

# Direct Measurement of Papillary Collecting Duct Sodium Transport in the Rat

## EVIDENCE FOR HETEROGENEITY OF NEPHRON FUNCTION DURING RINGER LOADING

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**ABSTRACT** It has been suggested that collecting duct sodium transport was inhibited by extracellular volume expansion. To directly evaluate this possibility, micropuncture of the papillary collecting duct of young rats was performed during hydropenia and Ringer loading. The possibility of heterogeneity of nephron function was evaluated during Ringer and hyperoncotic albumin loading by comparing the delivery of sodium to the end of the distal tubule of superficial nephrons with papillary base delivery.

During hydropenia ( $n = 14$ ), sodium delivery to the base averaged 0.95% of the filtered sodium load and reabsorption along the collecting duct was noted from base to tip in each collection pair averaging 0.80% of the filtered load.

During Ringer loading, sodium delivery to the base was markedly greater than in hydropenia, 11.8 vs. 0.95% of the filtered load ( $P < 0.001$ ). Yet, sodium reabsorption was also much greater, 6 vs. 0.8% ( $P < 0.001$ ).

In 13 paired collections, during Ringer loading, sodium delivery to the papillary base, 12.2% of the filtered load, was consistently greater than late distal tubular delivery from superficial nephrons, 8% ( $P < 0.005$ ). In contrast, reabsorption of sodium from late distal tubule to papillary base was found during albumin infusion, 6.2 vs. 3.1% ( $P < 0.001$ ).

Therefore, these studies demonstrate that: (a) the delivery of sodium to and reabsorption along the papillary collecting duct were markedly greater during Ringer loading than in hydropenia; (b) the amount of sodium delivered to the papillary base was greater than the de-

livery to the end of the distal tubule of superficial nephrons during Ringer loading, suggesting that deeper nephrons deliver more sodium to the collecting duct in this setting; and (c) the difference in sodium excretion between Ringer loading and hyperoncotic albumin infusion is due to events occurring between the late distal tubule of superficial nephrons and the base of the papillary collecting duct.

## INTRODUCTION

Recent studies from several laboratories have suggested that the collecting duct<sup>1</sup> (CD)<sup>2</sup> participates in the qualitative and quantitative regulation of sodium excretion (1-5).

It was, in fact, suggested that inhibition of sodium reabsorption along the CD was a major determinant of the magnitude of the natriuresis of extracellular volume expansion (1, 2). Similarly, Sonnenberg proposed that alteration in CD sodium transport was responsible for the deoxycorticosterone acetate-escape phenomenon (5). In all of these studies, the difference between end distal tubular delivery (obtained by micropuncturing late distal tubules of superficial nephrons) and the fractional urinary excretion of the electrolyte was utilized as an index of CD transport. It should be recognized, however, that this method can only be a valid marker of CD transport if the delivery of the electrolyte to the end of

<sup>1</sup> In this paper, the collecting duct includes the connecting tubule, cortical collecting tubule, and collecting duct proper. The possible differences in function in these segments will be discussed subsequently.

<sup>2</sup> Abbreviations used in this paper: CD, collecting duct; (TF/P)<sub>IN</sub>, tubular fluid to plasma inulin; (TF/P)<sub>Na</sub>, tubular fluid to plasma sodium.

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the distal tubule of superficial nephrons is representative of overall nephron function. In other words, the results obtained in these previous studies could be due to heterogeneity of nephron function with sodium delivery to the CD being greater in more inner cortical nephrons in the models in which the difference between fractional late distal delivery and urinary excretion of sodium diminished. To further investigate this possibility and to determine more directly sodium transport in the CD, direct puncture of the papillary CD of young rats was performed concomitant with collections of late distal tubular fluid of superficial nephrons during extracellular volume expansion.

## METHODS

Studies were performed on male and female Munich-Wistar rats weighing 50–125 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, W. 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. The preparation of the papilla was quite similar to that described by others (6, 7). The ureter was carefully dissected and opened at a point close to the pelvis and the papilla was then totally exposed by further dissection of the pelvic wall. The papilla was also continuously bathed in 37°C mineral oil and transilluminated with a small fiberoptic.

The animals were given a solution containing 10% inulin dissolved in Ringer solution at a rate of 10  $\mu$ l/min. While the inulin was equilibrating, proximal tubular transit time was performed and in some studies late distal tubular segments were localized with two or three injections of lissamine green (7  $\mu$ l of a 10% solution). Four distal segments localized by this technique were subsequently microdissected, and all were found to be in the last 20% of the distal tubule. This finding is quite comparable to our previous results in adult rats (1, 8).

