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J Clin Invest. 1973;52(2):386-397. https://doi.org/10.1172/JCI107195.

Research Article

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Micropuncture Studies of Sodium Transport in the Remnant Kidney of the Dog

THE EFFECT OF GRADED VOLUME EXPANSION

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ABSTRACT Proximal and distal tubule micropuncture studies were performed to examine the response to graded extracellular volume (ECV) expansion in 10 normal dogs (stage I), 11 dogs with a unilateral remnant kidney (stage II), and 7 dogs with a remnant kidney after removal of the contralateral kidney (stage III). Before ECV expansion in stage III, there was a suggestive reduction in proximal tubule as well as loop fractional reabsorption of sodium. After ECV expansion to 3% body weight proximal tubule reabsorption was depressed in all groups of animals, while little further inhibition was observed in this segment with additional expansion to 10% body weight. In contrast, the fraction of filtered sodium remaining in the distal tubule rose progressively in all three groups after graded ECV expansion, suggesting that the graded natriuretic response found in the final urine was largely due to a similar response in the loop of Henle rather than that in the proximal tubule. The distal tubule response of the remnant kidney in both stages II and III was greater than that in stage I. These data indicate that although enhanced sodium excretion per nephron in chronic renal failure may be related to uremia, its exaggerated response to ECV expansion is due, at least in part, to certain as yet unidentified intrarenal factors consequent to reduction in functioning renal mass.

This work was presented at the Canadian Society of Clinical Investigation 20 January 1971, Ottawa, Canada and published in *Clin. Res.* 1970. **18**: 750 (Abstr.)

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Received for publication 7 October 1971 and in revised form 5 April 1972.

INTRODUCTION

Renal handling of sodium in chronic renal disease is known to undergo certain characteristic alterations during the course of the disease process (1, 2). As the functioning renal mass is progressively reduced, the fraction of the filtered sodium excreted in the urine (fractional sodium excretion) on a constant dietary sodium intake increases proportionally so that its homeostasis is relatively well maintained even in the face of advanced renal failure. Also, the response of the chronically diseased kidney in fractional sodium and water excretion to extracellular volume (ECV)[1] expansion is greatly exaggerated (2). The mechanism for these functional alterations in chronic renal disease is not known. Using the models of unilateral pyelonephritis and remnant kidney in the dog, Bricker, Klahr, Lubowitz, and Rieselbach (1) postulated that the remaining nephrons in the diseased kidney are capable of normal functional activities under nonuremic environment since the functional parameters are nearly equal in both the diseased and the contralateral intact kidneys, and it is only when uremia supervenes that the observed functional adaptation takes place. Inherent in this theory is that no adaptation occurs in the nonuremic state. On the other hand, a small difference in sodium transport between the diseased and the contralateral kidneys was also

[1] *Abbreviations used in this paper:* ECV, extracellular volume; ECVE, extracellular volume expansion; FE, fractional excretion; GFR, glomerular filtration rate; TF/P, tubule fluid-to-plasma ratio; $U_{Na}V$, urinary sodium excretion rate.

observed (3). Gutmann and Rieselbach (4) demonstrated that this difference in sodium transport between the two kidneys was greatly exaggerated when sodium excretion was enhanced by ECV expansion with saline or by administration of potent diuretics such as furosemide, indicating a greater natriuretic response of the diseased kidney as compared to the contralateral kidney. Thus, functional changes of the diseased kidney may not always be related to uremia.

The tubule sites for these alterations in sodium transport in chronic renal disease have not been clearly defined. Some micropuncture studies in the rat revealed reduction in proximal tubule reabsorption (5, 6), while others disclosed no change in the proximal segment (7, 8). Also, relatively little is known about transport changes at more distal sites of the nephron in the chronically diseased models (9). One of the difficulties in micropuncture studies of the diseased models, used to demonstrate significant changes in transport parameters, is that comparison has to be made between groups of animals with considerable functional variation within the same group. Since the response of the chronically diseased kidney to ECV expansion is known to be greater than that of the normal kidney (2), this diuretic maneuver may help to exaggerate the differences in sodium transport between the two different groups of animals. Our present micropuncture studies were, therefore, designed to evaluate the responses in the proximal and distal tubule of the remnant kidney in the dog to graded ECV expansion, and these were compared with the corresponding values in the normal dog. Our results indicate that the exaggerated response of the remnant kidney to ECV expansion occurs mainly in the loop of Henle regardless of whether the contralateral kidney is removed. However, after removal of the contralateral kidney, evidence suggests the fractional proximal tubule reabsorption of the remnant kidney is reduced and this probably also contributes to the enhanced natriuretic response observed in the final urine.

METHODS

Micropuncture studies were performed in 28 mongrel dogs weighing 8–20 kg. The remnant kidney model with its three experimental stages was used. 10 normal dogs without any surgical manipulation before the study were used for stage I. Stage II included 11 animals in which unilateral remnant kidney was induced by ligating three fourth to five sixth of the main branches of renal artery in one kidney resulting in segmental infarction. In order to make the remnant kidney more accessible to micropuncture, the vessels were ligated so that the remaining renal mass would be located near the center of the kidney. The micropuncture experiments were carried out 2 wk after induction surgery. Stage III consisted of seven animals in which nephrectomy of the contralateral kidney was performed in stage II, 2 wk after the induction surgery. An additional week was allowed for azotemia to develop before the experiments. All animals were placed on a constant amount of dog diet, fed once daily. However, stage III dogs generally became anorexic after contralateral nephrectomy and did not always ingest all the food supplied. Since five of the seven stage III dogs appeared dehydrated as judged by weight loss of 0.3–0.8 kg, on one occasion these dogs received intravenous infusion of Ringer's lactate solution of 500 ml over a period of 2–3 h 3–4 days before the micropuncture studies. Nevertheless, some of these animals remained volume contracted with net weight loss of 0.2–0.5 kg at the time of the studies. In all animals, water was withheld overnight before the micropuncture experiments.

