Effects of Acute Volume Expansion and Altered Hemodynamics on Renal Tubular Function in Chronic Caval Dogs

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ABSTRACT It is well established that dogs with chronic partial constriction of the thoracic inferior vena cava develop sodium retention, ascites, and respond poorly to acute saline loading. A group of such chronic caval dogs, and a group of normal controls were studied during hydropenia, and again after acute saline loading by clearance and recollection micropuncture techniques. After volume expansion, the caval dogs excreted 52 μEq/min per kidney of sodium compared with 370 μEq/min per kidney for the normal controls. During hydropenia and after the saline infusions, single nephron filtration rates, fractional reabsorption of sodium within the proximal tubule, and proximal delivery of filtrate to the distal nephron were comparable in both groups of dogs.

Micropuncture of distal tubular segments confirmed that the loop of Henle was the major site for salt and water retention in the expanded caval dogs. Alteration of intrarenal hemodynamics by vasodilating one kidney and elevating systemic arterial blood pressure induced a normal natriuretic response in the saline-loaded caval dogs. Proximal tubular function remained unchanged and the loop of Henle appeared to be the major site responsive to these hemodynamic maneuvers. These same experiments in saline-loaded control dogs had no effect on function of the proximal or distal nephron and did not increase urinary excretion of sodium or water.

These experiments provide evidence that the loop of Henle is the major site for sodium retention in volume-expanded chronic caval dogs excreting minimal amounts of sodium.

INTRODUCTION

Normal dogs respond to acute volume expansion of the extracellular fluid (ECF)[1] with an immediate brisk natriuresis (1). This increased renal excretion of sodium is largely mediated through inhibition of fractional sodium reabsorption in the proximal tubule (2). Dogs with partial, chronic constriction of the thoracic inferior vena cava (chronic caval dog) develop salt and water retention, edema and ascites, and usually respond to acute saline loading with only, at best, a feeble natriuretic response (3, 4). It has generally been felt that the minimal sodium excretion observed in chronic caval dogs under conditions of acute saline loading is due to maintenance of sodium reabsorption in the proximal tubule (5).

The chronic caval dog has been widely used by Davis and associates (3, 6–8) as a reasonable model in which to study the pathophysiology of edema. These authors, and others (4, 9) have demonstrated that the development of sodium retention in this preparation cannot be correlated with depression of glomerular filtration (GFR) or renal plasma flow (RPF), increased renal venous pressure, altered renal nerve activity, or increased secretion of antidiuretic hormone (ADH) or adrenal cortical hormones. It has therefore been suggested that the sodium retention and the failure to respond to acute ECF volume expansion is due either to the elaboration of a nonaldosterone salt-retaining substance, or some

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[1] Abbreviations used in this paper: C_{in}, inulin clearance; ECF, extracellular fluid; GFR, glomerular filtration rate; P_K, plasma potassium concentration; P_{Na}, plasma sodium concentration; RPF, renal plasma flow; TF/P, tubular fluid/plasma ratio; U_K, urinary potassium concentration; U_{Na}, urinary sodium concentration; V_o, single nephron filtration rate.

subtle alteration in intrarenal physical forces which favors increased tubular transport of sodium (8, 10). These mechanisms were thought to be operative at the level of the proximal tubule (5).

The micropuncture experiments which demonstrated that the failure of caval dogs to excrete sodium in response to acute volume expansion was due to maintenance of fractional sodium reabsorption in the proximal tubule were carried out in dogs with acute rather than chronic constriction of the thoracic vena cava (5). Under these conditions, the major changes in systemic hemodynamics, and severe depression of GFR which occurred could easily have explained the observed data. Indeed, recently Auld, Alexander, and Levinsky (11) have demonstrated in the acute caval dog, that the earlier data reported by Cirksena, Dirks, and Berliner (5) were probably due to filtration effects. Auld et al. (11) have also demonstrated, by micropuncture techniques, that the proximal tubule of both the acute and chronic caval dog responds normally to volume expansion with depression of fractional salt and water transport and have inferred that in chronic caval dogs, the blunted natriuresis is due to sodium retention at a distal nephron site.

This present study was undertaken to clarify with the aid of micropuncture techniques, the functional profile of the proximal and distal nephron in chronic caval dogs with regard to sodium reabsorption.

METHODS

Acute experiments were performed on 18 control and 17 experimental dogs weighing between 14 and 24 kg and anesthetized with sodium pentobarbital (Nembutal). All animals were deprived of food and water overnight and received 10 mg desoxycorticosterone acetate (DOCA)[2] and 5 U Pitressin Tannate in oil by intramuscular injection on the morning of each experiment. The 17 experimental dogs were made to retain sodium and develop ascites by partially constricting the thoracic inferior vena cava with stout umbilical tape through a right thoracotomy incision under sterile conditions. When these animals had recovered from the surgery and developed palpable ascites some 3–20 days later, they were then subjected to experimentation. The average time from surgery to acute experiment was usually 5–7 days. In earlier experiments, sham-operated controls were used, but these did not differ from unoperated controls which were used in the later experiments. Both control and chronic caval dogs were kept in similar quarters and fed the same diet. Both groups were prepared for experiments in the same manner. All animals were intubated with cuffed endotracheal tubes and when necessary ventilated with a Harvard respiratory.[3] Two polyethylene catheters were inserted into the abdominal aorta through femoral arterial cannulations. One was connected to a mercury manometer and used to record blood pressure; the other was positioned above the renal arteries and used for periodic injections of lissamine green.

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[2] Kindly supplied as Percorten by Dr. A. Wassef, CIBA Company Ltd., Dorval, Quebec.

[3] Harvard Apparatus Co., Inc., Millis, Mass.

Through flank incisions, each ureter was cannulated to the level of the renal pelvis. Arterial blood samples were collected from a cannula placed in one carotid artery. Polyethylene catheters were placed into the jugular vein for the infusion of inulin and p-aminohippuric acid (PAH) into the brachial vein for the infusion of Ringer's solution, and into the abdominal vena cava to measure venous pressure at the level of the renal veins by saline manometry. A rectal thermometer was used to monitor body temperature, and when necessary the dogs were warmed with a heating pad. All infusions were administered with constant infusion pumps. Inulin and PAH were used as indices for glomerular filtration rate and cortical plasma flow respectively. These were given in water at 0.5 ml/min, at suitable concentrations so that after an appropriate prime, plasma inulin concentration was kept at 50–80 mg/100 cm^3, and plasma PAH concentration at 1.5–2 mg/100 cm^3. At least 45 min was allowed for equilibration. Clearance collections were generally from 15–40 min in duration during hydropenia and somewhat shorter after volume expansion, but never less than 6 min. Heparinized arterial blood samples were taken at the midpoint of each clearance period and immediately centrifuged to harvest the plasma. At least three clearance periods were taken during each phase of the experiment.

Micropuncture techniques. The left kidney was mobilized and prepared for micropuncture through a left flank incision by conventional techniques. The decapsulated area was kept covered with mineral oil at 37°C. Injections of 0.3–0.8 ml of 10% lissamine green were used to identify surface end-proximal tubules and distal tubules. Tubules to be punctured were marked with 1% nigrosine so they could be repunctured. The recollection micropuncture technique was used exclusively during these studies (2). An effort was made to puncture only end-proximal tubules. Sharpened micropipettes (o.d. = 7–15 μ) were placed into the tubules in as shallow an angle as possible and kept carefully centered. A large oil block was injected into the tubule and collection of fluid was made keeping the oil column stationary just downstream to the pipette tip. Where collection of a tubular sample required excess aspiration on the syringe, the sample of fluid was rejected. After collection of the sample, the pipette tip was blocked with a large column of mineral oil saturated with water, and the fluid inspected for red cells under the microscope. Most samples were usually cell-free, while occasionally a sample would contain just a few cells. If red cells were present to excess (more than five to six in the fluid sample) the collection was rejected. All samples were stored at 4°C and analyzed for inulin within 48 hr. Storage for this length of time did not interfere with inulin recovery or analysis. Because of their marked transparency, distal tubules were punctured while they still held lissamine green, but no collection was started until the column of dye was well out of the tubule. Single nephron filtration rate (V_o) was measured by collecting a timed sample of tubular fluid in the collecting pipette and measuring the volume in a calibrated, constant-bore, capillary glass tubing filled with silicone fluid. V_o was calculated by measuring the rate of flow of fluid into the collecting pipette and multiplying this value by the TF/P inulin (tubular fluid/plasma) ratio for the specific sample.