If the rat had a proximal tubular transit time greater than 14 s, undue retention of the dye in the distal tubule, or a mean arterial pressure less than 90 mm Hg it was discarded.

1 h after the inulin infusion had been started, initial samples were collected. The tubules were punctured with sharpened pipettes with tip diameters varying from 10 to 13  $\mu$ M for the CD collections and from 5 to 7  $\mu$ M for the distal tubular samples. A puncture was performed as far proximally in the papilla as possible (this will hereafter be called the base sample), and a column of mineral oil stained with Sudan black was injected. Since it was not possible to maintain the oil block just distal to the pipette at high tubular flow rates, all samples were collected without a block but the collection rate was always considerably slower than the flow of fluid within the collecting tubule. In addition,

the pipette tip was always directed against the flow of the tubular fluid. Samples from the end of the papilla (hereafter called the tip sample) were collected by the introduction of a pipette into the terminal orifice of the CD. 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. The distance between base and tip was measured with a micrometer eyepiece. The late distal tubular samples were obtained as previously described (1, 8). These collections were always preceded and (or) followed immediately by a papillary base collection.

The following three groups of studies were performed:

**Hydropenia** ( $n=8$ ). After preparation of the animal as described above, alternate collections were obtained from the base and tip of the papilla. It was not possible to collect late distal samples during hydropenia in these young rats because of the extremely low tubular flow rates.

**Ringer loading** ( $n=12$ ). After initial preparation, 10% body weight Ringer solution was given over 60 min. The infusion was then reduced to a rate slightly above urinary losses and papillary samples and distal tubular punctures were then alternately obtained.

**Hyperoncotic albumin infusion** ( $n=6$ ). After initial preparation, 0.6% body weight 25% albumin (Hyland Div., Travenol Laboratories, Inc., Costa Mesa, Calif.) was given over 40 min. At the completion of the infusion, an equilibration period of 20 min elapsed before micropuncture samples were obtained. During the equilibration and collection period, a maintenance infusion of Ringer solution was given at a rate slightly in excess of the urinary losses.

In all groups, blood was obtained at the beginning and end of the collection period. 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 (9) while the concentration of inulin in tubular fluid was measured by the method of Vurek and Pegram (10). Sodium concentration in distal tubular fluid was measured with an Aminco Helium Glow Flame 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.).

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

**Calculations.** (a) Fraction of filtered load of sodium (Na) 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) fraction of filtered load of sodium absorbed along papillary CD =  $(TF/P)_{Na/in} \text{ base} - (TF/P)_{Na/in} \text{ tip} \times 100$ .

## RESULTS

**Hydropenia.** Clearance results were obtained in four of the eight studies from the contralateral kidney. The contralateral glomerular filtration rate was 0.49 ml/min while the fractional sodium excretion was 0.04%. Table I and Figs. 1–3 summarize the micropuncture results.

The  $(TF/P)_{in}$  ratio rose in each of the 14 paired collections with a mean change from 65 at the base to 109

TABLE I  
Summary of Collecting Duct Micropuncture Studies

Model	(TF/P) <sub>I<sub>Na</sub></sub>		(TF/P) <sub>N<sub>a</sub></sub>		(TF/P) <sub>N<sub>a</sub>/I<sub>Na</sub></sub> × 100	
	Base	Tip	Base	Tip	Base	Tip
Hydropenia (n = 14)	65 ± 14*	109 ± 17†	0.43 ± 0.05	0.13 ± 0.02‡	0.95 ± 0.13	0.16 ± 0.02‡
Ringer loading (n = 25)	11 ± 1	21 ± 2‡	1.09 ± 0.05	0.98 ± 0.06§	11.8 ± 0.9	5.8 ± 0.6‡

\* Mean ± 1 SEM.

† P < 0.001 base vs. tip.

§ P < 0.01 base vs. tip.

at the tip,  $P < 0.001$  (Fig. 1). In addition, the  $(TF/P)_{Na}$  ratio decreased from base to tip in each pair with a mean change from 0.43 to 0.13,  $P < 0.001$  (Fig. 2). The sodium delivery to the base ranged from 0.18 to 1.79% of the filtered load of sodium with a mean value of 0.95 (Fig. 3). Reabsorption was noted from base to tip in each collection pair and averaged 0.8% of the filtered sodium load. The fractional reabsorption of the sodium load delivered to the base averaged 82%.

