Demonstration of a Hormonal Inhibitor of Proximal Tubular Reabsorption during Expansion of Extracellular Volume with Isotonic Saline

FLOYD C. RECTOR, JR., MANUEL MARTINEZ-MALDONADO, NEIL A. KURTZMAN, JACK C. SELLMAN, FRED OERTHER, and DONALD W. SELDIN
with the technical assistance of ALLEN C. NUNN

From the Department of Internal Medicine, The University of Texas Southwestern Medical School, Dallas, Texas and Veterans Administration Hospital, Dallas, Texas 75235

ABSTRACT Evidence for the elaboration of a hormonal inhibitor of renal tubular reabsorption in response to expansion of extracellular fluid volume was obtained by examining the effects of plasma from rats and dogs undergoing saline diuresis on the rate of proximal tubular reabsorption measured both directly by micropuncture techniques and indirectly by clearance techniques.

Intravenous infusion of plasma from saline-loaded rats and dogs, but not plasma from control animals, inhibited the intrinsic reabsorptive capacity of the proximal tubule (as estimated from the shrinking-drop technique) by 35%, and reduced fractional reabsorption (as estimated from the tubular fluid-to-plasma ratio) by 20%. In addition the natriuretic plasma increased urine flow, solute-free water clearance, and potassium excretion in rats with hereditary diabetes insipidus, indicating an increase in the delivery of filtrate out of the proximal tubule to the more distal diluting segments of the nephron.

The hormonal inhibition of proximal tubular reabsorption had an extremely rapid onset of action (within seconds after instillation into the tubular lumen) and a short duration of action (less than 30 min after cessation of an intravenous infusion). Inhibitory activity was lost from natriuretic plasma upon dialysis and could be recovered in the dialysate. Dialysates of natriuretic plasma, when injected directly into the tubular lumen, also inhibited proximal reabsorption, indicating an action on the luminal side of the cell.

INTRODUCTION

It is now abundantly clear that the augmented excretion of sodium which results from expansion of extracellular fluid (ECF) volume with isotonic saline is mediated, neither by an increase in glomerular filtration rate (GFR) nor by suppression of aldosterone secretion, but rather by some yet unidentified mechanism which inhibits the tubular reabsorption of sodium (1–4). The fact that saline infusion during water diuresis increases both solute-free water clearance (C_{H_2O}) and sodium excretion suggests that a major site of inhibition is the proximal tubule (3), an inference amply confirmed by micropuncture studies (5–8).

The mechanism whereby expansion of ECF volume reduces proximal tubular reabsorption has not yet been identified. Several investigators (1, 9–12) have suggested that the inhibiting influence may be mediated by the elaboration of a natriuretic hormone, whereas others (13–16) have invoked alterations in various physical factors, such as colloid osmotic pressure, blood pressure, renal

Dr. Kurtzman is a Special Fellow, Office of the Surgeon General, U. S. Army.

Address requests for reprints to Dr. Floyd C. Rector, Jr., Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Tex. 75235.

Received for publication 11 September 1967 and in revised form 27 November 1967

vascular resistance, and distribution of renal blood flow.

Cross-circulation studies, designed to demonstrate the presence of a natriuretic hormone, have yielded equivocal results (1, 4, 11, 12). The cross-circulation technique, however, is not ideally suited to examine for the presence of a natriuretic hormone. Micropuncture studies have shown that saline infusions suppress reabsorption primarily in the proximal tubule (5–8), and that much of the filtrate delivered out of this segment is reabsorbed distally, with only a portion of the increment appearing in the final urine. Under conditions of cross-circulation, the small increase in sodium excretion commonly observed might not accurately reflect the true magnitude of proximal tubular inhibition. On the other hand, such small increases in sodium excretion that do occur may well be the consequence, not of the cross-circulation of a humoral inhibitor, but rather of hemodynamic changes associated with increased renal blood flow and compositional changes associated with dilution of the blood, both of which are known to result from the cross-circulation procedure and are themselves capable of inducing natriuresis (15, 17, 18).

The present experiments were designed to obviate these difficulties by testing for the presence of a humoral inhibitor in plasma from animals undergoing saline diuresis (natriuretic plasma), not only by examining its effect on urinary sodium excretion, but rather by directly assessing its capacity to inhibit proximal tubular reabsorption. It was shown that an infusion of natriuretic plasma inhibited proximal tubular reabsorption, as judged by three different techniques: prolongation of the rate of reabsorption of a shrinking drop of saline during stopped flow; depression of the inulin tubular fluid to plasma ratio (TF/P) during free flow; and increased urinary excretion of free water and potassium in rats with hereditary diabetes insipidus. On the basis of these results the conclusion was drawn that expansion of extracellular volume causes the release of a humoral agent which inhibits sodium reabsorption in the proximal portions of the nephron.

METHODS

The effect of plasma from antidiuretic animals and from animals undergoing saline diuresis (natriuretic plasma) on proximal tubular reabsorption was assayed in three groups of rats. Groups I and II consisted of Sprague-Dawley rats, whereas group III contained Brattleboro rats with hereditary diabetes insipidus.[1] Rats in groups I and II were anesthetized with sodium pentobaribital and prepared for micropuncture as previously described (19). In order to measure GFR by a constant infusion technique the assay rats in groups I and II were subjected to right unilateral nephrectomy 1–3 wk before the acute experiment. In groups I and III GFR was measured with radioactive inulin as previously described (19). In group III GFR was calculated on the basis of both the inulin infusion and the urinary excretion, with good agreement between the two methods. In group II inulin was given at a rate of 2 mg/min to maintain a plasma concentration of approximately 100 mg/100 ml. To replace surgical loss of ECF volume occurring during the surgical procedure each rat was given 1 ml of bicarbonate-saline solution (25 mM $NaHCO_3$ + 110 mM NaCl). This amount of saline replacement is less than previously used (19) in order to avoid any possibility of overexpanding the assay animal and thus obscuring possible effects of natriuretic plasma on proximal reabsorption. Transit time was measured with lissamine green as previously described (19).