Proximal tubular transit time was taken from the capillary flush after a lissamine green injection until one tubule remained filled about a capillary cluster. Loop of Henle transit time was taken as the difference between the time taken for lissamine green to reach the distal tubules and the proximal tubular transit time. Tubular pressure was measured with

micropipettes (o.d. = 10–15 μ) connected to a water manometer. Pipette capillarity was measured in a pool of saline on the kidney surface with the manometer zeroed. Such values were almost always less than 1–2 cm H_2O. Pipettes with values greater than this were discarded. The pipettes were filled with 2% lissamine green, and the end point was taken when dye neither entered the tubule, nor fluid entered the pipette. Measurements were repeated three times in each tubule and were reproducible to within 0.5 cm H_2O. Values between tubules usually agreed to within 1–2 cm H_2O. No pipette was used to measure pressure more than three times.

A SAM-7BW pressure microtransducer [4] was inserted between the renal capsule and parenchyma well away from the puncture site. The subcapsular pressure so recorded on a Gilson two-channel polygraph was used as an index of renal interstitial pressure (12). The transducer sensitivity was such that changes of pressure of the order of 1 mm Hg could be detected. Reproducibility of readings was ±0.25%.

Experiments were performed according to two different protocols. (*a*) 12 control and 12 caval dogs were studied during hydropenia and again after volume expansion, with Ringer's solution. This solution had the following composition: Na^+, 143 mEq/liter; Cl, 119 mEq/liter; HCO_3^-, 24 mEq/liter; K^+, 3.7 mEq/liter; osmolality, 280 mOsm/kg. The infusions were warmed to body temperature before administration. Solution was infused at about 30 ml/min until 7–8% of the body weight had been administered. This usually took about 45 min; the rate was then decreased so that it equaled or was just above the urine flow rate (usually 4–6 ml/min). After a period of stabilization, clearance periods were begun; and micropuncture measurements repeated.

In the 12 dogs of each group, proximal tubules were always punctured. Only in eight control dogs and seven caval dogs were punctures of the distal tubule also carried out. (*b*) Six controls and five chronic caval dogs were prepared as usual, but in addition a needle was placed into the left renal artery and kept open with normal saline at 0.5 ml/min. These animals were first studied after volume expansion. After control measurements, acetylcholine bromide in saline was infused in suitable concentration at 0.5 ml/min to deliver 40–60 μg/min into the left renal artery. In control dogs, noradrenaline was infused at 4–20 μg/min to elevate arterial blood pressure some 15–40 mm Hg above base line values. While the left kidney was being dilated and with the blood pressure elevated, clearance and micropuncture measurements were repeated. Ringer's solution was continually infused throughout the entire experiment. In caval dogs, the same procedure was followed, except in sequential fashion so that studies could be made during the acetylcholine only, and acetylcholine and noradrenaline periods separately.

Analyses. Inulin in urine and plasma were measured by an anthrone method (13). Inulin in tubular fluid was measured by the microfluorometric technique of Vurek and Pegram (14) modified for 10 min boiling time.

Na^+ and K^+ were measured on a flame photometer with internal lithium standard. PAH was measured in earlier studies by the method of Smith, Finkelstein, Aliminosa, Crawford, and Graber (15), and in later experiments by the Autoanalyzer methods. Both techniques agree to within 3–5%.

Plasma proteins were measured by hand refractometry. Hematocrit readings were done on an Adams microhemato-

crit centrifuge (Readacrit).[5] Sodium in distal tubular samples were measured on a helium glow spectrophotometer.[6] The tubular samples were read within 24 hr, but no difficulties were encountered as long as the fluid was protected adequately at each end of the pipette with large oil blocks. To confirm the accuracy of these readings, 13 NaCl solutions were prepared (0.3–0.9%) in both the isotonic and hypotonic range and the Na^+ concentrations read by macroflame photometry. Portions of these samples were then aspirated into oil-filled pipettes and treated identically with tubular fluid samples. These portions were then analyzed within 24 hr by the helium glow spectrophotometer, and the values expressed as a ratio (helium glow/flame photometry). For the 13 samples the mean ratio was 0.995 ±0.01 (SEM). 11 samples of proximal tubular fluid kept for 24 hr had a mean TF/P Na^+ ratio of 1.000 ±0.01 (SEM).

Calculations. Clearances were calculated by standard formulas. Plasma sodium values were those of the period when determining TF/P Na^+ ratios, but plasma inulin values were interpolated graphically to time of puncture to determine TF/P inulin ratios. No Donnan correction was used in calculation of filtered loads of Na^+. Means for each parameter are means for individual clearance periods or tubules, except for Tables V–VIII where group means are calculated from the means from each dog. In fact there is no significant difference no matter how these results are expressed.

The *t* test is used for determining levels of significance. Only paired repunctures have been used in calculating the data.

RESULTS

The data recorded in this study compares 18 control dogs in whom acute saline loading induced a marked natriuretic response with 17 chronic caval dogs in whom comparable volume expansion induced only minimal increments in sodium excretion.

All chronic caval dogs used in these experiments had sodium retention and ascites. The mean abdominal vena caval pressure was 18.1 ±0.09 (SEM) cm H_2O compared with 4.9 ±0.24 cm H_2O for the control animals.

Plasma and urine composition. Table I summarizes data from 12 control and 12 chronic caval dogs studied during hydropenia. Although the mean plasma sodium concentration is significantly lower in the caval dogs reflecting the tendency to internal dilution, this lower value is due to mainly three or four dogs where the P_{Na^+} was of the order of 114–120 mEq/liter. Many of the chronic caval animals had P_{Na^+} values in the normal range. A similar situation holds true for the hematocrit values. The plasma protein concentration cannot be used as an acurate index for plasma volume expansion since the ascitic fluid generally contained a great deal of protein (2.5–3.5 g/100 ml).

The tendency for the chronic caval dog to retain so-

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[4] Scientific Advances, Inc., Columbus, Ohio.

[5] Clay Adams, division of Becton, Dickinson & Co., Parsippany, N. J.
[6] I am indebted to Dr. E. A. Lockhart and Dr. J. H. Dirks for these determinations.

Table I

Summary of Plasma and Urine Composition in Hydropenia

	P_{Na}**	P_K*§	PCV‖	Plasma protein	U_{Na}‡	U_K¶	$\dfrac{U_K}{U_K + U_{Na}} \times 100$
	mEq/liter	mEq/liter	%	g/100 ml	mEq/liter	mEq/liter	%
Control dogs	146.5 ±1.01	3.36 ±0.08	48.9 ±1.2	5.76 ±0.15	76.6 ±9.7	106.4 ±8.9¡	58.2 ±5.1
Caval dogs	139.6 ±2.5	3.59 ±0.14	42.6 ±0.96	4.50 ±0.12	12.0 ±2.05	75.9 ±10.8	86.4 ±3.6
Probability values	$P < 0.01$	NS	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.05$	$P < 0.01$

* P_{Na}, Plasma sodium concentration.
‡ U_{Na}, Urinary sodium concentration.
§ P_K, Plasma potassium concentration.
‖ PCV, Packed cell volume.
¶ U_K, Urinary potassium concentration.
Values are mean ± 1 SEM.

dium was highlighted by the very low urinary sodium values often of the order of less than 1 mEq/liter.

The ratio $U_K/U_K + U_{Na}$ has been used as a measure of Na^+-K^+ exchange at a distal nephron cation exchange site (16). Use of this approximation indicates the degree of augmented sodium transport at this site in chronic caval dogs.

Effects of acute volume expansion—clearance data. Table II summarizes clearance data obtained during hydropenia and again after volume expansion. There was no significant difference between experimental (left) and control (right) kidneys for both groups of dogs during either phase of the experiment. Thus, for each of the variables listed, mobilization of the left kidney for micropuncture did not compromise its function.

The arterial blood pressure was significantly lower in caval dogs during hydropenia ($P < 0.05$) and did not

change in response to saline loading. In control dogs, blood pressure rose significantly by 12.7% in response to volume expansion. Since caval dogs required about 50–60% of the anesthetic dose of pentobarbital used for induction in the control group, the possibility remains that the recorded values in caval dogs do not truly represent the state of peripheral hemodynamics, but rather may be influenced by the level of anesthesia.