Recollection micropuncture experiments were carried out in three phases in all animals. Tubule fluid samples were collected at the same puncture site during the control phase of hydropenia, a second phase of ECV expansion to 3% of body weight and a third phase of additional expansion to 10% of body weight. In the second phase, modified Ringer's solution containing Na 145, K 3.5, Cl 128.5, and HCO_3 20 meq/liter was infused at a rate of 12 ml/min over 30–45 min until the amount reached 3% of body weight. In the third phase, additional infusion of Ringer's solution to 7% body weight was given at a rate of 24 ml/min, so that the total ECV expansion amounted to 10% of body weight. In all phases, infusion of Ringer's solution was continued at an appropriate rate to match the urine flow. As stage III dogs excreted less urine than stage I and II animals during the period of acute ECV expansion, infusion of Ringer's solution equal to 10% of body weight in all groups of animals might have resulted in slightly greater degree of expansion in stage III. Therefore, the magnitude this difference in ECV expansion was estimated by obtaining the mean differences in total urine flow between stages I and III during the period of acute expansion. The mean cumulative difference in net volume expansion was 120–150 ml which was less than 10% of the total amount of fluid infused for volume expansion and, therefore, should not alter the experimental results especially in view of the fact that some of the stage III animals were slightly volume contracted.

Animals were prepared for micropuncture experiments in a manner previously described (10) and only a brief description will be given here. Dogs were anesthetized with intravenous injection of pentobarbital 30 mg/kg, followed by injection of small doses of sodium thiopental as necessary to sustain anesthesia. An endotracheal tube was inserted and adequate respiration was maintained with a Harvard respirator (Harvard Apparatus Co., Millis, Mass.). A jugular vein and a foreleg vein were cannulated for infusions of inulin and Ringer's solution, a femoral vein was cannulated for collection of blood samples and a femoral artery was cannulated for measurement of blood pressure. Through a suprapubic incision, the bladder was exposed and each ureter was catheterized for collection of urine. The left kidney was exposed by flank incision and prepared for micropuncture as has been described (10). The main renal artery was cannulated with a 27 gauge needle connected to PE 20 polyethylene tubing for injection of F, D, and C green no. 3 dye (Keystone Aniline and Chemical Co., Chicago, Ill.).

Tubule fluid samples were collected from a late or the last accessible site of the proximal tubule, as well as from the distal tubule. These were identified by injecting 0.1–0.2 ml of 5% F, D, and C green into the renal artery and timing the appearance of the dye to the surface tubules. An effort was made to obtain recollected samples for all three

phases from each puncture site. However, on occasion, only a pair of tubule fluid samples were collected in two phases and these were also included in the evaluation of the data. A long block of colored mineral or castor oil was maintained immediately distal to the puncture site to prevent retrograde collection. Approximately 30–80 nl of tubule fluid samples were collected for analyses of inulin and sodium in duplicate. Each sample was examined microscopically to exclude the presence of erythrocytes.

Standard clearance techniques with 15-min periods were employed and heparinized blood samples were obtained at midpoint of each period. Urine collection from the remnant kidney in the hydropenic phase was continued for 30 min for each period to minimize the error due to the dead space of catheter. Inulin in the tubule fluid was determined by the ultramicro-fluorometric method of Vurek and Pegram (11), and sodium and potassium by helium glow spectrophotometry (12). Inulin in the plasma and urine was measured by the anthrone method (13) and sodium and potassium by flame photometry. The precision of all the above measurements have been reported previously (14, 15). Concentrations of inulin, sodium, and potassium in the tubule fluid were matched for the corresponding values in the plasma interpolated from the most approximate plasma samples. Mean values for each experimental phase per animal were used for analyses of clearance and micropuncture data. Student's t test was performed for paired and unpaired comparison of various experimental data.

The micropuncture study of experimental renal disease is directed in part at observing changes in baseline proximal tubule reabsorption as reflected by tubule fluid-to-plasma (TF/P) inulin ratios in different groups of animals. One approach supporting the validity of group comparisons is to analyze control TF/P inulin data of the last accessible portion of the proximal tubule compiled from five observers in our laboratory in the last 2 yr. Table I indicates that each observer demonstrated adequate recollection/control TF/P inulin ratios during continued hydropenia, as values were not significantly different from 1.00 in each instance. The mean last proximal TF/P inulin expressed per tubule and per dog is also noted for each experiment. The overall mean of the five observers was 1.77±0.02 for 367 tubules and 1.77 ±0.03 for 116 dogs. The mean values for an individual observer were not different from the overall means per tubule or per dog. A comparison of TF/P inulin ratios in 34 pairs of recollection/control distal tubule fluid during hydropenia yielded a mean $(TF/P)_2/(TF/P)_1$ of 1.00±0.06 (SE). In addition 56 pairs of proximal tubule recollection during saline infusion showed the corresponding mean of 1.00±0.01 (SE) and that of 19 pairs of distal tubule recollection in diuretic phase 0.99±0.05 (SE).

RESULTS

The effect of graded ECV expansion was studied in the proximal and distal tubule of 10 stage I, 11 stage II, and 7 stage III dogs. Three-phase recollection micropuncture experiments were carried out during hydropenia, followed by ECV expansion to 3% body weight and then to 10% body weight. An example of such experiments in stage II is shown in Table II. Micropuncture data were obtained from the remnant kidney while clearance data were from both the remnant and contralateral control kidneys. In this experiment, glomerular filtration rate (GFR) was reduced in the remnant kidney to about one fifth of the contralateral kidney. There was a progressive increase in fractional sodium and water excretion after graded ECV expansion with the values in the remnant kidney consistently higher than those in the contralateral kidney. The graded diuretic response in the final urine was generally reflected in progressive reduction in distal TF/P inulin ratios and a progressive rise in distal TF/P sodium. However, the effect on proximal tubule reabsorption was observed only after 3% ECV expansion and no further reduction in its reabsorption occurred after additional expansion to 10% body weight.

