{Na/In}$ × 100
		%			%			%	
Nondiuretic (*n* = 6)	0.26±0.05‖	17.3±3.3	1.7±0.4	0.20±0.06	51.2±13.9	0.45±0.15	0.07±0.02	73.0±22.9	0.08±0.02
Ringer loading (*n* = 8)	0.45±0.05	7.1±0.4	6.9±0.7	1.11±0.06	11.3±1.4	10.7±1.1	1.07±0.05	21.2±1.6	5.40±0.6
Ringer + chlorothiazide (*n* = 7)	0.58±0.01	3.2±0.4	19.5±1.5	1.11±0.03	5.0±0.7	25.4±2.1	1.26±0.06	8.9±1.8	17.7±0.9
Ringer + furosemide (*n* = 8)	0.91±0.03	2.8±0.3	35.5±3.4	1.01±0.02	3.8±0.4	28.8±2.8	0.99±0.04	4.6±0.5	23.3±2.6

* Tubular fluid to plasma sodium ratio.
‡ Tubular fluid to plasma inulin ratio.
§ Tubular fluid to plasma sodium to inulin ratio.
‖ Values presented indicate the mean±SEM.

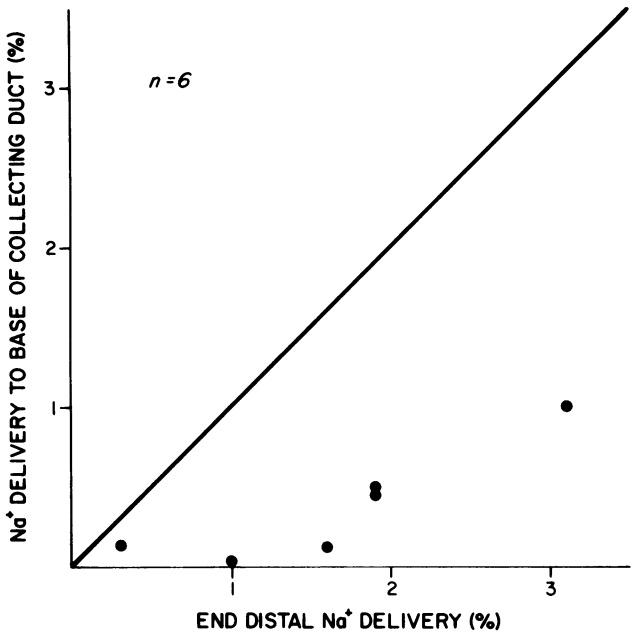

FIGURE 1 Comparison of superficial late distal tubule and papillary base sodium delivery during hydropenia.

proximal tubule of JMN. Because the reflection coefficient for sodium has been estimated to be approximately one in the descending limb of Henle's loop (8), the sodium delivery to the bend of the loop of JMN may be a reasonable marker of the sodium delivery out of the proximal tubule of these nephrons. It is recognized, however, that the data obtained from SN late proximal tubules and JMN loops may not be completely comparable because of sodium transport in the straight portion of the proximal tubule. The results of these studies are summarized in Table II and Fig. 3. In the hydropenic studies, the mean contralateral GFR and fractional sodium excretion were 0.75 ml/min and 0.05%, respectively. The SN late proximal tubular fluid to plasma inulin ratio (TF/P_{In}) averaged 2.76 indicating that 38% of the filtered load of sodium and water were delivered out of the accessible portion of the SN proximal tubule. There was almost identical delivery of sodium to the bend of Henle's loop (Table II and Fig. 3). The (TF/P_{In}) was higher than in SN, 6.64, but there was also a parallel rise in the tubular fluid to plasma sodium ratio $(TF/P)_{Na}$ to 2.33. Thus, 36% of the filtered sodium load was delivered to the bend of JMN, a value not different from the delivery out of the SN proximal tubule. As has been reported by others (9–11), JMN GFR was consistently higher than SN GFR, 39 vs. 25 nl/min, $P < 0.005$.