During hydropenia, rates of urine flow were significantly higher ($P < 0.05$) in caval dogs although sodium excretion was significantly reduced ($P < 0.01$). After volume expansion, both water and sodium excretion were significantly reduced in chronic caval dogs ($P < 0.01$).

For each group of dogs in hydropenia, there was no statistical difference in the values of GFR and C_{PAH}. Neither of these two parameters changed significantly

Table II

Summary of Blood Pressure and Clearance Data during Hydropenia and Volume Expansion

Group	Blood pressure	V*		$U_{Na}V$‡		C_{IN}§		C_{PAH}‖		F. F.¶	
		**E	C‡‡	E	C	E	C	E	C	E	C
	mm Hg	ml/min		µEq/min		ml/min		ml/min			
Controls n = 12		0.24	0.33	18.5	24.3	35.2	34.4	100.2	95.7	0.35	0.36
Hydropenia	102 ±3.3	±0.02	±0.06	±2.9	±3.4	±1.4	±1.5	±4.4	±3.8	±0.01	±0.01
Volume expansion	115 ±3.9	2.72	2.66	370	340	36.4	36.5	105.1	109.8	0.35	0.33
		±0.43	±0.31	±42.8	±31.3	±1.8	±1.7	±6	±9.4	±0.02	±0.03
Chronic caval dogs n = 12		0.42	0.50	3.0	4.1	33.8	35.5	91.6	93.2	0.37	0.38
Hydropenia	92.8 ±2.3	±0.08	±0.09	±0.60	±0.65	±2.6	±2.4	±8.1	±6.5	±0.01	±0.02
Volume expansion	92.5 ±2.5	1.62	1.34	52.7	63.3	32.2	36.2	82.6	83.5	0.39	0.43
		±0.09	±0.14	±8.3	±8.3	±2.2	±2	±7.8	±6.2	±0.01	±0.03

* V, Urinary flow rate; ‡ $U_{Na}V$, urinary sodium excretion; § C_{IN}, inulin clearance; ‖C_{PAH}, PAH clearance; ¶ F. F., filtration fraction; ** E, experimental (left kidney); ‡‡ C, control (right) kidney.
Values are mean ± 1 SEM.

in response to acute saline loading within each group. However, the tendency for C_{PAH} to fall with volume expansion in the caval dogs, made this parameter significantly lower in these dogs in this phase of the experiment in comparison with the control group ($P < 0.05$). This feature caused the filtration fraction to be significantly higher in caval dogs during volume expansion ($P < 0.05$). This increased filtration fraction however, was not translated into an increase in peritubular colloid osmotic pressure for the caval dog, since using Bresler's formula (17) and the appropriate plasma protein values, the efferent arteriolar protein concentration was calculated to be 5.22 g/100 ml for caval dogs, and 5.70 g/100 ml for the control group during acute volume expansion.

The degree of volume expansion was similar for both groups of dogs. Using the fall in hematocrit as an index of plasma compartment expansion, this parameter fell 22.5% in control dogs and 21% for caval dogs.

Micropuncture data: proximal tubules. As a validation of the recollection technique, 14 proximal tubules were punctured in four dogs during hydropenia. The ratio of the second puncture to the first was 0.997 ±0.01 (SEM). In mobilizing the kidney for micropuncture in caval dogs with ascites, small amounts of fluid were often lost from the peritoneal cavity during the dissection. To assess whether dehydration was occurring in the caval dogs as compared with the controls, hematocrits taken immediately after anesthetic induction and insertion of an arterial cannula were compared with hematocrit values taken during the first clearance period after the com-

pletion of surgery. In control dogs the ratio of the second hematocrit to the first was 1.07 ±0.02 and 1.06 ±0.03 for the caval dogs. These ratios are not significantly different from each other or from unity. Similarly, there was not difference in changes in plasma proteins before and after surgery. Table III summarizes the response of the proximal tubule to volume expansion. There is no significant difference in fractional reabsorption or superficial nephron filtration rate in both groups of drugs during hydropenia. Accordingly, there was no difference in distal delivery of filtrate in either group of dogs.

After volume expansion, TF/P inulin fell significantly ($P < 0.05$) in each group of dogs, but there was no significant difference in this variable between the two groups. In each group, saline loading caused marked increases in V_o ($P < 0.05$); 66% for the control group and 53% for the caval dogs, but these values were not significantly different. The comparable decreases in fractional reabsorption and increases in single nephron filtration rate for both groups of dogs after volume expansion, as well no change in whole kidney GFR means that distal delivery of glomerular filtrate is equivalent in control and experimental dogs in this phase of the experiment.

When group means for the micropuncture data were calculated from mean values for each dog rather than from individual tubules as above, the results and conclusions are no different. For the control group, TF/P inulin fell from 1.81 ≠ 0.03 in hydropenia to 1.43 ≠ 0.03 after volume expansion. ($P < 0.05$) while V_o rose from 64.3 ≠ 5.4 to 105.0 ≠ 8 nl/min. In the experimental ani-

TABLE III

Effects of Volume Expansion on Proximal Tubular Function

Group	Phase	TF/P Inulin*	Fractional reabsorption	Vo‡	Distal delivery	Tubular pressure	SCP§ Maximum	SCP§ Steady state	Transit time
			%	nl/min	nl/min per nephron	mm Hg	mm Hg	mm Hg	sec
Controls n = 12	Hydropenia	1.83 ±0.06 n = 45‖	45.3	60.8 ±4.4 n = 18	33.3	12.4 ±1.26 n = 28	—	19 ±2.7	20.1 ±0.36 n = 45
	Volume expansion	1.45 ±0.04 n = 45	31	101 ±7.4 n = 18	69.6	30.8 ±1.57 n = 28	47.7 ±3.1	26.9 ±4.5	20.6 ±0.45 n = 43
Caval n = 12	Hydropenia	1.85 ±0.06 n = 47	46	69.9 ±3.6 n = 21	37.7	11.8 ±1.16 n = 35	—	15.3 ±2.4	21.2 ±0.69 n = 31
	Volume expansion	1.51 ±0.06 n = 47	33.8	107 ±6.2 n = 21	71	24.0 ±0.93 n = 25	40.2 ±5.1	28 ±4.6	22 ±0.77 n = 31

* TF/P, tubular fluid to plasma ratio.
‡ Vo, single nephron filtration rate.
‖ n, number of nephrons.
§ SCP, subcapsular pressure.

mals, TF/P inulin fell from $1.83 \neq 0.04$ to $1.49 \neq 0.03$ $(P < 0.05)$ while V_o rose from $69.1 \neq 4.2$ to $107 \neq 6.9$ nl/min.

Tubular pressures as measured by saline manometry were not significantly different during hydropenia in both groups of dogs. After volume expansion, tubular pressure in controls rose by 144 and by 103% in the caval dogs. Thus, tubular pressure in caval dogs was significantly less $(P < 0.05)$ after acute saline loading.

During the course of rapid saline loading, subcapsular pressure rapidly increased to a maximal peak value, and then gradually decreased towards a lower steady-state value once the acute load had been completed. This observation has been previously noted (12). Micropuncture measurements of tubular pressures were taken during the steady-state phase of subcapsular pressure. During hydropenia, subcapsular pressure was not significantly different in the two groups. Immediately after the acute load of Ringer's solution, peak subcapsular pressure was significantly higher $(P < 0.05)$ in the control than in the caval dogs. However, during the steady-state phase, the values were not different. There was no response in transit time to the acute volume expansion in either group of dogs, and in each phase of the experiment the values did not significantly differ for each group. This suggests that there was no difference in tubular diameter for each group of dogs. Thus of all the measured and observed parameters, the caval kidney differed from the control kidney only in tubular pressure and peak subcapsular pressure after acute volume expansion. Grossly, the caval kidney looked normal and did not appear congested. The capsule stripped with as equal facility as in a control dog.

Fig. 1A shows the mass TF/P inulin data for both groups. The points are randomly scattered for each group of dogs.

Distal tubules. In eight control and seven chronic caval dogs, paired recollections were obtained from the distal tubule as well as from the proximal tubule, before and after acute saline loading.

To validate recollection in distal tubules, seven nephrons were repunctured during hydropenia in two normal dogs. The ratio of the second TF/P inulin to the first was 1.04 ± 0.02 (SEM). These results are comparable with those reported by other investigators working in the dog (18).