**Ringer loading.** In these studies, the contralateral glomerular filtration rate was 0.63 ml/min while the fractional sodium excretion rate was 4.4%.

As was the case in hydropenia, the  $(TF/P)_{I_{Na}}$  rose in each instance from base to tip with a mean change from 10.6 to 21.1 ( $P < 0.001$ ). The  $(TF/P)_{Na}$  fell slightly from 1.10 at the base to 0.98 at the papillary tip ( $P < 0.01$ ). The delivery of sodium to the papillary base ranged from 4.8 to 23.2% of the filtered load of sodium

and averaged 11.8% (Fig. 4). Reabsorption from base to tip was noted in each of the 25 paired collections with a mean value of 6.0% of the filtered sodium load. The mean fractional reabsorption of the sodium load delivered to the base was 51%. This was significantly less than the hydropenic value ( $P < 0.001$ ). Delivery of sodium to and reabsorption along the papillary CD were markedly greater during Ringer loading than hydropenia ( $P < 0.001$  for both values).

**Comparison of late distal and papillary base sodium delivery.** 13 pairs of distal tubular and papillary base collections were obtained during Ringer loading. In four of these observations, the papillary base was punctured before and immediately after the distal tubular puncture and the base values were averaged. Fig. 5 summarizes the findings. In 11 of the 13 paired collections the amount of sodium delivered to the papillary base exceeded the amount of sodium at the end of the distal tubule of superficial nephrons. This difference varied from 1.1 to 8.9% of the filtered load. In seven of these pairs,

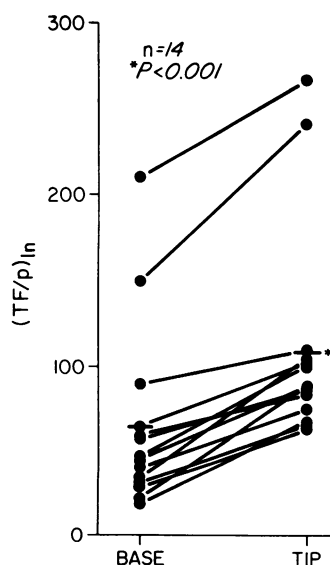


FIGURE 1 Comparison of  $(TF/P)_{I_{Na}}$  at the base and tip of the papillary collecting duct during hydropenia.

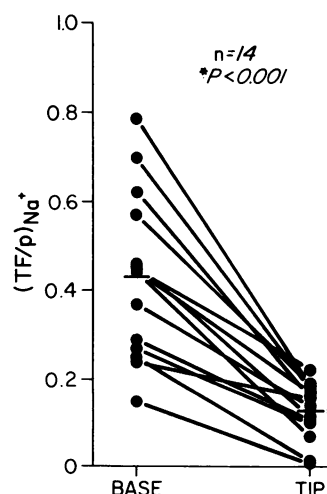


FIGURE 2 Comparison of  $(TF/P)_{Na^+}$  at the base and tip of the papillary collecting duct during hydropenia.

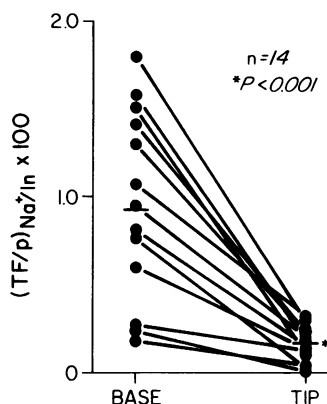


FIGURE 3 Comparison of the fractional sodium delivery  $[(TF/P)_{Na/In} \times 100]$  to the base and tip of the papillary collecting duct during hydropenia.

sodium delivery to the base exceeded end distal delivery by more than 5% of the filtered load. The mean base delivery, 12.3% of the filtered sodium load, was significantly greater than end distal sodium delivery, 8.0% of the filtered sodium load ( $P < 0.005$ ).