In the Ringer studies, contralateral GFR and fractional sodium excretion were 1.01 ml/min and 5.4%, respectively ($P < 0.01$ and <0.001 in comparison to hydropenia). When compared with hydropenic values there was a marked fall in the $(TF/P)_{In}$ to 1.64 and a rise in fractional sodium delivery out of the proximal tubules to 62% ($P < 0.001$ for both parameters). Similarly, fractional sodium delivery to the bend of Henle's loop significantly increased to 58% of the filtered load ($P < 0.005$). This increase was associated with a 56% decrease in the $(TF/P)_{In}$ ratio and only a 29% fall in the $(TF/P)_{Na}$ ratio when compared to hydropenia. As is shown in Fig. 3, SN and JMN sodium delivery were both clearly greater during Ringer loading than in hydropenic rats. In addition, sodium delivery out of the SN proximal tubule tended to be slightly greater than in JMN during Ringer loading although these values were not significantly different. Although JMN GFR was still higher than SN GFR, there was a tendency for the ratio of JMN to SN GFR to be lower in the Ringer studies than in hydropenia. In any case, there was no evidence from these studies to indicate that either fractional or absolute sodium transport was disproportionately inhibited in JMN, at least to the bend of Henle's loop during Ringer loading.

Ringer loading and chlorothiazide studies. The results of these studies are summarized in Table I and Fig. 4. Contralateral GFR and fractional sodium excretion averaged 0.85 ml/min and 17.8%, respectively. Late distal sodium delivery, 19.5% of the filtered

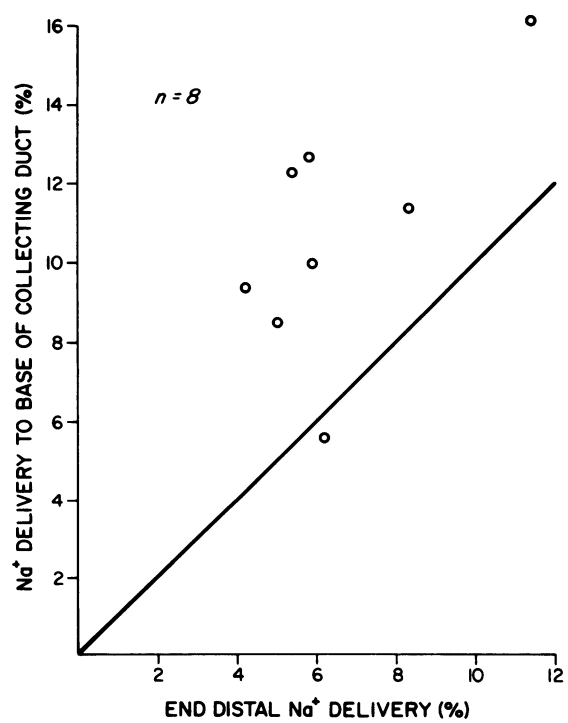

FIGURE 2 Comparison of superficial late distal tubule and papillary base sodium delivery during Ringer loading.

TABLE II
Comparison of Superficial and Juxtamedullary Sodium Delivery

	Superficial late proximal tubule			Juxtamedullary loop of Henle			
	$(TF/P)_{In}$*	Fractional Na delivery	Nephron GFR	$(TF/P)_{Na}$‡	$(TF/P)_{In}$	Fractional Na delivery	Nephron GFR
		%	*nl/min*			%	*nl/min*
Hydropenia (n = 7)	2.76±0.22§	38±3	25±2	2.33±0.11	6.64±0.53	36±2	39±4
P‖	<0.001	<0.001	NS	<0.01	<0.001	<0.005	NS
Ringer loading (n = 6)	1.64±0.05	62±2	32±5	1.72±0.14	3.03±0.27	58±4	40±5
P ¶	NS	NS	NS	<0.01	NS	NS	NS
Ringer loading + furosemide (n = 5)	1.79±0.06	57±2	29±4	1.24±0.06	2.43±0.23	52±4	37±8

* Tubular fluid to plasma inulin ratio.
‡ Tubular fluid to plasma sodium rate.
§ Values presented indicate mean±SEM.
‖ Hydropenia vs. Ringer loading.
¶ Ringer loading vs. Ringer loading + furosemide.

sodium load, was markedly higher than the value found during Ringer loading ($P < 0.001$). Yet, as is shown in Fig. 4, there was still net addition of sodium between late distal tubule and papillary base. The mean value of papillary base sodium delivery, 25.4% of the filtered sodium load, was significantly greater than late distal sodium delivery, $P < 0.01$. There was also marked reabsorption of sodium between papillary base and tip, the difference averaging almost 8% of the filtered load, $P < 0.001$.

In five additional studies in which chlorothiazide was given to hydropenic animals, net reabsorption of sodium between late distal tubule and papillary base was noted in each study with mean values of 3.3 and 2.1% of the filtered load, respectively, $P < 0.02$.