Table IV summarizes the data obtained from distal tubule micropuncture. There was no significant difference in the fraction of the water left unreabsorbed at approximately the mid-distal tubule for both groups of dogs during hydropenia. After acute saline loading, TF/P inulin fell significantly in each group $(P < 0.01$ controls and $P < 0.05$ cavals). However, TF/P inulin was significantly higher for caval dogs $(P < 0.05)$ in
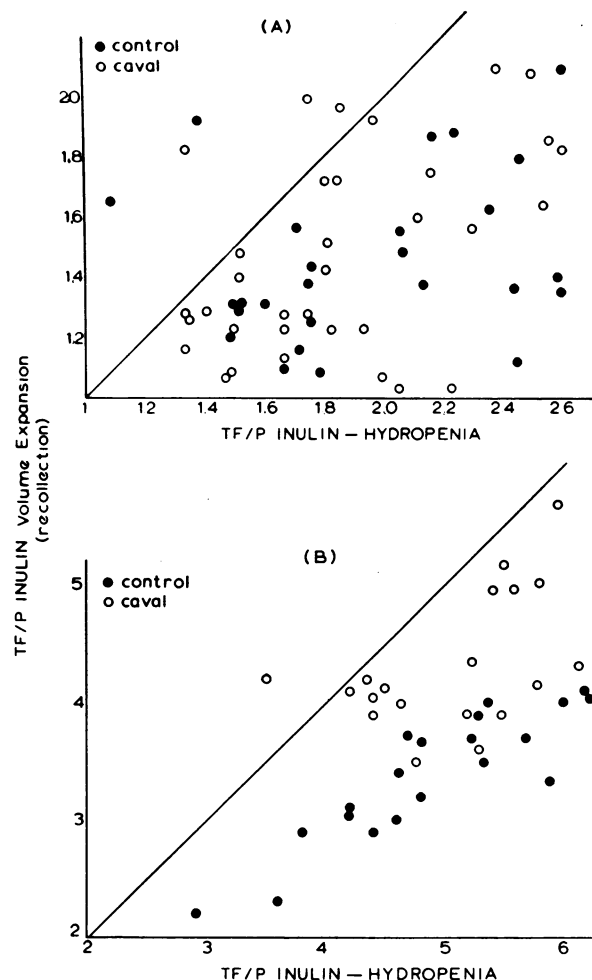

FIGURE 1 The effect of acute saline loading on recollection TF/P inulin ratios in control and caval dogs. A. Proximal tubule. B. Distal tubule. Each point is a paired recollection.

this phase of the experiments. Accordingly, the fraction of unreabsorbed water left in the distal tubule in caval dogs was significantly less after volume expansion than for control dogs.

TF/P Na^+ was significantly higher in control than in caval dogs during hydropenia $(P < 0.01)$. However, when TF/P Na^+ was factored by inulin ratio, there was no significant difference in the amount of sodium left in the distal tubule for either group of dogs. After volume expansion, TF/P Na^+ rose significantly $(P < 0.01)$ for each group, and in this phase of the experiment, the differences between the amount of sodium left unreabsorbed becomes quite apparent. In the control group 13.7% of the filtered sodium is left unreabsorbed, while only 8.9% is left in the distal tubule of caval dogs.

Fractional excretion of water rose by 8% in control dogs after ECF expansion and by only 2.57% in caval dogs. Fractional excretion of sodium rose by 5.73% in

TABLE IV

*Effects of Volume Expansion on Distal Tubular Function**

Parameter	Hydropenia		Volume expansion	
	Controls	Cavals	Controls	Cavals
TF/P inulin	5.10 ±0.21 (n = 20)‡	4.98 ±0.24 (n = 16)	3.43 ±0.20 (n = 20)	4.05 ±0.18 (n = 16)
	NS§		$P < 0.05$	
% water unreabsorbed	19.6	20	29	24.7
	NS		$P < 0.05$	
TF/P Na$^+$	0.40 ±0.03 (n = 20)	0.31 ±0.02 (n = 16)	0.47 ±0.02 (n = 20)	0.36 ±0.02 (n = 16)
	$P < 0.01$		$P < 0.01$	
TF/P Na × 100 Inulin, %	7.8 ±1.6	6.22 ±1.4	13.7 ±2.0	8.9 ±1.2
	NS		$P < 0.05$	
Fractional excretion water, %	0.67 ±0.12	1.24 ±0.08	8.67 ±0.10	3.81 ±0.09
	$P < 0.05$		$P < 0.05$	
Fractional excretion sodium, %	0.32 ±0.18	0.11 ±0.10	6.05 ±0.15	1.07 ±0.12
	$P < 0.05$		$P < 0.01$	

* Abbreviations as in previous tables.
‡ n, number of nephrons.
§ Probability values compare results between groups of dogs in each phase of hydropenia or volume expansion.

control animals and by less than 1% (0.96%) for the caval dogs.

Figs. 1B and 2 summarize the recollection data for inulin and sodium in the distal tubule. Unlike the random scatter for the inulin concentration ratios observed in the proximal tubule between control and experimental groups, the recollection inulin concentration ratios in distal tubules appears to be consistently higher for caval dogs. This is borne out in the mean TF/P inulin ratios.
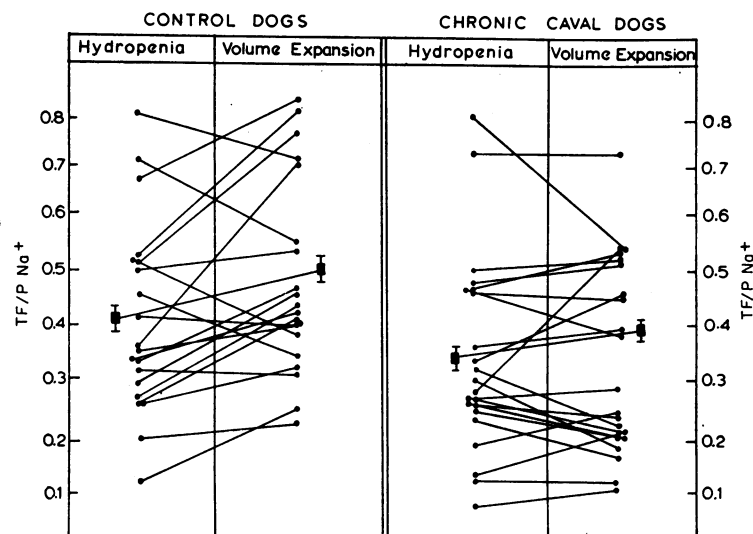
When group means for the distal micropuncture data

FIGURE 2 Paired distal tubule TF/P Na$^+$ ratios in hydropenia and after acute saline loading. The closed boxes and joining lines are the mean values ±1 SEM.

were calculated from mean values per dog rather than from individual tubules, as done above, the results and conclusions are no different than those shown in Table IV. For the control group TF/P inulin hydropenia was $5.12 \neq 0.23$ and this fell to $3.43 \neq 0.15$ after volume expansion ($P < 0.05$), while TF/P Na$^+$ rose from $0.39 \neq 0.03$ to $0.46 \neq 0.02$ ($P < 0.05$). In the caval dogs, TF/P inulin fell from $4.99 \neq 0.21$ to $4.01 \neq 0.16$ ($P < 0.05$) while TF/P Na$^+$ rose from $0.29 \neq 0.02$ to $0.37 \neq 0.02$ ($P < 0.05$).

After acute volume expansion, fractional reabsorption in the proximal tubule of control dogs was depressed by some 14.3%. However the increment of water left unreabsorbed in the distal tubule was 9.4% and the increment for sodium 5.9%. Thus the difference presumably had been reabsorbed by the loop, (assuming minimal reabsorption occurs in the pars recta and early distal tubule). The extra amount of water and sodium delivered to the collecting duct was almost totally excreted in the urine, since urinary excretion of water increased by 8% and urinary excretion of sodium by 5.73%. The situation for the chronic caval dogs was somewhat different however. In this group, the increment of unreabsorbed glomerular filtrate delivered from proximal tubule to the loop of Henle after acute volume expansion was 12.2%. In the distal tubule however, only 4.7% of this increment remained for water and 2.7% for sodium. Further reabsorption of sodium and water occurred in the late distal tubule and collecting duct, for urinary

excretion of water after saline loading increased by only 2.57%, and urinary excretion of sodium by 0.96%. Thus of the original increments in glomerular filtrate delivered distally from the proximal tubule after saline loading, 56% of the water and 40% of the sodium are delivered into the urine for control dogs, and only 21% of the water and 7.9% of the sodium are excreted in the urine of the caval dogs. The above data are summarized in Fig. 3.