Clearance data. Mean clearance data in all three stages of dogs are summarized in Table III. In stage I, results are expressed as the mean of two kidneys and mean

TABLE I

Summary of Mean Last Proximal TF/P Inulin in Normal, Hydropenic Dogs Collected by Five Observers

Observer	$(TF/P)_2/(TF/P)_1$ inulin	Mean TF/P inulin per tubule	Mean TF/P inulin per dog
J. D.	0.97±0.01	1.83±0.07 (33)*	1.84±0.09 (16)‡
B. E.	0.98±0.03	1.76±0.04 (163)	1.75±0.04 (47)
R. E.	1.01±0.01	1.65±0.07 (20)	1.67±0.09 (9)
M. L.	1.01±0.03	1.75±0.05 (51)	1.81±0.09 (19)
N. W.	0.98±0.02	1.79±0.04 (100)	1.75±0.06 (25)
Pool data		1.77±0.02 (367)	1.77±0.03 (116)

* Number of tubules.

‡ Number of dogs.

TABLE II

Representative Micropuncture and Clearance Data in Stage II

Kidney	GFR			FEH_2O			FE_{Na}		
	H	3% E	10% E	H	3% E	10% E	H	3% E	10% E
	ml/min			%			%		
CK	37.7	39.8	34.7	3.6	17.3	24.3	2.6	9.5	17.6
RK	7.3	8.2	8.0	5.7	22.0	35.4	5.2	12.9	27.2

Sample	TF/P_{IN}			TF/P_{Na}		
	H	3% E	10% E	H	3% E	10% E
Proximal tubule						
1	2.12	1.84	1.83	0.96	0.93	0.92
2	1.85	1.66	1.64	0.97	0.96	1.01
Distal tubule						
3	3.55	2.53	2.93	0.27	0.31	0.64
4	3.34	3.22	2.30	0.33	0.32	0.32
5	2.62	2.98	2.13	0.16	0.22	0.21
6	3.39	2.42	2.30	0.15	0.36	0.50

Abbreviations: CK, contralateral kidney; 3% E, 3% extracellular volume expansion; 10% E, 10% extracellular volume expansion; FE_{H_2O}, fractional excretion of water; FE_{Na}, fractional excretion of sodium; H, hydropenia; RK, remnant kidney; TF/P_{IN}, tubule fluid-to-plasma inulin ratio; TF/P_{Na}, tubule fluid-to-plasma sodium ratio.

GFR in the three experimental phases were comparable at 30–32 ml/min. Mean fractional excretion (FE) of sodium increased progressively for 1.6 in hydropenia to 3.6% after 3% ECV expansion and to 9.6% after 10% expansion. The corresponding mean FE water in the three phases were 1.8, 5.7, and 12.9%, respectively.

TABLE III

Summary of Clearnace Data

Expt. stage	Kidney	Expt. phase	GFR	V	FE_{H_2O}	$U_{Na}V$	FE_{Na}
			ml/min	*ml/min*	%	*μeq/min*	%
I	Mean	H	32.0±2.1	0.6±0.2	1.8±0.4	83.5± 16.4	1.6±0.3
(10 dogs)		3% E	31.1±2.2	1.7±0.3*	5.7±0.9*	158.7± 35.2*	3.6±0.6*
		10% E	30.2±2.1	3.9±0.4*	12.9±1.0*	411.9± 71.0*	9.6±1.3*
II	CK	H	38.9±3.2	1.0±0.3	2.4±0.7	96.6± 22.6	1.6±0.4
(11 dogs)		3% E	37.5±2.8	3.0±0.9*	8.7±2.3*	251.6± 73.7*	5.0±1.7*
		10% E	33.6±1.1	4.9±1.1*	16.7±2.8*	505.4±136.3*	11.8±2.8*
	RK	H	7.1±0.6‡	0.2±0.03‡	3.1±0.8	18.1± 3.7‡	1.9±0.3
		3% E	7.1±0.8‡	0.9±0.2*‡	12.6±2.3*‡	77.6± 21.1*‡	7.5±1.6*‡
		10% E	7.9±0.8*‡	1.9±0.3*‡	24.2±2.5*‡	202.1± 46.1*‡	17.3±2.5*‡
III	RK	H	6.9±1.3	0.6±0.1§	9.0±1.2§	74.5± 27.9	5.3±1.0§
(7 dogs)		3% E	8.5±1.9*	1.4±0.3*	19.2±2.5*	131.1± 36.7*	9.9±1.8*
		10% E	9.3±2.0*	2.5±0.5*	31.8±3.5*	264.9± 67.1*	20.4±4.2*

Abbreviations: Expt. stage, experimental stage; Expt. phase, experimental phase; V, urine flow; $U_{Na}V$, urine Na excretion; others as in Table I.

Values are mean ± SEM.

* Significantly different from preceding phase.

‡ Significantly different from corresponding value in the contralateral kidney.

§ Significantly different from corresponding stage II value of the remnant kidney. GFR values in stage I dogs represent only those of the micropunctured kidney.

These changes were similar to those in FE sodium and indicate that graded ECV expansion results in progressively greater diuretic response in stage I.

In stage II, GFR of the contralateral kidney had a mean of 38.9 ml/min in hydropenia which was not significantly altered at 37.5 and 33.6 ml/min after graded ECV expansion. Mean GFR of the remnant kidney were unchanged at 7.1 ml/min after 3% ECV expansion but increased slightly to 7.9 ml/min with 10% expansion. These stage II animals were nonuremic with mean blood urea nitrogen (BUN) of 16 mg/100 ml, mean plasma creatinine 0.8 mg/100 ml and mean femoral arterial pressure of 112 mm Hg, which were similar to those of the normal animals. Mean FE sodium of 1.6, 5.0, and 11.8% and FE water of 2.4, 8.7, and 16.7% of the contralateral kidney in stage II were not significantly different from the corresponding values in stage I. However, these values of the remnant kidney in the three phases at 1.9, 7.5, and 17.3% for sodium and 3.1, 12.6, and 24.2% for water were significantly higher than those of the contralateral kidney, with the exception of the values in hydropenia which did not attain statistical significance. This relationship is shown in Fig. 1 where such a comparison of mean FE sodium and FE water between the two kidneys in stage II is made. These data indicate that disproportionately greater response to ECV expansion occurs in the functionally reduced kidney as compared with the contralateral control kidney.