Ringer loading and furosemide studies. In these studies, contralateral GFR and fractional sodium excretion averaged 0.89 and 24.4%, respectively. As was the case in the chlorothiazide studies, sodium delivery

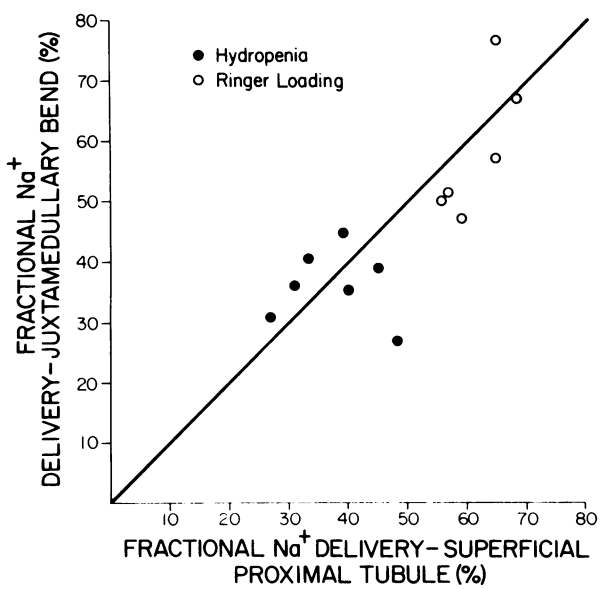

FIGURE 3 Comparison of fractional sodium delivery to the superficial late proximal tubule and juxtamedullary bend of Henle's loop during hydropenia and Ringer loading.

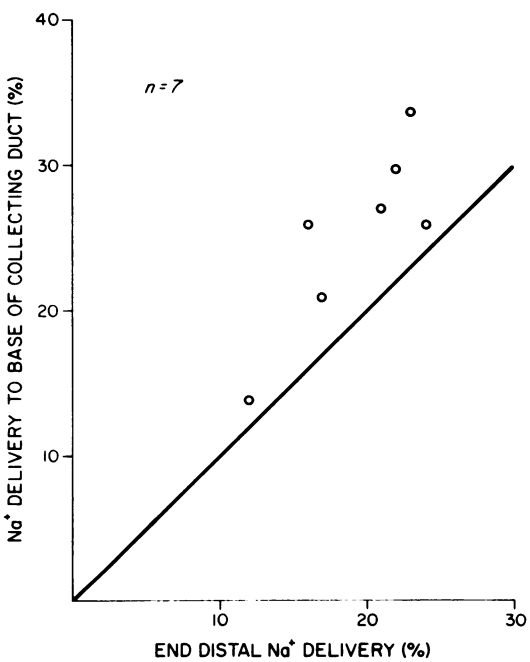

FIGURE 4 Comparison of superficial late distal tubule and papillary base sodium delivery during Ringer loading plus chlorothiazide.

to the late distal tubule was markedly increased vs. Ringer loading alone; 35.5 vs. 6.9% of the filtered load, respectively ($P < 0.001$). Yet, in contrast to the chlorothiazide studies, furosemide not only abolished, but reversed the sodium delivery pattern between late distal tubule and papillary base. As is shown in Fig. 5, there was net reabsorption of sodium between late distal tubule and papillary base in each of the eight studies with a mean difference of 6.7% of the filtered load ($P < 0.005$). There was also net reabsorption of sodium along the papillary collecting duct, the mean change being 5.5% of the filtered load ($P < 0.01$).

Comparison of SN and JMN sodium transport during Ringer loading plus furosemide. The reversal of the sodium delivery pattern between the late distal tubule and papillary base by furosemide suggested that sodium reabsorption differed in the ascending limb of JMN and SN during Ringer loading. It is important, however, to exclude an effect of this agent on sodium transport along more proximal nephron segments of SN and JMN during Ringer loading. As shown in Table II, fractional delivery of sodium to the end of the superficial proximal convoluted tubule averaged 57% of the filtered load compared to 52% delivered to the bend of Henle's loop of JMN. Neither of these values differed significantly from those obtained during Ringer loading alone. It should be noted that the TF/P_{Na} ratio at the bend of Henle's loop of JMN was significantly less than the corresponding value during Ringer loading alone (Table II). This finding probably reflects further papillary "washout" with furosemide and therefore decreased water abstraction along the thin descending limb. Although the TF/P_{In} after furosemide was not statistically less than that observed during Ringer loading alone, the fact that the fractional delivery of sodium did not differ between the two groups tends to substantiate this interpretation.