Effect of altered intrarenal hemodynamics. It has previously been reported that altering intrarenal hemodynamics may reverse the sodium retention observed in the chronic caval dog (19). To assess whether this reversal occurs primarily in the proximal or distal nephron, six control and five chronic caval dogs were studied by micropuncture techniques during steady state volume expansion, and again after the experimental kidney had been vasodilated and the systemic blood pressure elevated with intravenous infusions of noradrenaline. The control dogs were studied in two phases only, while the experimental animals were studied sequentially as outlined in Methods. Clearance data from the control dogs are summarized in Table V; and the micropuncture data in Table VI. The clearance data are from the left kidney only; the micropuncture data are from paired recollections in 20 proximal tubules and 14 distal tubules. Blood pressure was elevated by 17.9%, the same order of change which occurs with acute saline loading alone. C_{IN}, $U_{Na}V$, and the fractional excretion of sodium and
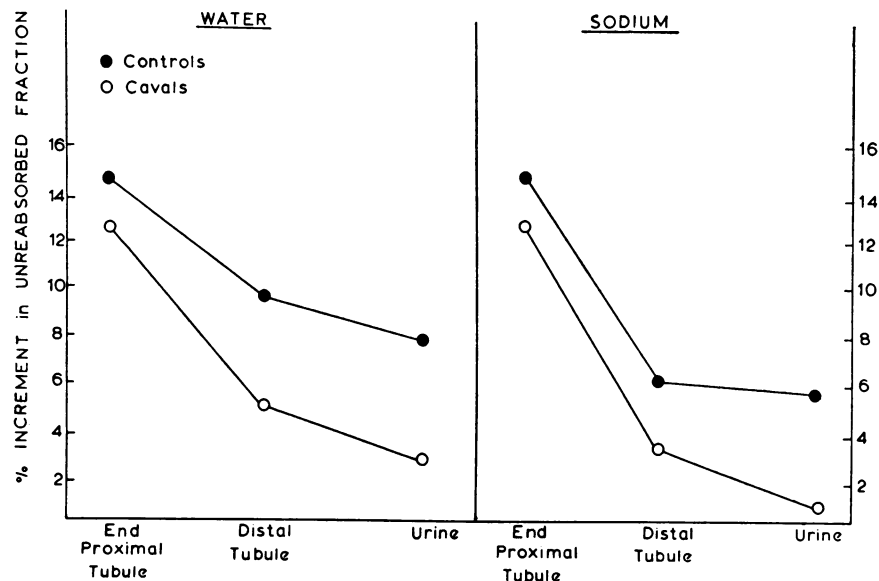

FIGURE 3 The increment in unreabsorbed water and sodium left in each nephron site and excreted in the urine after acute saline loading. The results for the proximal tubule are not significantly different between groups. End-proximal tubule segments were recognized by lissamine green passage. Note continuing reabsorption of sodium in late distal tubule and collecting ducts of caval dogs.

TABLE V

The Effects of Altered Hemodynamics on Renal Function in Expanded Control Dogs

Dog	Blood pressure C‡	E§	C_{IN} C	E	C_{PAH} C	E	$U_{Na}V$ C	E	*F.E.H₂O C	E	F.E. Na⁺ C	E
	mm Hg		*ml/min*		*ml/min*		*μEq/min*		%		%	
1	98	105	33	28	117	141	183	156	3.5	8	4.8	3.6
2	105	110	41	52	135	194	273	257	7.8	12.4	5.5	6.4
3	110	150	25	21	58	52	258	230	7.4	8.8	5.0	5.7
4	105	110	48	44	134	132	198	148	13.4	12.5	2.8	2.8
5	100	148	37	30	144	108	160	170	9.9	10.15	2.9	3.5
6	115	125	45	47	103	167	311	312	4.4	5.6	4.5	4.3
Mean	106	125	38	37	115	132	231	212	7.7	9.6	4.3	4.4
P value	<0.05		NS		NS		NS		NS		NS	

* F. E., fractional excretion; ‡ C, volume expansion; § E, volume expansion plus altered hemodynamics. Data are for left kidney only; each number is the mean of at least three clearance periods.

water were not significantly altered by the adjustment of hemodynamics. Although the mean C_{PAH} was not significantly elevated, the trend was for this variable to increase in most of the dogs. Presumably the superimposed constrictor effects of the noradrenaline minimized the full effect of the acetylcholine.

The infusion of acetylcholine and noradrenaline therefore had no further effect on renal function including

TABLE VI

*Effects of Altered Hemodynamics on Tubular Function in Expanded Control Dogs**

	Proximal tubule						Distal tubule					
	TF/P_{IN}		Transit time		TF/P_{IN}		$TF/P_{Na}⁺$		$TF/P_{Na/IN}$ × 100		Loop transit time	
Dog	C	E	C	E	C	E	C	E	C	E	C	E
			sec						%		*sec*	
1	1.55 (n = 3)‡	1.53	24	25	3.43 (n = 3)	3.46	0.39 (n = 3)	0.44	11.4	12.7	46	47
2	1.47 (n = 2)	1.44	19	21	3.93 (n = 2)	3.77	0.38 (n = 2)	0.40	9.7	10.6	46	45
3	1.36 (n = 3)	1.33	25	25	3.80 (n = 2)	3.76	0.41 (n = 2)	0.38	10.8	10.1	43	46
4	1.53 (n = 4)	1.52	17	15	3.03 (n = 2)	3.49	0.36 (n = 3)	0.40	11.8	11.5	45	44
5	1.33 (n = 4)	1.30	22	23	3.40 (n = 3)	3.51	0.34 (n = 2)	0.39	10.0	11.1	50	50
6	1.39 (n = 4)	1.30	18	17	3.41 (n = 2)	3.90	0.25 (n = 2)	0.31	7.4	8	47	45
Mean	1.44	1.40	21	21	3.50	3.65	0.36	0.39	10.2	10.8	46	46
P value	NS		NS		NS		NS		NS		NS	

* Abbreviations as in previous tables.
‡ n, number of nephrons; each number is a mean value.

TABLE VII

Effects of Altered Hemodynamics on Renal Function in Expanded Caval Dogs

Dog	Blood pressure $C\ddagger$	E_1	E_2	C_{IN} C	E_1	E_2	C_{PAH} C	E_1	E_2	$U_{Na}V$ C	E_1	E_2	F. E. H_2O C	E_1	E_2	F. E. Na^+ C	E_1	E_2
	mm Hg			*ml/min*			*ml/min*			*μEq/min*			*%*			*%*		
1	88	100	130	41	50	48	80	109	93	42	180	241	0.64	2.86	5.0	1.95	2.45	4.63
2	90	85	110	42	48	40	122	131	190	12	64	154	0.6	2.6	6.5	0.22	0.96	2.6
3	112	112	130	30	30	22	—	—	—	9	70	200	1.5	4.0	8	0.24	2.16	6.4
4	85	90	105	44	48	44	158	203	135	12	61	102	1.54	4.21	4.47	0.19	0.91	1.7
5	80	85	125	30	32	28	131	130	75	2	97	306	2.52	7.40	12.6	6.05	2.1	6.0
Mean	91	94	120	37	41	36	123	143	123	15	95	201	1.36	4.21	7.31	0.51	1.72	4.26
P value§	<0.05			NS			NS			<0.05			<0.05			<0.05		

* Abbreviations as in previous tables.
‡ C, volume expansion; E_1, volume expansion plus acetylcholine; E_2, E_1 + i.v. noradrenaline.
§ P values compare E_2 with control phase of volume expansion alone.

fractional reabsorption of salt and water in the proximal or distal tubule.

Clearance data from the left kidney only of the chronic caval dogs are shown in Table VII. Micropuncture data from 15 proximal tubules punctured in all three phases of the experiment are shown in Table VIII. Because of the paucity of distal tubules on the surface of the dog kidney, and because of the marked pulsatile movements often induced by the acetylcholine infusion, it was not always possible to puncture these tubular segments under all three conditions of the experiment. Accordingly, 11 distal tubules were punctured during volume expansion alone, and then repunctured in the last phase of the experiment. In the middle phase of volume expansion plus

acetylcholine only, only 6 of the 11 nephrons could be repunctured.