Group I. In the first group of 18 rats the intrinsic reabsorptive capacity of the proximal tubule was measured with the shrinking-drop technique of Gertz (20, 21). Five rats were infused with plasma from rats, while the remaining 13 received plasma from dogs. Four of the rats were given 5 mg of deoxycorticosterone acetate (DOCA) in oil intramuscularly 4 hr before the experiment.

In each assay rat 6–10 measurements were made during each of three periods (control, experimental, and recovery; see below for experimental protocol).

Group II. In the second group of 12 rats fractional reabsorption in the proximal tubule was calculated from the ratio of inulin concentration in tubular fluid and plasma [$(TF/P)_{In}$] at the end of the proximal convolution. The end of the proximal convolution was identified by the passage of lissamine green (7, 22). In each rat four samples of tubular fluid were collected in each of three periods (control, experimental, recovery).

Group III. In order to estimate the effect of natriuretic plasma on the delivery of filtrate out of the proximal tubule, experiments were carried out in six rats with hereditary diabetes insipidus (Brattleboro strain). In the absence of antidiuretic hormone (ADH) urine flow can be used as a rough approximation of the amount of fluid delivered out of the proximal portions of the nephron to the distal diluting segments. Urine volume (V), osmolar clearance (C_{osm}), solute-free water clearance (C_{H_2O}), and sodium and potassium excretion were measured during control, experimental, and recovery periods. Four to five 10-min urine collections from an indwelling bladder catheter were obtained in each period; arterial blood was obtained at the midpoint of each urine collection.

Experimental protocol. The same experimental protocol was used in all three groups of assay rats. The experiment was divided into three periods: control, experi-

[1] Courtesy of Dr. Heinz Valtin.

mental, and recovery. Each period consisted of an initial 30 min for equilibration and 30–45 min for shrinking-drop measurements or collection of tubular fluid and urine samples. Plasma was infused intravenously through the entire experiment at a rate of 0.02 ml/min. (Preliminary studies demonstrated that this amount of plasma by itself would not change proximal reabsorption.) During the first or control period plasma from control animals was infused. During the second or experimental period the control plasma was discontinued and natriuretic plasma was infused. During the third or recovery period natriuretic plasma was discontinued and the original control plsma reinfused. In an occasional rat the sequence was varied.

In two experiments 5 ml of natriuretic plasma was dialyzed through Visking cellophane membrane against 50 ml of isotonic bicarbonate-saline solution for 18–24 hr at 4°C. The dialyzed plasma was then infused into group I assay rats according to the standard experimental protocol. In an additional experiment, 5 ml of natriuretic plasma was dialyzed against an equal volume of distilled water for 18–24 hr at 4°C and the dialysate then infused into a group I rat at 0.04 ml/min during the experimental period.

In five other experiments 3 ml of antidiuretic plasma and 3 ml of natriuretic plasma were each dialyzed against 3 ml of isotonic bicarbonate-saline solution for 18–24 hr at 4°C. The plasma was discarded and the dialysate was then injected into the lumen of a proximal tubule and its reabsorptive rate measured with the shrinking-drop technique. In these experiments measurements were first made with isotonic bicarbonate-saline solution, then with dialysate of control plasma, next with dialysate of natriuretic plasma, and finally again with dialysate of control plasma.

Collection of plasma. For each experiment heparized arterial blood from three to four control rats was pooled. In another group of three to four rats, saline diuresis was induced by infusing bicarbonate-saline solution intravenously at 0.4 ml/min. When urinary sodium excretion rose to approximately 10% of the filtered sodium, the rats were bled directly from the aorta. The blood was centrifuged and the plasma stored at 4°C until the time of the experiment. Each batch of plasma could be utilized for two or three experiments. There was no difference in the results if a fraction of pooled plasma or plasma from individual rats was infused into the recipient rats.

In experiments utilizing dog plasma both control and natriuretic plasmas were obtained from the same donor animal. In the first three experiments (Table II, experiments 6, 7, 9), both control and natriuretic plasma were obtained from the femoral artery. In subsequent experiments all samples were obtained through a catheter implanted into a jugular vein. Heparinized saline filled the entire length of the catheter until the blood samples were obtained. After collection of control plasma, bicarbonate-saline solution was infused through a leg vein at 15 ml/min. When sodium excretion increased to approximately 10–15% of the filtered sodium, blood was obtained for the experimental period.

Analytic methods. The concentration of inulin in tubular fluid was measured by the method of Vurek and Pegram (23). Inulin in plasma filtrate and urine was measured by the anthrone method (24). The concentration of inulin-^{14}C was measured by adding samples of infusion solution, plasma, urine, and tubular fluids to 10 ml of Bray's solution and counting in a Packard Tri-Carb liquid scintillation counter (Packard Instruments Co., Inc., Downers Grove, Ill.). The details of this technique have already been described (19). Sodium and potassium in plasma and urine were measured by flame photometry with lithium as an internal standard. Urine and plasma osmolalities were measured by the technique of Ramsay and Brown (25).

Calculations. Fractional reabsorption was calculated from the $(TF/P)_{In}$ ratio with the expression,

$$\text{fractional reabsorption} = [1 - (P/TF)_{In}]. \quad (1)$$

The intrinsic reabsorptive capacity, K (expressed as milliliter reabsorbed per second per milliliter of tubular volume), was either measured directly with the split-drop technique and calculated from the expression (20, 21),

$$K = \frac{0.693}{t_{1/2}}, \quad (2)$$

where $t_{\frac{1}{2}}$ is the time required to reabsorb 50% of the injected column of saline from the tubular lumen, or estimated indirectly from the $(TF/P)_{In}$ and transit time (T) by the expression (7, 19, 22),

$$\log \left(\frac{TF}{P} \right)_{In} = \frac{KT}{2.3}. \quad (3)$$

The ratio of tubular volume ($\pi r^2 d$) to GFR/per nephron (Vo) was estimated from the expression (7),

$$\text{fractional reabsorption} = K \frac{\pi r^2 d}{Vo}, \quad (4)$$

where r is the tubular radius and d is the tubular length.