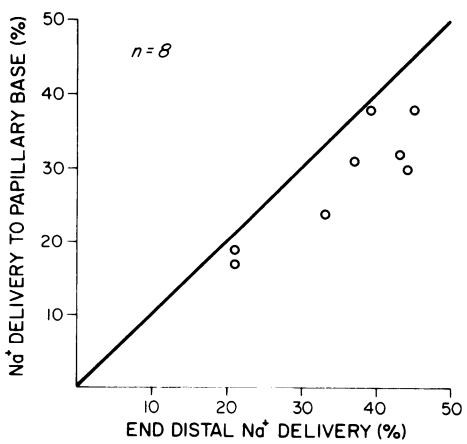

FIGURE 5 Comparison of superficial late distal tubule and papillary base sodium delivery during Ringer loading plus furosemide.

Determination of loop of Henle to vasa recta sodium concentration gradients. The results of these studies are summarized in Table III. In hydropenia, there was a constant gradient between thin ascending limb and ascending vasa recta with mean values of 331 and 262 meq/liter, respectively, $P < 0.02$. This concentration gradient of 69 meq/liter is quite similar to the values found by Johnston et al. (3). In contrast, the sodium gradient was abolished during Ringer loading, the mean values being 207 and 209 meq/liter in ascending limb and ascending vasa recta, respectively. The administration of furosemide to Ringer-loaded animals did not qualitatively alter the effect of Ringer loading. The sodium concentration in the loop of Henle and ascending vasa recta averaged 165 and 177 meq/liter, respectively. Thus, the gradient between thin ascending limb and ascending vasa recta was abolished in the latter two groups.

DISCUSSION

In a previous study from this laboratory, net addition of sodium was found between the end of the distal tubule of SN and the base of the papillary collecting duct during Ringer loading (1). The results of that study performed in young rats (50–125 g) were in-

TABLE III
Summary of Measurement of Papillary Structure Sodium Concentration

Model	No.	Sodium concentration		Gradient
		Ascending limb	Ascending vasa recta	
		meq/liter		
Hydropenia	1	298	243	+55
	2	338	229	+109
	3	262	242	+20
	4	295	208	+87
	5	463	388	+75
Mean±SEM		331±35	262±32	+69±15
Ringer loading	1	184	178	+6
	2	233	234	−1
	3	168	156	+12
	4	195	202	−7
	5	220	237	−17
	6	217	246	−29
Mean±SEM		207±10	209±15	−5±6
Ringer loading + furosemide	1	198	197	+1
	2	163	182	−19
	3	150	172	−22
	4	162	163	−1
	5	153	171	−18
Mean±SEM		165±9	177±6	−12±5

terpreted to indicate that sodium transport was inhibited to a greater extent in some segment of more inner cortical nephrons during volume expansion. The present work confirms this finding in larger rats (170–250 g) indicating that this phenomenon is not limited to immature animals. Further, in contrast to the findings during Ringer loading (Fig. 2), there was net reabsorption of sodium between the late distal tubule and papillary base in nonvolume-expanded animals (Fig. 1). In previous studies in which only late distal tubule sodium delivery and urinary sodium excretion were measured, it was suggested that collecting duct sodium transport was inhibited during Ringer loading (2, 12, 13). Yet, as is shown in Table I, the fraction of the filtered sodium load reabsorbed along the terminal collecting duct is much greater during Ringer loading than in hydropenia, 5.3 vs. 0.37% ($P < 0.001$). These results are also quantitatively similar to our previous results in young rats (1) and further enhance the view that the papillary collecting duct responds to an increment in sodium load during extracellular volume expansion by markedly increasing absolute sodium reabsorption.

Thus, the small difference between superficial late distal sodium delivery and urinary sodium excretion during Ringer loading is not because of diminished sodium transport in the terminal collecting duct but rather is related to a marked increase in the amount of sodium delivered to the most proximal portion of the accessible collecting duct. It even seems likely that the magnitude of this net addition is underestimated because an even longer segment of the papillary collecting duct is inaccessible to micropuncture. In any case, the remainder of this discussion will examine the determinants of this net addition of sodium between late distal tubule and papillary base during Ringer loading.