Blood pressure was elevated 26.3% in the last phase of the experiments as compared with the control phase. The mean blood pressure of 120 mm Hg was not significantly different from the 125 mm Hg observed in controls after noradrenaline infusion. C_{IN} and filtration fraction did not change significantly during the experiment. In response to unilateral infusion of acetylcholine, C_{PAH} of the experimental kidney increased significantly from 123 ±6 ml/min to 143 ±8 ml/min ($P < 0.05$). After the infusion of noradrenaline, C_{PAH} fell again to control values.

Sodium excretion increased by 80 μEq/min per kidney

TABLE VIII

Effects of Altered Hemodynamics on Tubular Function in Expanded Caval Dogs

Dog	Proximal tubule TF/P_{IN} C	E_1	E_2	Transit time C	E_1	E_2	Distal tubule TF/P_{IN} C	E_1	E_2	TF/P_{Na}^+ C	E_1	E_2	TF/$P_{Na/IN}$ C	E_1	E_2	Loop transit time C	E_1	E_2
							sec						*%*			*sec*		
1	1.45	1.47 (n = 3)	1.49	18	19	19	3.98 (n = 3)	3.94 (n = 2)	3.18 (n = 3)	0.37 (n = 3)	0.35 (n = 2)	0.43 (n = 3)	9.3	8.9	13.5	51	48	47
2	1.34	1.29 (n = 3)	1.38	19	19	21	4.10 (n = 2)	3.90 (n = 1)	3.05 (n = 2)	0.36 (n = 2)	0.38 (n = 1)	0.44 (n = 2)	8.8	9.8	14.5	45	47	46
3	1.51	1.54 (n = 3)	1.45	21	23	19	4.20 (n = 2)	4.11 (n = 1)	3.10 (n = 2)	0.40 (n = 2)	0.38 (n = 1)	0.42 (n = 2)	9.5	9.2	13.5	49	46	45
4	1.44	1.60 (n = 3)	1.39	17	16	18	3.80 (n = 2)	3.80 (n = 1)	3.17 (n = 2)	0.33 (n = 2)	0.40 (n = 1)	0.42 (n = 2)	8.7	16.5	13.3	53	48	45
5	1.46	1.50 (n = 3)	1.44	15	18	17	3.91 (n = 2)	3.79 (n = 1)	3.40 (n = 2)	0.34 (n = 2)	0.34 (n = 1)	0.39 (n = 2)	8.7	8.7	11.5	47	46	46
Mean	1.44	1.48	1.43	18	19	18.8	3.99	3.91	3.18	0.36	0.37	0.42	9.0	9.4	13.3	49	46.2	45.8
P value		NS			NS				<0.05			<0.05			<0.05			<0.05

* Abbreviations as in previous tables.

in response to renal vasodilatation, and increased another 187 μEq/min per kidney when blood pressure was elevated. Thus, although renal vasodilatation alone improved the fractional excretion of salt and water, it was not until perfusion pressure had been increased that these two parameters reached normal levels. Micropuncture data reveal that TF/P inulin remained unchanged in the proximal tubule during alteration of intrarenal hemodynamics (1.44 ±0.03 vs. 1.48 ±0.08 vs. 1.43 ± 0.06). There was no significant change in transit time, though intratubular pressure increased significantly in the acetylcholine phase.

TF/P inulin in the distal tubules during volume expansion was 3.99 ±0.22, representing some 25% of filtered water left unreabsorbed at that site. This value is comparable with the value reported (24.7%) for the group of 12 caval dogs studied in response to volume expansion only, and significantly different ($P < 0.05$) from the 28.5% left unreabsorbed in the control dogs undergoing equivalent volume expansion. During the infusion of acetylcholine into the left kidney, TF/P inulin fell to 3.91 ±0.03, but this change was not significant. However only six nephrons are involved. When blood pressure was elevated with the noradrenaline infusions, TF/P inulin fell significantly to 3.18 ±0.19 ($P <$

0.05). This represented 31.4% of filtered water left unreabsorbed, i.e., altering intrarenal hemodynamics had increased the increment of unreabsorbed water by 6.4%. In response to acetylcholine, fractional water excretion increased by 2.85% ($P < 0.05$) and increased another 3.11% ($P < 0.05$) when blood pressure was elevated for a total increase of 5.96% over that observed in the volume-expanded state alone. Thus, the increment of water delivered to the collecting ducts during acetylcholine and noradrenaline infusions is virtually completely excreted. TF/P Na^+ remained unchanged in the first two phases of the experiment, but increased to 0.42 ($P < 0.05$) in the last phase. There was a significant increment in the unreabsorbed fraction of Na^+ in the distal tubule by 3.9% after infusion of acetylcholine and noradrenaline. Fractional excretion of urinary sodium had increased significantly in each phase of the experiment, and in the final phase an additional 3.48% was appearing in the urine. Thus, virtually all of the increment in sodium delivered to the late distal tubule and collecting ducts in the third phase of the experiment was excreted. These data are summarized in Fig. 4.

The transit time of lissamine green through the loop of Henle during steady-state volume expansion was significantly slower ($P < 0.05$) in chronic caval dogs.

WATER · · · · · · · · · · SODIUM

● —● CONTROLS —Expanded + Ach. + NOR-ADR.
O- -O CAVALS —Expanded + Ach.
O—O CAVALS — Expanded + Ach + NOR-ADR.

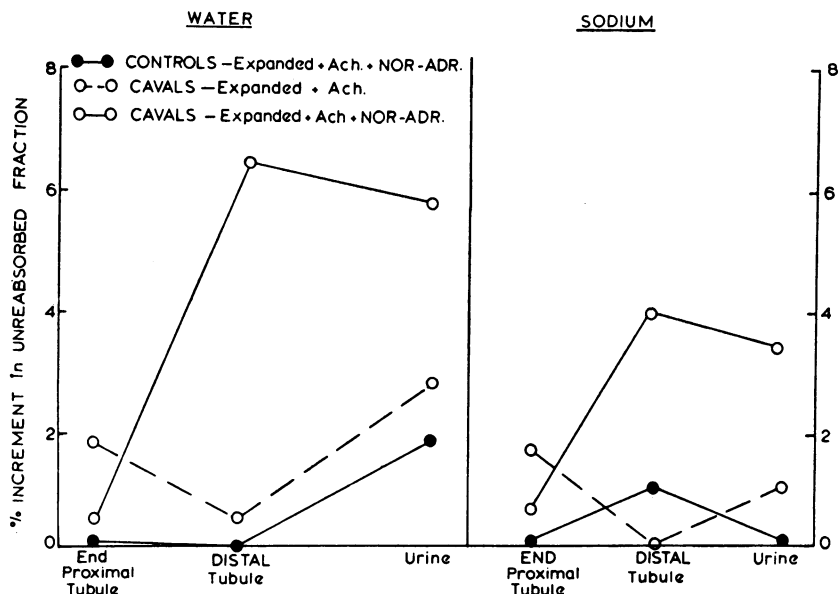% INCREMENT in UNREABSORBED FRACTION

FIGURE 4 The effect of altering renal hemodynamics on fractional rejection of sodium and water at each nephron site and in the urine of saline-loaded control and experimental animals. All per cent increments are compared with the saline-loaded state as a base line. Hemodynamic maneuvers produce no significant effects in the controls; full natriuretic and diuretic response in caval dogs require both increased renal vasodilatation and elevation of perfusion pressure. Altered hemodynamics have reversed the sodium and water retention previously observed in the expanded caval dogs in the loop of Henle and in the late distal tubule-collecting duct.

(49 ±0.3 vs. 46 ±0.40 sec). This variable became normal 46.2 ±0.28 sec ($P < 0.05$) in response to acetylcholine infusion and remained constant in the last phase of the experiment.

DISCUSSION

In the present study, as in previously reported investigations (4), the blunted natriuresis of saline-loaded chronic caval dogs cannot be explained on the basis of a reduction in the filtered load of sodium. In the present series of experimental animals, GFR was comparable with that observed in control dogs, but mean plasma sodium concentration was significantly lower by some 5%. The mean filtered load of sodium however, was not different in control and caval dogs. This tendency to hyponatremia was reflected mainly by very low P_{Na}* values in four dogs ($P_{Na} = 114$–120 mEq/liter). Normonatremic chronic caval dogs responded no better to volume expansion than their hyponatremic counterparts. In any event, the tendency to lower filtered loads in chronic caval dogs was partially offset by the significant reduction in plasma proteins (22% lower than controls) which could have altered the Donnan ratio.