In seven stage III dogs, 7 days after the removal of the contralateral kidney, mild azotemia developed with mean BUN of 49 mg/100 ml, mean plasma creatinine of 2.6 mg/100 ml, and mean femoral arterial pressure of 120 mm Hg. Some of the stage III dogs remained volume contracted despite infusion of Ringer's lactate 500 ml before the experiments and this was reflected in reduced mean sodium excretion rate ($U_{Na}V$) in hydropenia at 75 μeq/min as compared with the combined two kidney value of 176 μeq/min in stage I and 115 μeq/min in stage II. Mean GFR in the seven stage III dogs increased progressively from 6.9 to 8.5 and then to 9.3 ml/min during the three phases. Mean FE sodium and FE water in hydropenia were high at 5.3 and 9.0%, respectively, which were higher than the values in the remnant kidney of stage II, reflecting the salt-losing nature of the kidney with markedly reduced renal mass. After graded ECV expansion to 3% and 10% body weight, although mean FE sodium of 9.9 and 20.4%, and mean FE water of 19.2 and 31.8% in stage III were slightly higher than the corresponding values in the remnant kidney of stage II, the differences were not statistically significant. However, these values were significantly higher than those in stage I and those of the contralateral kidney in stage II, indicating enhanced response of the remnant kidney to graded ECV expansion in both stages II and III.

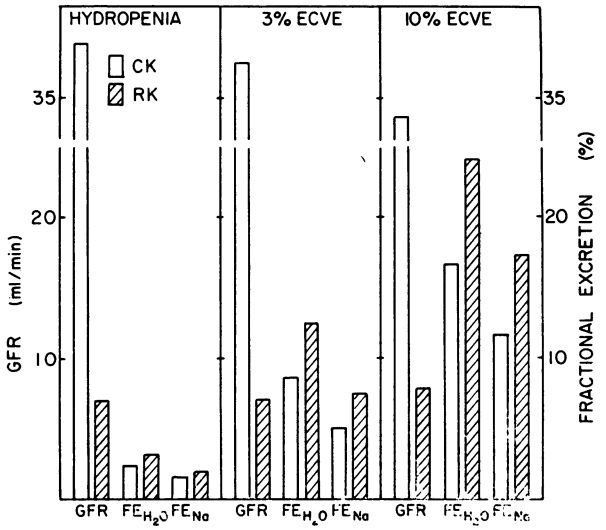

FIGURE 1 Comparison of GFR, and FE of water and sodium between contralateral kidney (CK) and remnant kidney (RK) in stage II. ECVE, extracellular volume expansion.

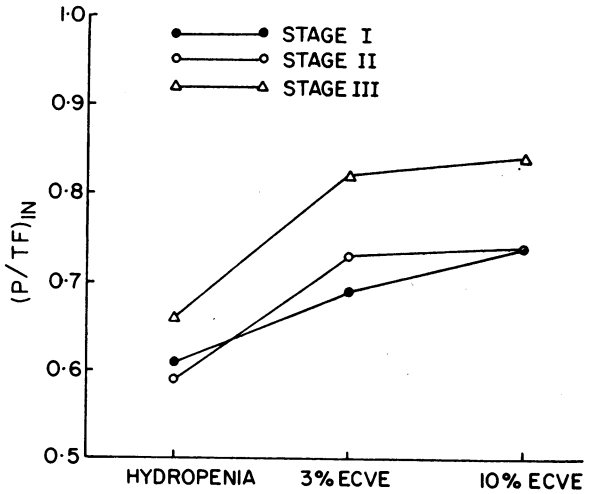

FIGURE 2 Effect of graded ECVE on the fraction of filtered water remaining in the proximal tubule in the three groups of dogs.

Micropuncture data. Mean micropuncture data in both proximal and distal tubules in all three stages of dogs are summarized in Table IV. In stage I, the mean proximal TF/P inulin was reduced from 1.67 in hydropenia to 1.49 after 3% ECV expansion, but did not change further at 1.40 after 10% expansion. A similar pattern of changes in mean proximal TF/P inulin was observed in stages II and III with the values in the three phases at 1.82, 1.41, and 1.36 for stage II, and 1.49, 1.23, and 1.22 for stage III. These changes in proximal tubule transport in response to graded ECV expansion are il-

TABLE IV

Summary of Micropuncture Data in Proximal and Distal Tubule

Expt. stage	Expt. phase	Proximal tubule		Distal tubule	
		TF/P_{IN}	TF/P_{Na}	TF/P_{IN}	TF/P_{Na}
I	H	1.67±0.09	1.00±0.03	3.94±0.30	0.19±0.02
(10 dogs)	3% E	1.49±0.09*	1.03±0.03	2.93±0.16*	0.26±0.03*
	10% E	1.40±0.10	1.03±0.04	2.41±0.14*	0.34±0.03*
II	H	1.82±0.11	0.97±0.02	4.26±0.55	0.23±0.04
(11 dogs)	3% E	1.41±0.09*	0.99±0.02	2.74±0.21*	0.32±0.04*
	10% E	1.36±0.07	1.00±0.02	2.28±0.22*	0.52±0.06*‡
III	H	1.49±0.09‡	0.99±0.02	2.77±0.25‡	0.25±0.05
(7 dogs)	3% E	1.23±0.06*	0.98±0.03	2.08±0.18*‡	0.30±0.03
	10% E	1.22±0.08	0.97±0.03	1.86±0.10*	0.53±0.03*