Two possibilities can be invoked to explain this phenomenon. First, it is possible the net sodium secretion occurs along the collecting duct system proximal to the papillary base. Second, Ringer loading may inhibit sodium reabsorption to a greater extent in JMN than in SN.

Regarding the first possibility, sodium secretion during Ringer loading would have to occur along the cortical collecting tubule because significant net sodium reabsorption occurs along the papillary collecting duct. If this were the case, it is likely that sodium secretion would occur down a concentration gradient from interstitium to tubular lumen, probably via the intercellular pathway. Yet, Tischer and Yarger (14) have previously presented evidence which suggests that the intercellular pathway of the cortical collecting tubule is less permeable than that of the papillary collecting duct. In addition, in vitro studies by Burg et al. (15) and Helman and co-workers (16) describe a relatively high electrical resistance along the cortical collecting tubule. Isotopic studies of unidirectional sodium flux indicate that very little back leak occurs along this nephron segment (17). It should also be pointed out that in the present studies, the gradient for sodium addition, as judged from the TF/P_{Na} ratio of fluid entering the collecting duct, is greatest in hydropenia (Table I), a model in which net reabsorption occurred between the late distal tubule and papillary base. Finally, the observation that furosemide abolishes the pattern of net sodium addition between these sites would require an effect of this agent on the cortical collecting tubule. There is no evidence, however, that furosemide alters sodium transport along this nephron segment. In fact, Burg et al. found that this diuretic failed to effect either net sodium transport or the transepithelial electrical potential in the isolated cortical collecting tubule (18). Thus, even though we cannot totally exclude collecting tubule sodium secretion with absolute certainty, the bulk of experimental evidence makes this possibility highly unlikely and therefore favors heterogeneity of nephron sodium transport as the explanation for the net addition between the late distal tubule and papillary base during Ringer loading.

The first group of studies evaluating this finding was designed to attempt to compare sodium transport in the proximal tubule of SN and JMN (Table II and Fig. 3). Although it is not possible with micropuncture techniques to directly compare sodium delivery in the superficial and juxtamedullary proximal tubule, it is possible to measure the sodium delivery to the last accessible segment of the proximal convoluted tubule of SN and the amount of sodium delivered to the bend of Henle's loop of JMN. Even if sodium transport were comparable in the convoluted portion of SN and JMN, these two measurable parameters would still seemingly be different if significant sodium transport occurred in the pars recta and (or) descending limb of SN. In studies with the isolated tubular perfusion technique, sodium and water movement in the straight portion of the proximal tubule have been found to be $\cong$½–⅓ of the values obtained from segments of the convoluted tubules per unit length (19–22). In further in vitro studies, however, no sodium transport was found to occur along the descending limb of Henle's loop (8). In addition, the reflection coefficient for sodium was found to be approximately one in this latter segment (8). Thus, if these in vitro data are reasonably applicable to the in vivo setting, there may be modest net sodium transport between the late proximal convoluted tubule and the bend of Henle's loop. Yet, as is shown in Table II and Fig. 3, no significant difference was noted in either hydropenia or Ringer loading between SN late proximal and JMN bend sodium delivery. These findings

are quite comparable to observations obtained by Jamison et al. during hydropenia and the administration of furosemide alone (4). Whether this lack of difference between SN late proximal and JMN bend sodium delivery indicates that pars recta sodium transport is quite small in the rat in vivo or that sodium transport in the proximal convoluted tubule of JMN nephrons is less than in SN cannot be determined with the present data. Yet, several points are worthy of note. As is shown in Figs. 1 and 2, net addition of sodium occurred between the late distal tubule and papillary base during Ringer loading but not during hydropenia. Yet, the relationship between SN late proximal and JMN bend sodium transport remained the same during hydropenia and Ringer loading although in the latter model the absolute delivery rate increased markedly in both groups of nephrons. Thus, there is no evidence from these data that sodium transport proximal to the bend of Henle's loop of deep nephrons was disproportionately inhibited during Ringer loading when compared to hydropenic values. From Table II it is also apparent that absolute sodium delivery did not increase disproportionately in JMN during Ringer loading. In fact, there was a tendency for GFR to increase to a greater extent in SN with volume expansion although this alteration was not significant. In any case, there is no evidence from these data that either fractional or absolute sodium delivery to the bend of Henle's loop of JMN was disproportionately increased during Ringer loading. Yet, because of the constraints of this method, small differences in SN and JMN proximal delivery during Ringer's loading can not be totally excluded.