The data presented in this report also confirm and extend the micropuncture studied recently reported by Auld et al. (11). These investigators reported that the response of the proximal tubule in saline-loaded chronic caval dogs is normal and inferred that the distal nephron is the site for sodium retention.

In this study, it has been determined that both during hydropenia and after acute volume expansion, the proximal tubule of the chronic caval dog functions normally with regard to fractional and absolute sodium reabsorption, and the volume of filtrate delivered to the distal nephron. This conclusion however, derived from micropuncture data obtained from superficial nephrons, is predicated upon the assumption that the measurements made are valid. These measurements include determination of the free-flow TF/P inulin ratios and single nephron filtration rates (V_o).

To the extent that a conscious effort was made to puncture only end-proximal tubules as identified by lissamine green injections, the data concerning fractional sodium reabsorption are probably comparable between control and experimental animals. To the extent however, that the punctured segments identified by lissamine green do not represent a fixed anatomical portion of the entire proximal convolution, it is possible that small, but significant differences in fractional reabsorption of salt and water could be obscured by an unrecognized random pattern to the tubular punctures. Although no comparable data exist for the dog, Rector, Sellman, Martinez-Maldonado, and Seldin (20) have shown by micropuncture and microdissection techniques that the

end-proximal tubule in rats as identified by lissamine green injections corresponds to 55–65% of the total length of the proximal tubule. If this were also the situation in dogs, then conclusions concerning fractional reabsorption in both groups of dogs are probably valid.

A more serious problem is that of measuring V_o. Since this measurement involves integration of tubular fluid volume flow and TF/P inulin at the puncture site, then within the limitations of the analytical and collection techniques, the values during hydropenia are comparable between control and chronic caval dogs. The measurements of V_o obtained after volume expansion however, were gathered from superficial tubules previously punctured in the hydropenic state. In the face of constancy of whole kidney GFR, superficial nephron filtration rate rose to an equivalent degree (about 60%) in each group of dogs. This observation suggests that acute saline loading caused a redistribution of glomerular filtrate within the renal cortex. Because absolute reabsorption of salt and water in superficial proximal tubules is not significantly different, then delivery of filtrate from proximal tubules to corresponding distal nephrons is similar in both control and caval dogs. This implies that sodium retention at a distal nephron site is the cause of the reduced sodium excretion in expanded caval dogs. Redistribution after saline loading however, implies heterogeneity of function between superficial cortical and deeper juxtamedullary nephrons. The data derived from micropuncture therefore, need not be representative of function in these deeper nephrons. However, since cortical nephrons form the major proportion of nephron population, and in the presence of postexpansion redistribution would appear to receive a greater proportion of filtrate then estimates of nephron function based on cortical nephrons are unlikely to produce large errors. Under these circumstances, it would not appear unreasonable to compare function in the proximal and distal superficial tubules with composition of the final urine. The possibility exists, that after saline loading nephron function is in fact homogeneous, and that redistribution is an artifact of micropuncture methodology. Recently, the validity of the V_o measurement in repunctured nephrons in saline-loaded dogs has been questioned. Stein et al. (21) first concluded that acute saline infusion in dogs will cause redistribution of plasma flow to the renal cortex and raise V_o by 33%, while whole kidney GFR increased by only 4%. This group of investigators later reversed themselves (22) and reported that V_o increases in saline-loaded dogs only if proximal tubules are repunctured; when previously untouched nephrons are tested, there is no evidence for redistribution. These authors concluded that repuncturing nephrons after saline loading causes spurious results by altering flow dynamics within the proximal

tubule. Auld, Alexander, and Levinsky (23) have confirmed these results in dogs, but Schneider, Lynch, Dresser, and Knox (24) report that V_o does not differ from hydropenic values in saline-loaded dogs no matter whether repunctured or fresh nephrons are tested. A similar controversy regarding redistribution of renal plasma flow exists between investigators studying rats (25, 26).

Although more precise information is required before this hotly debated controversy of methodology is resolved, the problem need not invalidate the conclusion that distal delivery out of the proximal tubule is equivalent in saline-loaded control and caval dogs. Using an experimental preparation and protocol virtually identical with that employed in this study, Auld et al. (11) initially reported that saline loading produces cortical redistribution of filtrate to an equivalent degree in both control and chronic caval dogs. After eruption of the present controversy concerning methodology, these authors have repeated their studies testing both fresh and repunctured nephrons after acute volume expansion (23). Their results are in agreement with previous suggestions (22) that nephron function remains homogeneous after saline loading and that redistribution is possibly an artifact of the repuncture technique. However, no matter whether fresh or repunctured nephrons were tested after saline loading in the chronic caval dogs, V_o under each set of conditions was always comparable with values obtained in the control groups. Thus, no matter whether acute saline loading induces redistribution or not, it appears legitimate to conclude that distal delivery in caval dogs is not different from a control population. This is strong inferential evidence that the distal nephron is the site for sodium retention.

The measured variables of proximal tubular transit time, renal subcapsular pressure and intratubular pressure were similar in both groups of dogs during hydropenia. On a priori grounds one might have anticipated, because of the increased renal venous pressure that both intratubular and interstitial (subcapsular) pressures would be elevated in the chronic caval dogs. The fact that GFR, RPF, and filtration fraction were normal in hydropenia would tend to rule out a change in intrarenal vascular resistance as the adaptive mechanism returning interstitial and intratubular pressures to normal. In view of the findings of LeBrie and Mayerson (27) that elevation in renal venous pressure cause marked increases in the flow of renal lymph, it seems reasonable to conclude that the renal interstitial and tubular pressures were rendered normal in the caval dogs due to increased lymph flow acting to remove excess peritubular fluid.

During hydropenia, steady-state intratubular pressure in the proximal tubules were significantly less than

the simultaneously recorded subcapsular pressure. These differences disappeared after saline loading. These arithmetic differences in pressure should not be taken to mean that pressure gradients exist between interstitium and tubules. These variations are undoubtedly methodological in origin, since different techniques are employed to measure each pressure variable. In a recent study, Levy and Levinsky (28) using the same techniques, could find no difference between subcapsular and intratubular pressures.

The thesis that the proximal tubule functions normally in hydropenia and responds normally to volume expansion in chronic caval dogs raises several problems. The first, namely that small but real differences exist but cannot be determined due to limitations of technique has already been discussed. Secondly, while the results of this study agree with those reported by Auld et al. (11) they are in conflict with the results of Kaloyanides, Cacciaguida, Pablo, and Porush (16). These latter investigators performed clearance studies in chronic caval dogs under conditions of water and hypotonic saline diuresis and concluded that these animals retain sodium all along the nephron, including the proximal tubule. In these studies, the authors expanded both control and caval dogs to an equivalent degree and had no difficulty in establishing a maximal water diuresis in the experimental animals. Accordingly, the lower results for V/GFR cannot be explained on the basis of unrecognized back-diffusion of water across the collecting ducts due to excess amounts of antidiuretic hormone (ADH) in the edematous caval dogs.

The different results regarding the proximal tubule obtained in these experiments and in those of Kaloyanides et al. (16) may be partially explained on the basis of differences in techniques. The present study was carried out under conditions of dehydration, whereas the clearance studies alluded to were carried out under conditions of maximal water diuresis. Strictly speaking therefore, the conditions of both experiments are not truly comparable. In the present study, tubular fluid was obtained from nephron segments proximal to the pars recta. While there is not reason to believe that significant salt and water reabsorption occurs in the pars recta (29), and even less reason to believe that sodium transport in this segment would be different in caval dogs, this remains a theoretical possibility to explain differences in the results.