RESULTS

Effects on intrinsic reabsorptive capacity (group I). The effects of plasma from control animals and from animals undergoing saline diuresis are shown in Tables I and II; experiments using plasma from rats are listed in Table I and those using plasma from dogs are listed in Table II. The administration of natriuretic plasma increased sodium excretion modestly in five experiments, considerably in one (Table I, experiment 1), and not at all in one (Table I, experiment 5). Urine flow rose only slightly. GFR was not significantly altered by the natriuretic plasma in any experiment.

The effects of control and natriuretic plasmas on the intrinsic reabsorptive capacity of the proximal tubule are also shown in Tables I and II.

Preliminary experiments revealed that plasma from control animals had no effect on intrinsic reabsorptive capacity. The average $t_{\frac{1}{2}}$ of 11.4 sec and K of 0.062 sec^{-1} obtained during control periods (Tables I and II) are similar to those obtained in assay rats not infused with donor plasma. The infusion of plasma from animals undergoing saline diuresis uniformly prolonged the $t_{\frac{1}{2}}$ and decreased K. In preliminary experiments changes in proximal reabsorption were noted within 10–15 min after starting the infusion of natriuretic plasma, and maximal effects were attained within 20–30 min; upon discontinuing the infusion of natriuretic plasma the inhibitory effects on proximal reabsorption were completely dissipated within 20–30 min. For this reason the equilibration period of 30 min was utilized in all of the experiments in Tables I and II. Plasma from natriuretic rats increased $t_{\frac{1}{2}}$ to an average value of 20.1 sec and inhibited K by an average of 40% (Table I). Plasma from natriuretic dogs inhibited K by an average of 33% (Table II). Upon discontinuing the infusion of natriuretic plasma and reinfusing the original control plasma, $t_{\frac{1}{2}}$ and K returned to or towards the control values in every experiment. In experiment 17 (Table II) in an additional experimental period natriuretic plasma inhibited K to the same degree as in the first experimental period, and in the final recovery period K returned to the control level. In experiment 18, despite reversal of the sequence in which natriuretic and control plasmas were infused, K was still 43% lower during the infusion of natriuretic plasma than during the administration of control plasma. Pretreatment of the assay animals with DOCA (Table I, experiments 4, 5; Table II, experiments 13, 14) did not alter the response to either control or natriuretic plasma.

Average transit time (T) through the proximal tubule fell from a control value of 12.5 sec to 10.6 sec during the administration of natriuretic plasma and then rose to 12.2 sec during the recovery period. Per cent of reabsorption, calculated from the K and T in each experiment (equation 3), fell from a control level of 53 to 35% during the infusion of natriuretic plasma and rose again to

TABLE I

Effect of Natriuretic Rat Plasma on Intrinsic Reabsorptive Capacity of the Proximal Tubule

Rat No.	Reabsorptive half-time ($t_{1/2}$)*	Intrinsic reabsorptive capacity		GFR	Urine flow	Sodium excretion	Transit time (T)
		K	Inhibition				
	sec	sec^{-1}	%	*ml/min per g*	*µl/min*	*µEq/min*	*sec*
1. Control	11.1 ± 0.28	0.062		4.73	6	0.27	12.5
Experimental	16.7 ± 0.18	0.042	33	4.81	59	10.89	8.2
Recovery	10.6 ± 0.16	0.065		4.94	14	5.36	11.6
2. Control	14.9 ± 0.15	0.046		5.28	5	0.69	10.3
Experimental	19.8 ± 0.14	0.035	25	5.36	11	1.46	9.9
Recovery	15.1 ± 0.15	0.046		5.12	3	0.64	11.5
3. Control	12.4 ± 0.28	0.058		7.12	3	0.07	12.5
Experimental	20.8 ± 0.16	0.033	42	7.68	5	0.36	10.6
Recovery	10.7 ± 0.29	0.065		7.91	2	0.07	13.0
4.‡ Control	12.2 ± 0.24	0.057		5.98	4	0.10	11.9
Experimental	25.3 ± 0.53	0.027	52	5.80	4	0.21	9.0
Recovery	10.4 ± 0.11	0.067		5.17	3	0.10	11.5
5.‡ Control	12.3 ± 0.23	0.056		6.45	5	0.63	14.0
Experimental	22.8 ± 0.35	0.030	49	6.89	6	0.65	14.0
Recovery	11.0 ± 0.24	0.063		6.97	7	0.64	14.0

K, intrinsic readsorptive capacity; GFR, glomerular filtration rate.

* Mean ± standard error.

‡ Assay animal pretreated with DOCA.

TABLE II

Effect of Natriuretic Dog Plasma on Intrinsic Reabsorptive Capacity of the Proximal Tubule