Abbreviations as in Tables I and II.
Values are mean±SEM.
* Significantly different from preceding phase.
‡ Significantly different from corresponding value in preceding stage.

lustrated in Fig. 2 where the fractions of filtered water remaining in the late proximal tubule were plotted. As can be seen, a rise in the remaining fraction was observed for all three stages of dogs after 3% ECV expansion, but relatively little further inhibition of fractional fluid reabsorption occurred with additional expansion to 10% body weight. It is also noticed that fractional reabsorption in the proximal tubule in stage III during hydropenia was reduced as compared to that in stage II, suggesting that removal of the contralateral kidney may alter proximal tubule transport. However, the difference in fractional reabsorption in the proximal tubule between stages I and III did not attain statistical significance. When mean stage III TF/P inulin in hydropenia were compared to the overall control mean of Table I, a highly significant difference was obtained ($P < 0.01$).

In the distal tubule, mean TF/P inulin fell progressively in all three stages after graded ECV expansion. These effects of 3% and 10% expansion on distal TF/P inulin in 11 stage II dogs are shown in Fig. 3, where the values of all samples are plotted. At both levels of ECV expansion, the majority of these points fall below the line of identity, indicating a reduction in fractional fluid reabsorption proximal to the distal tubule puncture site. Since the distal tubule in the dog is poorley permeable to water under most conditions (16), the change in fluid reabsorption must have occurred mainly in the loop of Henle. Mean distal TF/P inulin in hydropenia, and after 3% and 10% ECV expansion as shown in Table IV were 3.94, 2.93, and 2.41 for stage I and these were similar to those in stage II at 4.26, 2.74, and 2.28. However, mean distal TF/P inulin in stage III at 2.77, 2.08, and 1.86 were generally lower than the corresponding values in stages I and II, with significant differences in the first two phases. These data suggest slightly reduced fractional reabsorption of water along the loop

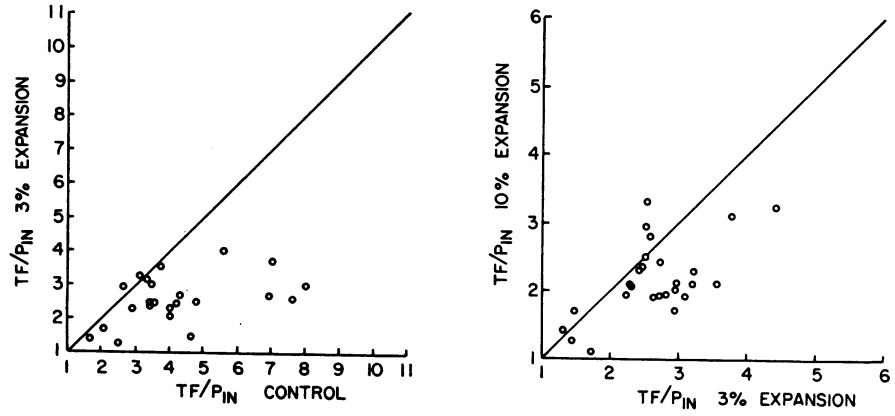

FIGURE 3 Effect of graded ECVE on distal TF/P inulin ratio in stage II.

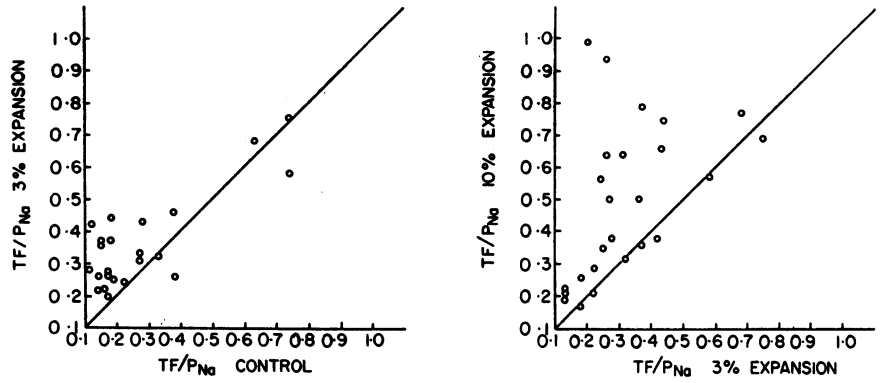

FIGURE 4 Effect of graded ECVE on distal TF/P sodium ratio in stage II.

of Henle in stage III, which probably reflects the similar reduction in fractional proximal tubule reabsorption in this group. Mean distal TF/P sodium increased progressively after graded ECV expansion in all three stages. These changes in stage II dogs are shown in Fig. 4 where distal TF/P sodium values of all samples are plotted. The majority of the points are scattered above the line of identity with both 3% and 10% expansion, indicating consistent rise in distal TF/P sodium after these experimental maneuvers. The magnitude of the rise in mean distal TF/P sodium was similar in all three stages after 3% ECV expansion, while after 10% expansion, its rise from 0.32 to 0.52 in stage II and from 0.30 to 0.53 in stage III was significantly greater than that in stage I from 0.26 to 0 34 (Table IV). In Fig. 5, mean fractions of filtered sodium remaining in the distal tubule and final urine were calculated and plotted. After graded ECV expansion greater rise in the distal tubule remaining fraction was observed in the remnant kidney in both stages II and III as compared to that in stage I. The sharp increase in the slope which occurred in stages II and III after 10% ECV expansion in the absence of additional reduction in proximal tubule reabsorption speaks strongly for its effect on reabsorption in the loop of Henle. Also, the slopes of increase in the remaining fraction in the urine in all three stages of animals were very similar to those in the distal tubule, suggesting that the enhanced natriuresis in the final urine was a result of greater reduction in reabsorption at a site proximal to the distal tubule.