Lastly, one must consider that the hematocrit in the caval dogs was some 13% lower than in control dogs. This implies expansion of the plasma compartment rather than blood loss, since hematocrit values in sham-operated dogs were normal. This degree of plasma expansion is roughly equivalent to that obtained when an average-sized dog (12–15 kg) is expanded by 3% of

the body weight. At least two groups of investigators (30, 31) have indicated that this degree of volume expansion is sufficient to cause maximal fall in proximal TF/P inulin. Therefore, for the degree of volume expansion existing in the caval dogs, the observed TF/P inulin values during hydropenia are inappropriately high. It were as if the caval dogs either could not recognize that they were volume-expanded, or else could not expand to the enlarged ECF space in the usual fashion. Alternatively, it is also possible that the proximal tubule is responding to other factors existing in states of edema formation, e.g. reduced flow to a critical site acting as a volume receptor—this factor tending to mediate increased sodium reabsorption by the proximal tubule. The observed TF/P inulin ratio during hydropenia may thus represent a balance of forces tending to act on sodium transport in different directions. From this point of view, and not considering ECF volume expansion alone, the level of fractional reabsorption in the proximal tubule need not be inappropriate. The recent observations of Beuntig and Earley (32) bear on this point. These investigators demonstrated in rats that by combining a reduction in the solute load presented to an individual nephron and by aortic clamping—each of these variables by themselves influencing fractional reabsorption of the proximal tubule in opposite directions—then glomerulotubular balance could be maintained i.e., the tubule was responding to a balance of forces. A similar balance of forces is operative in the proximal tubule of saline-loaded dogs undergoing acute constriction of the thoracic vena cava (11). This maneuver during hydropenia tends to increase fractional reabsorption of sodium. Acute saline loading however, then depresses sodium transport so that during acute caval constriction and volume expansion, TF/P inulin in the proximal tubule is of the same order of magnitude as is observed during the original hydropenic condition.

The inference that the distal nephron is the site of sodium retention in expanded chronic caval dogs is borne out by the micropuncture data. The increment in water and sodium left behind at the site of distal tubular micropuncture after large infusions of saline were significantly greater in normal control than in the chronic caval dogs. This implicates the loop of Henle, and possibly the early distal tubule as the major site for salt and water retention. While the late distal tubule and collecting duct in normal dogs behaved like a conduit excreting almost entirely the increment of sodium and water presented into the urine, this segment in the experimental animals participated in further slight degrees of salt and water reabsorption. The behavior of the collecting duct in normal dogs is in agreement with recent observations made by Dirks and Seely (33) in saline-loaded dogs.

During hydropenia, differences in sodium and water reabsorption in the loop of Henle could not be demonstrated between the two groups of dogs, despite significant differences in urinary excretion of sodium, and no difference in delivery of sodium into the loop. Two explanations are possible. Firstly, the site of sodium retention during this phase of the experiment could be beyond the area of the distal tubule tested by micropuncture. Secondly, since the differences in fractional reabsorption required to cause significant minute-to-minute alterations in urinary excretion of sodium are so small, the most likely explanation is that the techniques employed cannot differentiate small differences between the control and experimental dogs. This is borne out by the fact that when the distal nephron is stressed with an increased load of filtrate after acute saline loading, differences in sodium and water transport in the loop of Henle now become readily apparent. Similar circumstances have recently been reported by a group of investigators studying distal nephron sodium transport in the dog with a remnant kidney (31).

The mechanism whereby the loop of Henle appears to retain salt and water in chronic caval dogs remains unknown. Estimates of cortical and medullary Na^+ $- K^+ATPase$ reveal no differences in the level of this enzyme in four control and four chronic caval dogs;[7] and the altered sodium transport does not therefore appear to involve this mechanism.

The experimental data reported here are in agreement with a previous report by Friedler, Belleau, Martino, and Earley (19) that altering intrarenal hemodynamics in volume-expanded caval dogs may induce a marked natriuretic response. The present experiments indicate that the distal nephron and especially the loop of Henle is the site responsive to these hemodynamic forces. After renal vasodilatation and elevation of perfusion pressure, the fraction of sodium and water left unreabsorbed in the distal tubule of the caval dogs rose to normal limits, indicating alteration of sodium transport within the loop. The infusion of the vasoactive drugs also depressed reabsorption of sodium and water within the late distal tubule-collecting duct, converting these segments into "conduits" as in the control group of dogs. In this latter group of dogs there was no significant effect of the infusion on fractional reabsorption in any nephron segment, and no effect on urinary excretion of water or sodium.

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[7] Cole, C., J. M. Mittelman, and M. Levy. Unpublished observations.

It remains a theoretical possibility that the infusion of the acetylcholine caused redistribution of renal plasma flow in the chronic caval dogs with an increment in the delivery of filtrate to the superficial distal nephrons. If transport in the loop remained unchanged, the unreabsorbed fraction of sodium and water at the distal tubular site of puncture would increase. Several lines of evidence indicate that this is not a likely explanation. Firstly, Stein, Reineck, Osgood, and Ferris (34) have recently demonstrated by micropuncture techniques that acetylcholine infusions in dogs does not cause elevation of superficial V_0. Although it is possible that redistribution could occur in the caval dogs, the observation that proximal tubule transit time remained constant in the face of unchanging fractional reabsorption in this nephron site makes this possibility somewhat unlikely. Lissamine green transit times changed significantly only in the loop of Henle after renal vasodilatation. Secondly, the major increments in excretion of sodium, and unreabsorbed fraction left at the distal tubule did not occur in the caval animals until perfusion pressure had been elevated with noradrenaline—a maneuver not likely to increase superficial V_0.

The observation that the major increment in sodium excretion in expanded caval dogs does not occur until perfusion pressure is increased suggested that distal nephron sodium transport could be influenced by hemodynamic maneuvers. Micropuncture data confirm that reabsorption of sodium and water in the loop of Henle was not maximally depressed until arterial blood pressure had been elevated. These findings are in agreement with recent observations in the rat by Bank, Aynedjian, Bansal, and Goldman (35) and Stumpe, Lowitz, and Ochwadt (36) and in saline-loaded hypertensive patients by Buckalew, Puschett, Kintzel, and Goldberg (37). These investigators have demonstrated that sodium transport by the ascending limb of the loop of Henle may be depressed by either acute or chronic elevations of arterial blood pressure.

The mechanism whereby alteration of intrarenal hemodynamics influences sodium transport in the loop is not readily apparent from the present experiments. Other workers have suggested the importance of either intrarenal humoral factors (35, 36) or that the transmission of increased perfusion pressure to vasa rectae will interfere with the peritubular removal of reabsorbate in a manner similar to that thought to be operative for the proximal tubule (38).

The observation in these experiments that loop transit time (lissamine green) decreased in the expanded caval dogs after the acetylcholine infusion in the face of constant delivery of filtrate from the proximal tubule would suggest a decrease in volume of the loop. This decrease in volume would suggest that the acetylcholine

infusions had increased peritubular volume, but inhibitory effects on sodium transport did not become operative until blood pressure was elevated.

Schneider, Dresser, Lynch, and Knox (39), studying the dog with an A-V fistula as an experimental model for chronic sodium retention and edema, have recently reported that sodium reabsorption in the proximal tubule of such animals is normal and have also inferred that the major site for sodium retention is in the distal nephron. These observations together with the data reported in this paper, raise the possibility that in the commonest clinical situation involving chronic sodium retention, i.e. the systemic edema states, the loop of Henle may be the major nephron segment responsible for the increased sodium reabsorption. In the various clinical edema states, it has long been tacitly assumed that the major site for sodium retention is the proximal tubule (40). The major type of evidence for this comes from the clearance studies of Bell, Schedl, and Barrter (41) in patients with congestive heart failure and from Schedl and Barrter (42) in patients with cirrhosis and ascites. These investigators demonstrated that the infusion of mannitol into edematous patients was more efficacious in augmenting free water clearance than in normal controls, and interpreted their findings to mean that there was hyperreabsorption of filtrate in the proximal tubules of the patients with edema. However, in a recent micropuncture study, Seely and Dirks (18) have demonstrated that at least in the dog, mannitol has only a minimal effect in the proximal tubule and exercises its major osmotic effect in the loop of Henle. As these authors point out, an inhibitory effect of mannitol in the descending limb of the loop is not necessarily incompatible with clearance studies which have demonstrated that mannitol infusions increase free water clearance. In view of this observation, the clearance studies of Bell et al. (41) and of Schedl and Barrter (42) could be reinterpreted to imply that the major site of salt and water retention in edematous patients is in the loop of Henle.

Although it is premature to de-emphasize the role of the proximal tubule in the sodium retention of edema, it seems clear from the studies reported here, that in more chronic states of altered sodium balance, the loop of Henle may play a significant and perhaps predominant role.

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