Rat No.	Reabsorptive half-time ($t_{1/2}$)*	Intrinsic reabsorptive capacity		GFR	Urine flow	Sodium excretion	Transit time (T)
		K	Inhibition				
	sec	*sec*$^{-1}$	%	*ml/min per g*	*µl/min*	*µEq/min*	*sec*
6. Control	10.1 ± 0.26	0.069					14.5
Experimental	12.2 ± 0.17	0.057	17				14.5
Recovery	10.5 ± 0.15	0.066					16.0
							14.5
7. Control	9.4 ± 0.13	0.074					9.0
Experimental	18.3 ± 0.17	0.038	49				6.8
Recovery	10.4 ± 0.14	0.067					9.0
8. Control	10.6 ± 0.16	0.065		7.03	7	0.47	11.8
Experimental	17.9 ± 0.20	0.039	41	7.41	8	0.99	8.5
Recovery	9.4 ± 0.21	0.074		7.52	7	0.81	10.0
9. Control	11.8 ± 0.15	0.059		6.87	3	0.17	14.0
Experimental	20.0 ± 0.48	0.027	55	6.78	9	0.65	10.2
Recovery	11.4 ± 0.25	0.061		7.00	6	0.32	14.0
10. Control	12.8 ± 0.23	0.053					12.3
Experimental	16.1 ± 0.18	0.043	20				11.5
Recovery	12.2 ± 0.39	0.057					13.0
11. Control	11.8 ± 0.13	0.059					15.5
Experimental	13.0 ± 0.14	0.053	10				14.5
Recovery	11.2 ± 0.11	0.062					13.5
12. Control	11.8 ± 0.31	0.059					13.0
Experimental	16.2 ± 0.28	0.043	27				11.8
Recovery	11.0 ± 0.26	0.063					14.5
13.‡ Control	11.5 ± 0.50	0.060					10
Experimental	16.6 ± 0.46	0.042	31				8
Recovery	11.4 ± 0.42	0.061					10
14.‡ Control	11.5 ± 0.41	0.060					13
Experimental	15.7 ± 0.38	0.044	27				11.8
Recovery	11.8 ± 0.41	0.059					14.5
15. Control	13.2 ± 0.32	0.053					10.0
Experimental	27.0 ± 0.37	0.033	31				10.0
Recovery	15.1 ± 0.26	0.046					10.0
16. Control	10.0 ± 0.22	0.069					15.0
Experimental	13.8 ± 0.24	0.050	28				12.5
Recovery	9.8 ± 0.22	0.071					15.0
17. Control	10.6 ± 0.13	0.065			4	0.47	11.8
Experimental	17.9 ± 0.36	0.039	41		13	1.73	8.5
Control	9.4 ± 0.22	0.074			13	1.32	9.0
Experimental	18.3 ± 0.35	0.038			13	2.53	6.8
Recovery	10.4 ± 0.24	0.067			16	2.32	9.0
18. Experimental	14.5 ± 0.16	0.048					12.0
Control	8.1 ± 0.10	0.086	43				14.0
Experimental	14.6 ± 0.12	0.047					12.0

* Mean ± standard error.

‡ Assay rat pretreated with DOCA.

TABLE III

Effect of Natriuretic Plasma on Per cent Reabsorption in the Proximal Tubule

Rat No.	GFR	Sodium excretion	Transit time	$(TF/P)_{In}$*	Proximal reabsorption	
	ml/min per kg	*μEq/min*	*sec*		%	*% change*
Experiments using rat plasma						
1. Control	4.70	0.28	12.0	196. ± 0.26	49	
Experimental	4.57	0.58	7.9	1.18 ± 0.09	15	−70
Recovery	5.52	0.99	8.3	1.58 ± 0.16	37	
2. Control	3.38	0.25	11.2	2.42 ± 0.09	59	
Experimental	3.29	0.30	8.5	2.09 ± 0.08	52	−12
Recovery	3.29	0.08	11.7	2.88 ± 0.14	65	
Experiments using dog plasma						
3. Control	4.84	0.17	11.8	3.33 ± 0.42	70	
Experimental	5.89	0.13	10.0	2.32 ± 0.30	57	−19
Recovery	4.45	0.10	11.0	2.78 ± 0.09	64	
4. Control	4.55	0.76	13.0	2.41 ± 0.08	59	
Experimental	4.56	0.74	7.9	1.72 ± 0.05	42	−29
Recovery	4.83	0.30	10.8	2.41 ± 0.23	59	
5. Control	4.87	0.44	9.5	2.16 ± 0.02	54	
Experimental	4.99	0.31	8.5	1.84 ± 0.10	46	−15
Recovery	5.02	0.13	9.5	2.17 ± 0.16	54	
6. Control	4.98	1.01	15.0	2.13 ± 0.18	52	
Experimental	5.00	1.44	10.1	1.68 ± 0.06	41	−22
Recovery	5.85	1.03	9.2	1.88 ± 0.13	47	
7. Control	5.96	0.94	9.8	2.00 ± 0.15	50	
Experimental	4.90	1.14	9.1	1.64 ± 0.06	39	−22
Recovery	5.33	1.71	9.7	2.08 ± 0.10	52	
8. Control	9.76	0.31	11.3	2.10 ± 0.08	52	
Experimental	7.44	2.40	8.8	2.21 ± 0.36	55	+6
9. Control	7.00	0.09	12.0	2.00 ± 0.15	50	
Experimental	7.00	0.34	11.0	1.84 ± 0.26	46	−8
Recovery	6.90	1.57	12.3	1.88 ± 0.02	47	
10. Control	6.23	0.42	13.0	2.71 ± 0.39	67	
Experimental	5.98	1.10	11.7	2.31 ± 0.11	57	−15
Recovery	6.05	0.84	10.9	2.54 ± 0.37	61	
11. Control	7.64	0.09	16.6	2.61 ± 0.21	62	
Experimental	7.13	0.28	10.3	2.38 ± 0.17	58	−6
Recovery	6.57	0.38	11.0	2.50 ± 0.16	60	
12. Experimental	4.66		11.6	2.36 ± 0.08	58	
Control	5.55		13.0	3.21 ± 0.06	69	−57
Experimental	3.70		12.1	2.26 ± 0.07	56	

$(TF/P)_{In}$, ratio of inulin concentration in tubular fluid and plasma.

* Mean ± standard error.

52% during the recovery period; the average depression in per cent of reabsorption was 34%. The ratio of tubular volume to GFR per nephron, $\pi r^2 d/Vo$, also calculated from K and T (equations 3 and 4), was not altered by the natriuretic plasma.

Effect of per cent reabsorption (group II). In the second group of experiments (Table III) the administration of natriuretic plasma did not produce any significant increase in sodium excretion that could be attributed to the effects of the plasma. In those experiments in which sodium excretion did increase during the experimental period, it did not return to the control level during the recovery period. GFR was not changed by the natriuretic plasma.