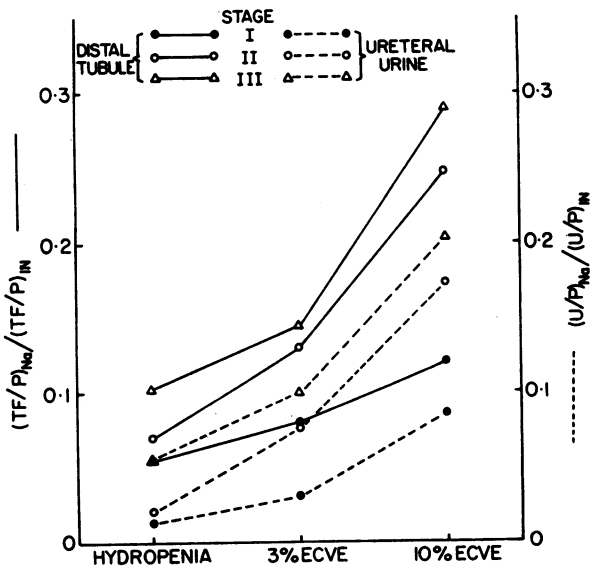

FIGURE 5 Comparison of the effect of graded ECVE on the fraction of filtered sodium remaining in the distal tubule with its effect on that in the ureteral urine.

Since these data are very suggestive of altered loop transport under our experimental conditions, mean fractional sodium reabsorption in the loop of Henle was calculated in terms of its segmental sodium load. This was obtained by extrapolation of our proximal tubule data, assuming the end proximal TF/P inulin in hydropenia to be 2.0 (14). These values are depicted in Fig. 6. During hydropenia, segmental fractional reabsorption of sodium in the loop in stage II was similar to or only minimally less than that in stage I, while the more prominent reduction in loop reabsorption in stage III was probably related in part to increased segmental load secondary to reduction in proximal tubule reabsorption in this group. After 3% ECV expansion, fractional loop reabsorption of sodium in both stages II and III was considerably less than that in stage I, while after 10% expansion this difference was markedly increased. The magnitude of reduction in fractional loop reabsorption in response to graded ECV expansion was similar for both stages II and III and roughly proportional to the level of expansion. A modest but progressive reduction in fractional loop reabsorption was also noticed in stage I after graded expansion. The similarity in the exaggerated loop response to ECV expansion between stage II and III remnant kidneys suggests that the altered response

is mainly due to reduction in functioning renal mass per se rather than the uremic environment.

DISCUSSION

Although altered renal transport of sodium in chronic renal failure is well known and has been the subject of intensive investgation (1–9, 17–19), the manner by which this change in renal handling of sodium takes place at various sites of the nephron has not been elucidated. Micropuncture studies performed in the past using various experimental models have centered mostly on the transport changes in the proximal tubule (5–8) and rarely in the distal segments (9). Bank and Aynedjian (7, 9) studied renal tubule transport in bilaterally pyelonephritic rats during osmotic diuresis and showed no significant difference in proximal tubule reabsorption between the diseased kidney and the normal kidney of the control rats, whereas fractional fluid reabsorption in the distal tubule was significantly reduced in the former. Hayslett, Kashgarian, and Epstein (8), using the shrinking drop technique in partially nephrectomized rats, also found no change in reabsorptive half time in the proximal tubule. Lubowitz, Purkerson, Rolf, Weisser, and Bricker (6), on the other hand, observed reduction in proximal tubule bicarbonate reabsorption in uremic, pyelonephritic rats but failed to demonstrate changes in proximal tubule fluid reabsorption. However, reduction in the latter parameter was shown in the remnant kidney of saline-infused uremic rats on high salt diet (5). The difficulty associated with these micropuncture studies on renal tubule transport in chronic experimental models lies in the fact that comparison of transport changes has to be made in different groups of animals. Since considerable variation in transport patterns within the same group of animals frequently exists, it is rather difficult to demonstrate statistically significant differences in transport parameters between different experimental groups except with large population groups. In our experiments, the three groups of animals were challenged with graded ECV expansion in order to create greater differences in renal sodium transport for comparison. This effect was observed in the remnant kidney of both stages II and III which showed greater tubule response to ECV expansion as compared to that of stage I. Another problem associated with the study of renal sodium transport in uremic animals is the tendency for these animals to become volume contracted as a result of poor dietary sodium intake and renal sodium wasting, and correction for this ECV contraction may not be carried out with ease. In our studies, although an attempt was made to restore ECV in stage III dogs by infusion of Ringer's lactate solution before the experiments, some of these animals probably remained volume contracted since mean absolute rate of urinary sodium excretion was less than that in stages I and II. Theoretically, if the sodium intake remains constant, reduction of renal mass should result in reduced sodium excretion and retention of sodium until ECV is expanded and/or an adaptive change in fractional sodium reabsorption by the remnant kidney leads to a new state of sodium balance. In our stage III animals, it is likely that sodium intake was lower than that in stages I and II, resulting in relatively less excretion of sodium. Therefore, our results demonstrating significant reduction in fractional proximal tubule reabsorption in hydropenic stage III dogs as compared to that in stage II, but not in stage I, should be regarded as only suggestive evidence for such a change in sodium transport in this segment. However, an equal sodium intake in stage III as in stages I and II would only augment and not reduce the difference in proximal tubule transport demonstrated in our studies.

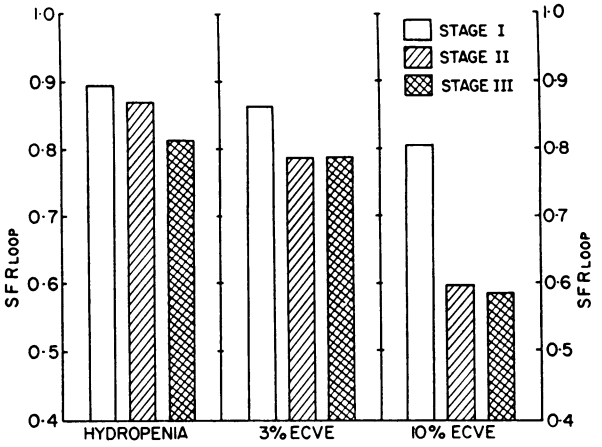

FIGURE 6 Corrected segmental fractional reabsorption of sodium in the loop of Henle (SFR_{Loop}) before and after graded ECVE.