Jamison and Lacy have previously compared sodium and water delivery to the bend of Henle's loop of JMN during hydropenia and Ringer loading in 55–95 g rats (23). Fractional water delivery was increased, but sodium delivery was not consistently altered by extracellular volume expansion. Whether these apparent discrepancies are because of differences in experimental design, the age of the rats or other factors is not known.

The next series of experiments was designed to evaluate the effect of chlorothiazide on the pattern of sodium delivery between late distal tubule and papillary base during Ringer loading. Clearance and micropuncture studies have suggested that the main sites of action of chlorothiazide are in the cortical portion of the ascending limb of Henle's loop and the distal convoluted tubule (24, 25). If Ringer loading inhibited sodium transport in this chlorothiazide-sensitive portion of JMN to a greater extent than in SN, administration of this diuretic may reduce or abolish the net addition pattern. Yet, as shown in Fig. 4, this was not the case. Thus, it seems unlikely that the chlorothiazide-sensitive portion of the distal nephron is responsible for the net addition of sodium between late distal tubular and papillary base during Ringer loading.

In contrast to chlorothiazide, furosemide not only abolished but reversed the sodium delivery pattern between late distal tubule and papillary base during Ringer loading. As is shown in Fig. 5, there was net reabsorption between late distal tubule and papillary base in each of eight studies with a mean difference of 6.7%. This phenomenon was not just a consequence of the marked increase in distal delivery because it can be seen that there was overlap in this parameter with the chlorothiazide experiments in which net addition of sodium between late distal tubule and papillary base was consistently noted (compare Figs. 4 and 5). Thus, the administration of furosemide, which presumably inhibited sodium chloride transport in the thick ascending limb of Henle's loop of all nephrons (18), reversed the pattern of sodium delivery between late distal tubule and papillary base seen with Ringer loading alone.

There are obviously a number of possible explanations for these findings. Because, as discussed above, there is evidence that furosemide is without an effect on the cortical collecting tubule, and the present data in our group VI animals (see Methods) exclude a proximal effect (Table II), it seems reasonable to interpret these studies to suggest that sodium transport in the loop of Henle of JMN is inhibited to a greater extent than in SN during expansion of the extracellular fluid volume. The mechanism responsible for this heterogeneity of nephron function cannot be clearly delineated from the present work. Yet, from the known functional and anatomical characteristics of the loop of Henle of SN and JMN, one possible model can be constructed. This proposal is schematically demonstrated in Fig. 6. The ascending loop of Henle of SN in the rat is composed only of a thick segment which begins at or slightly before the bend of the loop (26). This usually occurs at the border of the outer and inner zones of the medulla in the rat. The thick ascending limb then ascends into the cortex until it comes in apposition with the parent glomerulus and then becomes the distal convoluted tubule. In contrast, the ascending limb of JMN is composed of both a thick and thin segment. The thin ascending limb begins in the papillary portion of the inner medulla. The transition to the thick segment occurs at approximately the same point that the SN thick ascending limb is formed (26). The JMN thick ascending limb also ascends into the cortex and becomes the distal tubule in an area in close proximity to the parent glomerulus. Because both the SN and JMN thick ascending limb originate at approximately the same place, the total length of the thick ascending limb tends to be greater in SN.