During control periods the $(TF/P)_{In}$ averaged 2.42 and per cent of reabsorption averaged 58%. The administration of natriuretic plasma reduced the $(TF/P)_{In}$ in 11 out of 12 experiments; in one of these (experiment 9) the $(TF/P)_{In}$ did not rise during the recovery period. During the experimental period $(TF/P)_{In}$ averaged 1.96 and per cent of reabsorption averaged 47%; per cent of reabsorption was depressed an average of 19%.

The average transit time (T) fell from 12.3 sec during the control period to 9.6 sec during the experimental period and rose to 10.4 sec during the recovery period. The intrinsic reabsorptive capacity, K, calculated from the $(TF/P)_{In}$ and transit time (equation 3), was depressed an average of 22% by the natriuretic plasma. The ratio of tubular volume to GFR per nephron, also calculated from the $(TF/P)_{In}$ and T (equations 3 and 4), was not altered during the experimental periods.

Effect on water and solute excretion in rats with diabetes insipidus. To determine the effect of natriuretic plasma on the delivery of filtrate out of the proximal tubule, six studies were performed

TABLE IV

*Effect of Natriuretic Rat Plasma on Water and Solute Excretion in Rats with Hereditary Diabetes Insipidus**

Rat No.	Urine osmolality	Urine flow	C_{osm}	C_{H_2O}	Sodium excretion	Potassium excretion	GFR
	mOsm/kg	*μl/min*	*μl/min*	*μl/min*	*μEq/min*	*μEq/min*	*ml/min per kg*
1. Control	254	8.0	7.0	1.0			
Experimental	123	42.0	17.8	24.2			
Recovery	195	12.0	8.0	4.0			
2. Control	165	13.0	8.3	4.7	0.24	0.31	
Experimental	85	60.7	20.5	40.2	1.31	0.56	
Recovery	196	24.0	17.0	7.0	0.36	0.12	
3. Control	231	25.0	19.8	5.2	0.40	0.11	5.36
Experimental	189	84.0	51.6	32.4	2.92	1.84	5.16
Recovery	235	45.0	35.5	9.5	1.11	0.60	5.20
4. Control	215	4.2	3.2	1.0	0.33	0.19	
Experimental	126	38.8	15.8	23.0	1.99	0.50	
Recovery	171	21.1	12.3	8.8	0.21	0.06	
5. Control‡	228	11.7	8.3	3.4	0.16	0.14	
Experimental	162	87.6	45.8	41.8	1.08	1.16	
Recovery	239	33.3	24.2	9.1	0.45	0.54	
6. Control‡	195	38.5	26.3	12.2	0.66	0.05	4.98
Experimental	145	143.6	66.4	77.2	4.21	3.09	5.19
Recovery	195	42.5	30.3	12.3	0.17	0.40	5.00

C_{osm}, osmolar clearance; C_{H_2O}, solute-free water clearance.

* All values are the average of three to four collection periods.

‡ Control plasma was obtained from rats with hereditary diabetes insipidus.

in rats with hereditary diabetes insipidus. As shown in Table IV urine flow rose sharply in every experiment during the infusion of natriuretic plasma, increasing from an average flow of 16.7 μl/min during control periods to 76.1 μl/min during the experimental periods. Although there was a significant drop in urine flow during the recovery period, the average value remained at levels almost twice the control values. Since the urine flow of 31.3 μl/min during recovery is close to the flow before the rats were anesthetized, it is possible that the relatively low flow at the beginning was the consequence of anesthesia.

The marked rise in urine volume in all experiments was principally due to an increase in C_{H_2O}, which rose from a control value of 4.6 μl/min to a value of 43.2 μl/min during the experimental period. As in the case of urine flow, although there was a sharp drop in C_{H_2O} during recovery, the value was about twice that observed during control periods. To exclude the possibility that the control plasma might have had much higher levels of ADH than the natriuretic plasma, thus causing the lower urine flow and C_{H_2O} during control and recovery periods than during the experimental periods, two experiments (Table IV, experiments 5 and 6) were performed with control plasma taken from rats with hereditary diabetes insipidus. The differences between control and natriuretic plasmas in these two experiments were the same as those in the other four experiments.

Natriuretic plasma also increased solute excretion in these rats. C_{osm} rose from a control level of 12.1 μl/min to an experimental value of 33.1 μl/min and fell to 20.1 μl/min during the recovery period. There was a modest increase in sodium excretion in all experiments, with a fall towards control levels during the recovery periods. Similar changes in potassium excretion were also noted. In the two experiments in which it was measured GFR was not changed.

Characteristics of the hormonal inhibitor of proximal reabsorption. Natriuretic plasma retained its inhibitory activity after standing at room temperature for 3–4 hr. As seen in Fig. 1 it also retained its activity when stored at 4°C for as long as 30 days. Inhibitory activity did not appear in control plasma after standing at room temperature for 3–4 hr or after storage at 4°C for several days. Thus the substance appears to be relatively stable. The full extent of its heat stability, however, has not yet been ascertained.

As shown in Table V the inhibitory activity was lost when natriuretic plasma was dialyzed against large volumes of Ringer's bicarbonate solution. When natriuretic plasma was dialyzed against equal volumes of Ringer's bicarbonate solution, inhibitory activity was observed when the dialysate was infused intravenously. Dialysate of control plasma had no effect. These studies, showing loss of the inhibitor from natriuretic plasma during dialysis and its recovery in the dialysate, indicate that the substance is probably a small molecular weight, water-soluble compound.