There is additional information which further support our observation on the present studies. In another series of micropuncture experiments on the transport of remnant kidney in the dog, we have shown a similar reduction in fractional proximal tubule reabsorption in stage III even though no correction for fluid deficit was made in these animals (20). The fact that reduced fractional proximal tubule reabsorption could be demonstrated in more severely volume contracted animals in stage III further strengthens the conclusion derived from our observation. Furthermore, the base line mean TF/P inulins of both series in stage III are highly significant when compared to the larger series of control values shown in Table I. Also, Dirks, Wong, and Evanson (21) have demonstrated a reduction of similar order of magnitude in proximal tubule reabsorption in the remnant kidney of stage II dogs immediately after clamping of the contralateral renal artery. Since our stage III dogs also underwent nephrectomy of the contralateral

kidney, the reduced fractional proximal tubule reabsorption observed in stage III in the present studies is consonant with the findings after contralateral renal artery clamping. All these observations support the theory extended by Bricker (22) that proximal tubule sodium reabsorption is reduced when functioning nephron population is greatly diminished.

Although the mechanism by which reduction in fractional proximal tubule reabsorption may occur in the kidney with reduced renal mass is entirely unknown, certain factors should be taken into consideration. First, it is possible that an adjustment in renal hemodynamics may occur in the reduced kidney, leading to the relative increase in the perfusion of each nephron and this may result in changes in physical factors within the peritubular capillaries of the superficial proximal tubule in favor of reduction of fractional reabsorption (23, 24). However, such a hemodynamic alteration within the cortex of stage III remnant kidney has not been convincingly demonstrated.[2] Second, adaptive increase in GFR per nephron in stage III (1, 22) may result in reduced fractional proximal tubule reabsorption if the absolute reabsorption remains unchanged. However, if the maintenance of glomerulo-tubule balance is an inherent nature of the proximal tubule under the normal conditions, then the observed fall in fractional reabsorption even with unchanged proximal reabsorption still implies a deviation from the expected behavior of this nephron segment under the experimental conditions. Moreover, evidence in the literature (25) suggests that increased GFR per nephron cannot be solely responsible for the observed reduction in fractional sodium reabsorption in stage III animals. Further studies are necessary to examine the changes in single nephron filtration rate in chronic reduction of renal mass. Third, Bricker (22) has postulated that a natriuretic humoral factor exists in uremic plasma that acts directly in the proximal tubule to inhibit sodium reabsorption. Although our present studies do not provide information as to the significance of such a humoral substance, the finding of diminished proximal tubule reabsorption in the remnant kidney immediately after clamping of the contralateral renal artery (21) may be explained by alternative mechanisms. Since these dogs were not uremic immediately after contralateral "nephrectomy," the observed reduction in proximal tubule reabsorption could not be related to uremia per se but rather to certain as yet unidentified intrarenal mechanism(s). However, it remains possible that the reduction of fractional proximal sodium reabsorption observed in both acute and chronic reduction in nephron mass is not mediated by the same factors. The role of elevated plasma urea in our stage III dogs must then be evaluated. Kauker, Lassiter, and Gottschalk (26) observed no reduction in fractional proximal water reabsorption in the rat when plasma urea was elevated to 39 mM and care was taken not to expand ECV. Nevertheless, we observed recently that fractional proximal sodium reabsorption was reduced after acute infusion of urea in normal dogs in which volume expansion was rigorously avoided.[3] Additional stage III experiments assessing the effect of restoring plasma urea concentration to normal on proximal reabsorption may be necessary to clarify this point.

It is of interest that graded ECV expansion to 3% and 10% of body weight in both normal and remnant kidneys did not result in progressive reduction in fractional proximal tubule reabsorption, and the greater natriuresis observed after 10% ECV expansion was largely due to its additional effect in the distal nephron since no further response was observed in the proximal tubule. The demonstration by Davis, Walters, and Murdaugh (27) in the dog that the magnitude of reduction in proximal tubule reabsorption remained constant at various levels of ECV expansion is consistent with our findings. On the other hand, micropuncture study in the rat by Brenner and Berliner (28) showed progressive response in proximal tubule reabsorption to graded ECV expansion. Nevertheless, the magnitude of natriuresis observed in the final urine with higher levels of ECV expansion could not be accounted for solely by its effect on the proximal tubule, also suggesting a distal site of inhibition of sodium transport. Increasing evidence in the literature supports the concept that sodium transport at a distal site, especially in the loop of Henle, is important in determining the degree of natriuresis that occurs in the final urine. Thus, inhibition of proximal tubule reabsorption alone by intravenous infusion of hyperoncotic albumin and dextran did not result in significant natriuresis (29). The fact that almost all of the diuretics studied by micropuncture techniques have their site of action in the loop of Henle (14, 15, 30, 31) also underscores the important role of the distal nephron in producing diuresis. In their clearance study in the dog during hypotonic saline infusion, Eknoyan, Suki, Rector, and Seldin (32) concluded from the changes in free water clearance that massive expansion of ECV inhibits sodium reabsorption in the ascending limb of Henle's loop. Although this has not been supported by microperfusion studies in the rat (33, 34), our findings that additional reduction in fractional sodium reabsorption occurred in the distal nephron, especially in the loop, in re-

[2] Carriere, S., N. L. M. Wong, and J. H. Dirks. Redistribution of renal blood flow in acute and chronic reduction of renal mass. *Kidney Int.* In press.