As noted in the Methods section, late distal tubular flow rate in hydropenia was too low in these small rats for adequate collection. Therefore, to contrast the results of the Ringer loading studies with another experimental model, a comparison was made of late distal tubular and papillary base sodium delivery in rats given hyperoncotic albumin ( $n = 10$ ). In the albumin studies sodium delivery to the late distal tubule of superficial nephrons, 6.2% of the filtered load, was not significantly different from that in the Ringer studies, 8.0%, confirm-

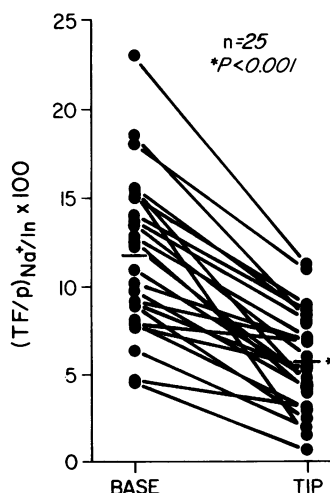


FIGURE 4 Comparison of the fractional sodium delivery to the base and tip of the papillary collecting duct during Ringer loading. Sodium delivery to and reabsorption along the papillary collecting duct were markedly greater during Ringer loading than hydropenia ( $P < 0.001$  for both values).

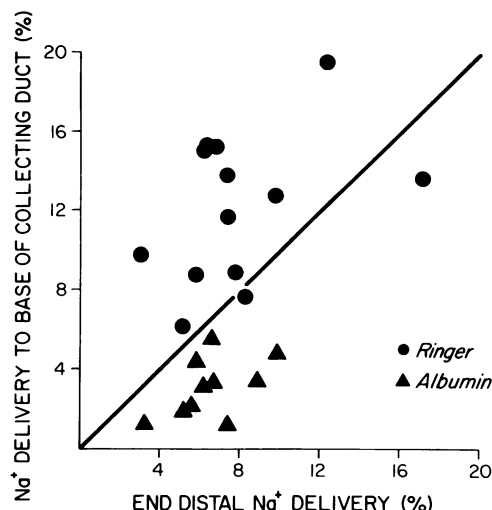


FIGURE 5 Comparison of the fractional sodium delivery to the end of the distal tubule of superficial nephrons vs. papillary base sodium delivery. ●, Ringer loading; ▲, hyperoncotic albumin infusion.

ing similar previous findings from our laboratory (1). In contrast to the results of the Ringer studies, however, there was net reabsorption of sodium from the late distal tubule to the papillary base in each of 10 collection pairs, 6.2 vs. 3.1% respectively,  $P < 0.001$  (Fig. 5). The fraction of the filtered sodium load excreted (papillary tip delivery), 1.4%, was also significantly less than the sodium delivery to the papillary base ( $P < 0.005$ ). Thus, at comparable sodium delivery to the late distal tubule of superficial nephrons, there was net addition of sodium at the papillary base in the Ringer studies (+ 4.2% of the filtered sodium load) and net

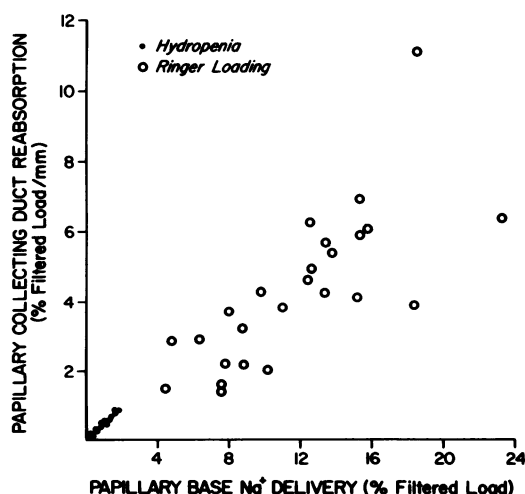


FIGURE 6 Comparison of the fractional delivery of sodium to the papillary base vs. reabsorption along the papillary collecting duct.

reabsorption in the hyperoncotic albumin experiments ( $-3.1\%$  of the filtered sodium load).

## DISCUSSION

In the present study, net reabsorption of sodium and water was noted along the papillary CD of young rats during both hydropenia and Ringer loading. In addition, the delivery of sodium to and reabsorption along the papillary CD were markedly greater during extracellular volume expansion than during hydropenia (compare Figs. 3 and 4).