The isolated tubule technique has been utilized to

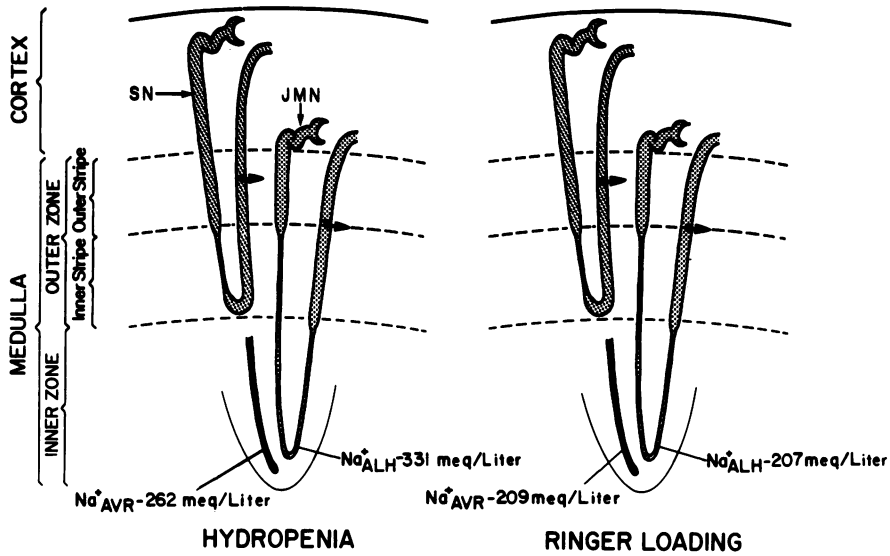

FIGURE 6 Proposed model to explain heterogeneity of Na^+ transport during Ringer loading. → denotes active salt transport in thick ascending link of both SN and JMN. In hydropenia (left panel), a sodium concentration gradient exists between thin ascending limb (ALH) and ascending vasa recta (AVR), 331 vs. 262 meq/liter. During Ringer loading, however, this gradient is abolished. Thus, sodium transport would be markedly decreased in the thin ascending limb of JMN because transport in this segment presumably occurs totally by passive means.

characterize the transport characteristics of the thick and thin ascending limb. Burg and Green (27) and Rocha and Kokko (28) demonstrated that the thick ascending limb actively transports chloride and that sodium movement is secondary to this active transport process. Furosemide inhibits active transport in this nephron segment and consequently reduces the net transport of sodium chloride (18). In contrast, there is no evidence for active transport of sodium or chloride in the thin ascending limb, but the nephron segment is permeable to both ions (29).

In the formulation of passive models developed to explain the urinary concentrating mechanism, one of the critical points is the presence of a sodium concentration gradient between the thin ascending limb and the medullary interstitium (30, 31). Recently, Johnston and associates have, in fact, directly demonstrated the presence of such a gradient (3), and the present data confirms this finding. As shown in Table III and Fig. 6, sodium concentrations of 331 and 262 meq/liter were found in ascending limb and vasa recta, respectively. Thus, passive sodium transport may occur along the thin ascending limb as a consequence of the development of this concentration gradient. During Ringer loading, however, the circumstances are quite different. The concentration gradient normally found during hydropenia is the consequence of the accumulation of urea in the medullary interstitium and the abstraction of water out of the descending limb of Henle's loop. During extracellular volume expansion, blood flow may markedly increase in the renal medulla leading to a "washout" of the interstitial hypertonicity (32). Indeed, Ringer loading abolished the sodium concentration gradient (Table III and Fig. 6) and thus may inhibit passive sodium transport in the thin ascending limb. This alteration will have no effect on sodium reabsorption in the thick ascending limb which presumably occurs as a consequence of active transport. In fact, numerous micropuncture studies have demonstrated a marked increase in absolute sodium transport in the superficial ascending limb during volume expansion (12, 23). Thus, because the ascending limb of SN is totally composed of a thick segment whereas the JMN contains both a thick and thin portion, it seems possible that Ringer loading may disproportionately alter sodium transport in this latter group of nephrons by reducing reabsorption in the thin limb and thereby decreasing total ascending limb sodium reabsorption. After the administration of furosemide, sodium chloride transport in Henle's loop will be markedly inhibited in both groups of nephrons. Because Ringer loading, per se, has already inhibited thin ascending limb reabsorption, the quantitative effect of this agent will be greater in SN than in JMN, thus obviating the net addition pattern seen with Ringer loading alone. In this regard, it should be pointed out that furosemide did not reestablish the gradient for sodium reabsorption in the thin ascending limb (Table III). Furthermore, the net reabsorption between late distal tubule and papillary base seen in the furosemide-Ringer studies may be a result of the combination of equivalent delivery of sodium to the distal portion of

SN and JMN coupled with significant sodium reabsorption along some portion of the medullary collecting duct proximal to the papillary base.

The quantitative contribution of this phenomenon to the natriuresis of Ringer loading seems substantial. The average net addition in these and our previous studies is ≅5% of the filtered sodium load and clearly represents a minimal estimate (1). Only the sodium reabsorptive capacity of the papillary collecting duct prevents a much greater increase in sodium excretion in this setting. It should also be emphasized that if the above formulation is correct, it may have relevance in other circumstances, such as drug-induced renal vasodilatation.

ACKNOWLEDGMENT

This work was supported in part by National Institutes of Health grant AM-17387.

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