[3] Edwards, B. R., A. Novakova, R. A. L. Sutton, and J. H. Dirks. Micropuncture study of effects of acute urea loading on proximal tubular reabsorption in the dog kidney. *Am. J. Physiol.* In press.

sponse to 10% ECV expansion in the dog is consistent with their observation. The modest reduction in fractional loop reabsorption in the normal kidney after massive ECV expansion in our studies was further supported by our observation of marked depression of sodium reabsorption in the remnant kidney with similar expansion. This reduction in fractional loop reabsorption of sodium could be due to direct effect of ECV expansion or secondary to increase in sodium delivery to the loop thereby exceeding its reabsorptive capacity. The fact that fractional loop reabsorption was reduced progressively with graded ECV expansion, while no additional effect was observed in the proximal tubule speaks for its direct effect on loop transport. Although the small increase in the remnant kidney GFR which occurred after 10% ECV expansion cannot account for the rather dramatic change in fractional loop reabsorption, the possibility of a greater increase in GFR of the superficial nephrons leading to increased sodium delivery to the loop without change in proximal tubule fractional reabsorption cannot be entirely excluded. Functional heterogeneity between the superficial and deep nephrons has been observed in the rat (35, 36) but inconsistent results have been reported in the dog (37–39). Also, an increase in superficial nephron GFR after ECV expansion has not been clearly documented in the dog (37). In view of the technical difficulties involved in determination of single nephron GFR (40), the question of whether ECV expansion exerts direct effect on loop sodium reabsorption must await further study with careful measurement of this parameter. Whatever the mechanism involved, our observation of reduced fractional loop reabsorption after massive ECV expansion strongly supports the concept that sodium transport in the loop of Henle plays an important role in determining the magnitude of natriuresis induced under various diuretic conditions.

The enhanced response of the remnant kidney to ECV expansion in the absence of uremia is of significance since the altered renal transport of sodium in chronic renal disease has generally been attributed to the effect of uremia (1, 2, 18). The observation by Gutmann and Rieselbach (4) that a greater increase in fractional sodium excretion after ECV expansion occurred in the pyelonephritic or remnant kidney in stage II dogs is consistent with our clearance data in the same stage. These observations indicate that functional alteration of chronic renal failure in regard to its exaggerated response to ECV expansion is attributable, at least in part, to reduction in functioning renal mass rather than solely to uremia. Since our graded loading studies in the normal dog suggest that massive ECV expansion enhances sodium excretion by further depression of fractional reabsorption in the loop of Henle, it is not surprising that the exaggerated response of the remnant kidney also occurred in this segment. The magnitude of reduction in fractional loop reabsorption of sodium corrected for its segmental load was comparable in the remnant kidney regardless of whether the contralateral kidney was removed, indicating similar nature of altered sodium transport in the loop of Henle. Therefore, the seemingly greater natriuretic response of the remnant kidney in stage III than that in stage II was, although not statistically significant, more likely the result of reduced proximal tubule reabsorption or increased GFR per nephron (1) or both in stage III animals.

The mechanism by which the remnant kidney responds to ECV expansion with greater fall in fractional loop reabsorption is not clear since little is known about factors which modulate sodium reabsorption in this segment. However, evidence in both clearance and micropuncture studies (41–43) indicates that sodium reabsorption in the loop is reduced when perfusion pressure of the kidney is increased by systemic hypertension. These observations suggest that, as in the proximal tubule (23, 24), physical factors in the peritubular capillaries also affect loop reabsorption. The changes in physical factors in the peritubular capillaries of the Henle's loop cannot be critically estimated without direct measurement and any discussion of the possible role of these factors in enhanced response of the remnant kidney to ECV expansion remains only speculative. Although the blood pressure in both stage II and III dogs was not elevated in our studies, peritubular capillary pressure of the loop may be increased in the remnant kidney if medullary blood flow is increased in the presence of elevated interstitial pressure after ECV expansion. The small size of the remnant kidney surrounded by firm, infarcted tissue may greatly impair its distensibility in response to ECV expansion, resulting in greater increase in intrarenal pressure. Also, redistribution of intrarenal blood flow in the remnant kidney in favor of enhanced medullary blood flow may occur and contribute to the changes in physical factors due to ECV expansion. Further studies with precise measurement of these parameters are required to clarify the possible mechanism of functional changes in the remnant kidney in response to ECV expansion.

It was also noted in the present studies that fractional reabsorption of sodium in the loop of Henle during hydropenia was less in stage III as compared to that in stages I and II. This finding is of interest since functional adaptation in renal handling of sodium in chronic renal failure has also been suggested to occur in the distal part of the nephron (8, 9). Although the reduced proximal tubule reabsorption as well as increased GFR per nephron (1, 22), which may both occur in chronic renal failure, may well contribute to the change in fractional

loop reabsorption observed in our hydropenic stage III study, its similarity to the pattern of transport changes which occurred after ECV expansion leads one to suspect that a direct inhibition of loop reabsorption was also involved. If this was the case, extrarenal factors are also likely to be responsible for the reduction in loop reabsorption in hydropenic stage III dogs, as a negligible change was found at this site of the remnant kidney in stage II. In this connection, azotemia may play a role for the functional alteration in the loop since infusion of urea without significant ECV expansion has been observed to inhibit loop as well as proximal tubule reabsorption.[3] The possibility that a natriuretic humoral factor (44) may be implicated in inhibtion of loop reabsorption also cannot be excluded. Clearly, the precise role of various extrarenal as well as intrarenal factors in functional alteration of chronic renal failure remains to be elucidated.

ACKNOWLEDGMENTS

The authors wish to express gratitude to Mrs. Christiane Schwab-Manzini, Mrs. Diane Carbonneau, Mrs. Miriam Satin, and Mrs. Erika Kurlents for their expert technical assistance.

This work was supported by grants from the Medical Research Council (Canada) MA-1915.

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