It has been suggested that sodium reabsorption along the CD was inhibited by expansion of the extracellular fluid volume (1, 2). In these free-flow micropuncture studies in the rat, collecting duct transport was evaluated by comparing the fractional delivery of sodium to the end of the distal tubule of superficial nephrons with fractional urinary sodium excretion. It was found that the delivery of sodium to the end of the distal tubule of superficial nephrons was comparable during Ringer and hyperoncotic albumin loading; yet sodium excretion was threefold greater in the Ringer studies (1). Similarly, end distal sodium delivery was not different between Ringer-loaded normal rats and animals that had been on a low salt diet for 2 wk while sodium excretion was much less in the low salt rats (2). Sonnenberg also reported that CD transport was inhibited during whole blood infusion and deoxycorticosterone acetate escape in the rat (4, 5). Yet, the interpretation of all these studies in which an indirect measurement of CD transport was utilized must be made with caution, since this technique is valid only if the delivery of sodium to the end of the distal tubule of superficial nephrons is representative of overall nephron function. The results of the present study suggest that this is not the case. As is shown in Fig. 5, there was a greater fraction of the filtered load of sodium delivered to the papillary base than to the end of the distal tubule of superficial nephrons during Ringer loading. This difference averaged 4% of the filtered load but was as great as 8.5% of the filtered load.

There are two possible explanations for this finding. First, it may be that there is net addition of sodium from the renal interstitium into the CD proximal to the base of the papilla. Since there was marked net reabsorption in the last portion of the CD, this would mean that net sodium transport in the opposite direction to that in the papillary CD would have to occur in the cortical collecting tubule and(or) outer medullary collecting segment. Although there are definite physiologic differences between the various portions of the CD (11), it seems unlikely that the net addition of sodium occurred in this manner. According to current concepts, the movement of sodium from interstitium to CD lumen would likely occur by means of a paracellular pathway (12,

13). Tisher and Yarger recently demonstrated in the rat that the tight junction of the cortical collecting tubule and outer medullary CD was impermeable to lanthanum while the tight junction in the papillary CD segment was freely permeable to the marker (14). The findings of Tisher and Yarger would suggest that circumstances which lead to back diffusion of sodium into the tubular lumen of the CD system would be most readily detectable in the papillary CD. Yet, marked reabsorption of sodium was noted by direct micropuncture in this nephron segment during Ringer loading (Fig. 4). Thus, although not excluded, it does not seem likely that the net addition of sodium from the end of the distal tubule to the papillary base was due to leakage of sodium from the renal interstitium.

Second, the delivery of sodium to the CD may have been greater from more inner cortical nephrons than the superficial nephrons accessible to micropuncture. If this possibility is correct, a consideration must be given to the age of the animals used in this study, since individual nephron function may be quite different in young rats. It is well known, however, that renal maturation follows a centrifugal pattern with the oldest nephrons being at the corticomedullary junction and the youngest in the subcapsular layer (15, 16). It would seem surprising, therefore, that a reduction in the intrinsic capacity of inner cortical nephrons to reabsorb sodium would be apparent only in young rats, since inner cortical nephrons are better developed than those of the outer cortex in rats of this age. In addition, we have performed two additional micropuncture studies during Ringer loading in Munich-Wistar rats weighing 180 and 205 g. Late distal tubular sodium delivery was 4.2 and 5.40%, while papillary base Na delivery was 9.4 and 12.3% of the filtered sodium load, respectively. Thus, the findings in the present studies do not seem to be dependent upon the age of the animals studied.

There are a number of other possible explanations for a greater suppression of sodium reabsorption in more inner cortical nephrons during extracellular volume expansion. First, Daugharty et al. in the rat (17) and Bruns et al. in the dog (18) found that the filtration fraction fell to a greater extent in inner cortical nephrons during Ringer loading. Thus, assuming that the magnitude of the decrease in peritubular capillary oncotic pressure is a determinant of reabsorption in the proximal tubule (19, 20), distal delivery of filtrate may be greater in deeper nephrons. Second, Kawamura et al. (21) and Jacobson and Kokko (22) have recently demonstrated basic electrophysiologic differences between the proximal convoluted and straight portions of superficial and juxtamedullary nephrons. It is possible that these and(or) some other intrinsic differences in the transport characteristics of superficial and juxtamedul-

lary nephrons may account for heterogeneity of nephron function during Ringer loading. Third, it has been suggested that washout of medullary solute may influence sodium transport in some portion of the distal nephron (23). This effect should be more marked in inner cortical nephrons, especially in the rat, since the loop of Henle of superficial nephrons does not descend into the inner medulla in this species.