Site of action of the proximal inhibitor. The ability to recover the inhibitor in dialysates of

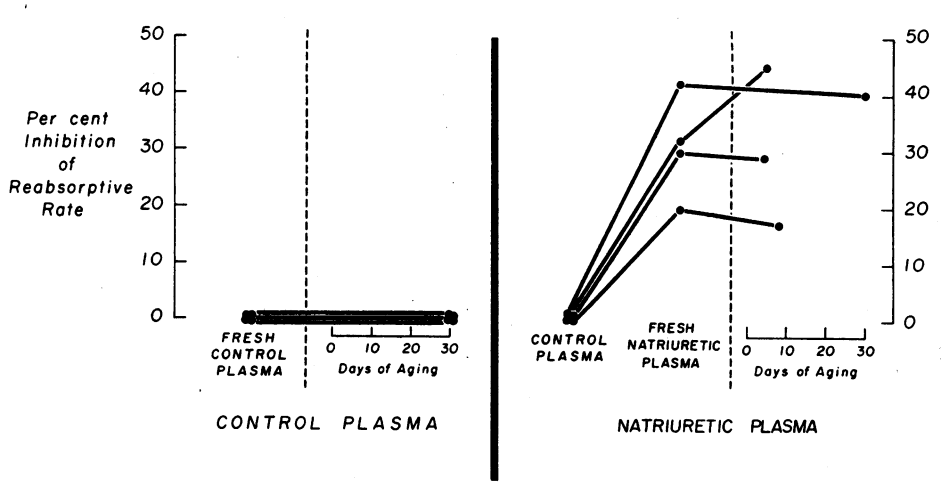

FIGURE 1 Effect of storage at 4°C on inhibitory activity of control and natriuretic plasma.

TABLE V

Effect of Dialysis on Inhibitory Activity of Natriuretic Plasma

Expt. No.	Reabsorptive half-time ($t_{1/2}$)	Intrinsic reabsorptive capacity	
		K	Inhibition
	sec	sec^{-1}	%
Dialysis against large volumes of fluid			
1. Control plasma	11.0	0.063	
Natriuretic plasma	15.2	0.045	28
Dialyzed natriuretic plasma	11.3	0.061	3
Control plasma	11.0	0.063	
2. Control plasma	10.8	0.064	
Natriuretic plasma	16.5	0.042	34
Dialyzed natriuretic plasma	11.1	0.062	
Control plasma	11.0	0.063	
Dialysis against equal volumes of fluid*			
3. Control plasma	11.3	0.61	
Natriuretic plasma	17.0	0.41	35
Dialyzed natriuretic plasma	10.8	0.64	0
Dialysate	16.6	0.42	34
Control plasma	10.8	0.64	

* Dialysate infused intravenously at 0.04 ml/min. Plasma infused intravenously at 0.02 ml/min.

TABLE VI

Intratubular Action of Dialysates of Natriuretic Plasma

Expt. No.	Reabsorptive half-time ($t_{1/2}$)	Intrinsic reabsorptive capacity	
		K	Inhibition
	sec	sec^{-1}	%
1. Ringer's solution	10.8 ± 0.32	0.064	
Dialysate-control plasma	9.6 ± 0.20	0.072	
Dialysate-natriuretic plasma	17.7 ± 0.24	0.039	45
Ringer's solution	9.1 ± 0.40	0.076	
2. Ringer's solution	15.0 ± 0.42	0.046	
Dialysate-control plasma	15.5 ± 0.54	0.045	
Dialysate-natriuretic plasma	20.3 ± 0.61	0.034	24
Ringer's solution	15.0 ± 0.57	0.046	
3. Ringer's solution	11.3 ± 0.38	0.061	
Dialysate-control plasma	12.2 ± 0.24	0.057	
Dialysate-natriuretic plasma	20.2 ± 0.58	0.034	43
Ringer's solution	11.5 ± 0.21	0.061	
4. Dialysate-control plasma	11.5 ± 0.16	0.060	
Dialysate-natriuretic plasma	16.2 ± 0.09	0.043	29
Dialysate-control plasma	11.5 ± 0.08	0.060	
5. Dialysate-control plasma	11.0 ± 0.89	0.063	
Dialysate-natriuretic plasma	16.3 ± 0.31	0.043	33
Dialysate-control plasma	11.0 ± 0.46	0.063	

natriuretic plasma made it possible to determine whether the substance could exert an effect when placed in contact with the luminal membrane of the tubule cell. As shown in Table VI dialysates of control plasma, when injected directly into the tubular lumen, were reabsorbed at the same rate as Ringer's bicarbonate solution. In contrast, dialysates of natriuretic plasma were reabsorbed at a much slower rate. The dialysates of natriuretic plasma inhibited K by an average of 35%, a degree of inhibition similar to that observed when either natriuretic plasma or dialysates were infused intravenously. These studies, therefore, clearly indicate that the substance can act from the luminal side of the cell.

It was surprising that the intratubular injection of dialysate, which should produce very high local concentrations, gave only the same degree of inhibition as the intravenous infusions. To pursue the quantitative aspects of this problem further the effect of serially diluting jugular venous plasma and dialysates was examined. As shown in Fig. 2 the inhibitory effect of natriuretic plasma infused intravenously was almost completely lost when the plasma was diluted 1:4 with Ringer's bicarbonate. In three other experiments natriuretic plasma infused intravenously lost all activity at a 1:2 dilution. In one of these experiments inhibitory activity was obtained when the diluted plasma was infused at twice the rate (0.04 ml/min). In contrast, dialysate injected into the tubular lumen produced the same inhibitory effect (approxi-

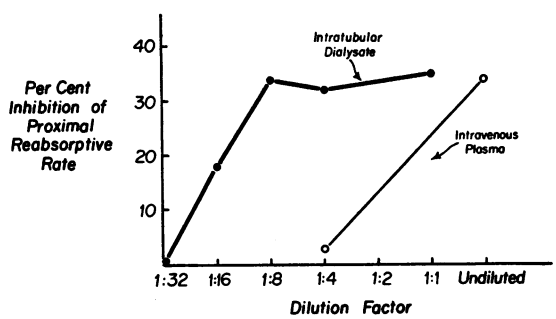

FIGURE 2 Effect of concentration of hormonal inhibitor on intrinsic reabsorptive capacity of the proximal tubule. The dialysis of plasma against an equal volume of Ringer's bicarbonate results in a concentration of inhibitor in the dialysate of exactly half that of undialyzed plasma. For this reason the horizontal axis is adjusted so that undiluted dialysate has a dilution factor of 1:1.