In a previous study from this laboratory, a comparison was made of the segmental analysis of sodium transport along the nephron during Ringer loading and hyperoncotic albumin infusion (1). Superficial late distal tubular sodium delivery was not different between the two groups, yet sodium excretion was three times greater in the Ringer studies. Quite comparable results were found in the present study. Late distal sodium delivery was again similar in the two groups (Fig. 5), while fractional sodium excretion (determined from papillary tip collections) was 5.6 and 1.3% in the Ringer and albumin groups, respectively. It was previously suggested that the difference in sodium excretion between the two groups was due to altered CD sodium transport (1). Yet, in the present work in which direct papillary CD sodium transport was evaluated, absolute sodium reabsorption during Ringer loading was markedly increased in comparison to hydropenic values and was also greater than during hyperoncotic albumin infusion (6 vs. 1.9% of the filtered sodium load),  $P < 0.001$ . Also, there was net addition of sodium from the superficial late distal tubule to the papillary base in the Ringer studies and net sodium reabsorption in the hyperoncotic albumin studies with the difference in sodium delivery to the papillary base between the two groups being 8.6% of the filtered sodium load ( $P < 0.001$ ).

As discussed in the previous section, the net addition of sodium between late distal tubule and papillary base in the Ringer studies seems more likely due to greater delivery of the cation to the CD from more inner cortical nephrons. Although other possibilities deserve further consideration, it is attractive to speculate that the difference in sodium delivery to the papillary base in these two groups is due primarily to depressed sodium transport in more inner cortical nephrons during Ringer loading.

This proposal is not contradictory to our previous observations utilizing the  $^{22}\text{Na}$  microinjection technique (1). The nuclide was injected in a late distal tubule and urinary recovery was evaluated in animals given either Ringer solution or hyperoncotic albumin. The recovery of the nuclide was markedly greater in the Ringer studies, suggesting the possibility of depressed CD transport in this setting. Yet, as was stated previously (1), a similar increased recovery of  $^{22}\text{Na}$  would

occur if deep nephron sodium delivery to the CD was markedly increased and overall CD transport exhibited some form of saturation kinetics. In other words, if CD transport is not linear at all rates of sodium delivery, an increased sodium load from deeper nephrons would decrease the fractional reabsorption of the nuclide along the length of the CD and increase recovery of the  $^{22}\text{Na}$  in the final urine.

In comparing sodium reabsorption along the papillary CD during hydropenia and Ringer loading, several points are worthy of note. Fractional reabsorption of the sodium load delivered to the papillary base was consistently higher in the hydropenic studies, 82 vs. 51% ( $P < 0.001$ ). The absolute fraction of the filtered load of sodium reabsorbed in the CD, however, was seven times greater in the Ringer studies, 6 vs. 0.8% ( $P < 0.001$ ). Thus, fractional reabsorption decreased, but absolute sodium reabsorption in the CD markedly rose during Ringer loading in association with an increased delivery rate of sodium to the papillary base. Fig. 6 compares papillary base sodium delivery with the fraction of the filtered load reabsorbed along the papillary CD. Up to a fractional delivery of approximately 12% of the filtered sodium load, the relationship is fairly linear. With the exception of one aberrant point, reabsorption tended to plateau above this delivery rate. These findings are qualitatively comparable to the results of Diezi et al. (6).

Although these findings do not exclude the possibility that extracellular volume expansion may alter CD sodium transport, absolute sodium reabsorption is clearly increased in this setting when compared to hydropenic values.

Thus, these results suggest that the natriuresis seen with Ringer loading is due, at least in part, to markedly increased sodium delivery from more inner cortical nephrons and that the terminal portion of the CD plays an important quantitative role in reducing the magnitude of the natriuresis. These findings are in contrast to two recent studies by Sonnenberg (24, 25) in which a modification of the microcatheterization technique of Jarausch and Ullrich (26) was utilized to measure CD sodium reabsorption during Ringer loading. In these two studies, either no net reabsorption (24) or even net addition of sodium was found along the papillary CD during Ringer loading. Yet, even aside from the marked technical differences between the microcatheterization technique and direct papillary micropuncture, the basic protocol in those two studies was quite different from that in the present work. In the first study, the rats had been on a markedly increased sodium intake for 1–3 wk before study (24). This type of regimen has been suggested to modify the distal nephron response to acute Ringer loading (27). In the second study, the degree of

acute extracellular expansion was markedly greater than in the present study, with fractional sodium excretion increasing to values as great as 30% (25). Further studies are needed to determine whether these apparent differences are a function of the model utilized to expand extracellular volume or of the technique of measuring CD sodium transport.

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