mately 35%) when diluted 1:2 and 1:4 as with the undiluted dialysate; activity was less at a 1:8 dilution and was completely gone at a 1:16 dilution. Since the inhibitor is diluted by a factor of 2 in the process of dialysis, the undiluted dialysate is equivalent to a 1:1 dilution of plasma and the 1:2, 1:4, 1:8, and 1:16 dilutions of dialysate are equivalent respectively to 1:4, 1:8, 1:16, and 1:32 dilutions of plasma. The intratubular injection, therefore, appears to result in the delivery to the reabsorptive site of about eight times more inhibitor than does the intravenous infusion. The studies with the dilutions of dialysate (Fig. 2) indicate that the process of inhibition exhibits saturation characteristics with a maximum inhibition of approximately 30–40%. For this reason, both the intravenous infusion of plasma and the intratubular injection of dialysate produce the same inhibitory effect despite the much higher local concentrations attained with the intratubular injections.

DISCUSSION

Previously we have suggested that fractional reabsorption in the proximal tubule is regulated by the interaction of two variables: the intrinsic reabsorptive capacity, K, and the ratio of tubular volume of GFR per nephron [$\pi r^2 d$/Vo (7, 19, 22)]. Recent studies in our laboratory (7) have shown that expansion of ECF volume with isotonic saline in rats depresses fractional reabsorption in the proximal tubule by reducing K 33% and $\pi r^2 d$/Vo 25%. We proposed that the decrease in K might be due to some hormonal inhibitor, whereas the decrease in $\pi r^2 d$/Vo might be mediated by changes in renal interstitial volume secondary to alterations in the hydrostatic and oncotic pressures in the peritubular capillary bed (7). Indeed, Earley, Martino, and Frielder (15) have shown that experimental maneuvers which modify hydrostatic and oncotic pressures in the postglomerular capillaries markedly influence sodium excretion.

The present experiments clearly demonstrate that a hormonal inhibitor of proximal reabsorption is elaborated in response to expansion of ECF volume with isotonic saline. Three lines of evidence support this conclusion. First, infusions of plasma from animals undergoing saline diuresis inhibited the intrinsic reabsorptive capacity of the proximal tubule, as measured by the shrinking-drop technique. The fact that dialysates of natriuretic plasma placed directly into the tubular lumen also inhibited intrinsic reabsorptive capacity unequivocally establishes a direct inhibitory action on the tubular epithelium rather than any indirect effects secondary to either compositional changes in blood or alterations in renal hemodynamics. Second, infusions of natriuretic plasma reduced $(TF/P)_{In}$ ratios, indicating a net reduction of proximal reabsorption. Third, infusions of natriuretic plasma increased both urine flow and cation excretion in rats with hereditary diabetes insipidus. Since minimal amounts of water are reabsorbed in the distal diluting segments of the nephron in the absence of ADH, the rise in urine flow can be used as evidence of accelerated delivery of filtrate out of the proximal portions of the nephron. Although there was a significant increase in sodium excretion in all six experiments (Table IV), most of the increased sodium delivered out of the proximal tubule was reabsorbed distally, resulting in augmented C_{H_2O}. In addition, potassium excretion rose also, indicating that a portion of the sodium delivered out of the proximal tubule was reabsorbed distally in exchange for potassium. All three types of studies, therefore, indicate that plasma from animals undergoing saline diuresis contains a humoral agent capable of inhibiting the intrinsic reabsorptive capacity of the proximal tubule, thereby producing a net suppression of proximal reabsorption and increasing the delivery of filtrate to more distal portions of the nephron.

The exact mechanism by which this hormone inhibits proximal reabsorption cannot be ascertained from the present studies. However, certain characteristics of its action can be described. First, the material acts both when infused intravenously and when injected directly into the tubule. This finding does not necessarily mean that the hormone reaches the transport site through both surfaces of the tubular cell. Substances such as digitalis, vasopressin, and aldosterone, which are known to influence transport across epithelial structures (frog skin, toad bladder, and isolated collecting ducts), have been demonstrated to gain access to their site of action through only one surface of the epithelial cell, even though the final site of action may be on the opposite surface. If the renal tubular cell behaves like these isolated epithelial structures,

it is probable that the tubular surface is the portal of entry, since the material injected intratubularly could have had contact with only the luminal surface of the cell. The material injected intravenously would not be limited to the blood surface of the renal tubular cell. Since it is dialyzable, it can doubtless pass through the glomerulus and thus would have access to the luminal surface of the tubule.

The second important characteristic of the inhibitory action on the proximal tubule is that the humoral agent exhibits saturation kinetics. The studies utilizing serial dilutions of dialysate (Fig. 2) indicate that a maximal inhibition of 35–40% is maintained over about an eightfold concentration range. As the dialysate is diluted by greater than 1 : 8 the inhibiting effect is rapidly lost. The saturation kinetics explain the finding that the infusion of natriuretic plasma at a very low rate (0.02 ml/min) inhibited the intrinsic reabsorptive capacity to the same degree (Tables I and II) as that observed in rats undergoing massive saline diuresis (7, 8, 26), where the circulating level of the inhibitor has been shown, in unpublished studies, to be very much higher. Indeed, preliminary studies in our laboratory suggest that there is a rough correlation between the level of circulating hormone and the magnitude of ECF volume expansion. The significance of such a variation in plasma levels is not clear, however, since the present studies would indicate that maximal inhibition is achieved at very low plasma concentrations and that any further rise would have little additional effect. It is possible, on the other hand, that if the hormone acts only from the luminal side of the cell and is either reabsorbed or inactivated by the tubular epithelium, variations in plasma concentration might be of physiologic importance by determining the length of the tubule inhibited.

Finally, the luminal inhibition has a very rapid onset of action, within seconds after its instillation into the tubular lumen. In this respect it differs from aldosterone, where a lag of at least 0.5 hr occurs before transport is stimulated, presumably because aldosterone stimulates the synthesis of an intermediate agent, perhaps a specific protein, which directly influences transport (27, 28). The rapid onset of action of the hormonal inhibitor suggests that it has a more direct effect and does not act by stimulating the synthesis of an active intermediary. Its duration of action is comparatively short, lasting no more than 20–30 min after the discontinuation of the intravenous infusion of natriuretic plasma. Sustained natriuresis, therefore, to the extent that it is mediated by the humoral inhibitor, would seem to require persistent expansion of ECF volume to insure continued production.

The elaboration of a potent hormonal inhibitor of proximal reabsorption in response to ECF volume expansion would explain some, but not all, of the changes observed during saline diuresis. The infusion of natriuretic plasma inhibited the intrinsic reabsorptive capacity to the same degree as did massive saline infusions (7), yet fractional reabsorption during free flow was reduced only 20% in contrast to the 50% depression observed during saline diuresis. This discrepancy is probably the consequence of two factors. First, because of higher plasma concentrations of hormone the entire length of the proximal tubule might be maximally inhibited during saline diuresis, but not during infusion of natriuretic plasma. Since for technical reasons most shrinking-drop measurements are made in the earlier portions of the proximal convolution, this discrepancy might not be detected. Second, saline infusions reduced the ratio of tubular volume to GFR per nephron by approximately 25%, whereas the infusion of natriuretic plasma had no effect on tubular geometry. Since the absolute rate or proximal reabsorption has been shown to vary directly with tubular volume (19–22),[2]

[2] The influence of tubular geometry also affords an attractive explanation for the different effects of the hormone and another potent inhibitor of proximal reabsorption, furosemide. As judged by the shrinking-drop technique, furosemide inhibits the intrinsic reabsorptive capacity of the proximal tubule to the same extent as does the hormone (29). Yet furosemide does not depress net proximal reabsorption, whereas the hormone does. The explanation, doubtless, revolves about the potent inhibition of reabsorption in the ascending limb by furosemide (30, 31). In consequence, large amounts of fluid issue into the collecting duct, generating internal hydronephrosis, which, in turn, causes proximal tubular dilatation, thereby overcoming the inhibitory effect of the drug (29). The hormone, at least in the concentrations resulting from the infusion of natriuretic plasma, exerts little, if any, distal effect, so that internal hydronephrosis does not occur. Therefore, the inhibition of intrinsic reabsorptive capacity is not masked by the enhancing effects of tubular dilatation, and net inhibition of proximal reabsorption results.

the reduction of $\pi r^2 d/Vo$ during saline diuresis could account for the greater suppression of fractional reabsorption. The fall in $\pi r^2 d/Vo$ is probably the consequence of an increased interstitial volume secondary to the combined effects of decreased plasma protein, reduced renal vascular resistance, and increased blood pressure (7, 14, 15) which occur during massive saline infusions but not during the infusion of small amounts of natriuretic plasma.

Another major difference between the effects of the hormone and saline diuresis is that, despite significant inhibition of proximal reabsorption, the infusion of natriuretic plasma failed to increase sodium excretion appreciably. This finding clearly indicates the importance of inhibition of reabsorption beyond the proximal tubule in promoting saline diuresis. Evidence from both C_{H_2O} studies (32, 33) and micropuncture experiments (34) indicate that the expansion of ECF volume suppresses sodium reabsorption in the distal nephron. There are several possible explanations as to why the infusion of saline, but not the infusion of small amounts of natriuretic plasma containing the hormone, inhibits distal reabsorption. It is possible that the hormone inhibits sodium reabsorption along the entire length of the nephron, but that, owing to the low circulating levels achieved by the plasma infusions, the hormone is reabsorbed from the tubular fluid and does not reach sufficiently high concentrations distally to inhibit the tubule. In contrast, in animals infused with saline far higher concentrations of hormone circulate in the blood, much of which escapes proximal reabsorption and achieves sufficiently high luminal concentrations distally to produce maximal inhibition. Even in the presence of high circulating levels, however, the hormone would probably account for only a fraction of the inhibition of distal reabsorption. Hemodynamic factors, such as increased medullary blood flow with washout of the hypertonic medullary interstitium (35) and redistribution of filtrate from deep nephrons with high capacity loops to superficial nephrons with low capacity loops (16, 36) probably account for most of the reduction of the total effective reabsorptive capacity of the distal nephrons during saline diuresis.

It would appear, therefore, that although a potent inhibitor of proximal reabsorption is released during ECF volume expansion, its full physiologic effect is not expressed unless it is accompanied by the associated renal hemodynamic changes that occur during saline diuresis. These changes might potentiate the effect of the hormone by altering the geometry of the proximal tubule, by increasing medullary blood flow, and by redistributing filtrate from deep nephrons with high capacity loops to superficial nephrons with low capacity loops.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the American Heart Association and in part by training grant 5 TI HE 5469 from the National Institutes of Health, U. S. Public Health Service. Dr. Martinez-Maldonado performed his work as a trainee of the U. S. Public Health Service, grant 5 TI HE 5469.

REFERENCES

1. DeWardener, H. E., I. H. Mills, W. F. Clapham, and C. J. Hayter. 1961. Studies on the efferent mechanism of sodium diuresis which follows the administration of intravenous saline in the dog. *Clin. Sci.* **21**: 249.
2. Levinsky, N. G., and R. C. Lalone. 1963. The mechanism of sodium diuresis after saline infusion in the dog. *J. Clin. Invest.* **42**: 1261.
3. Rector, F. C., Jr., G. Van Giesen, F. Kiil, and D. W. Seldin. 1964. Influence of expansion of extracellular volume on tubular reabsorption of sodium independent of changes in glomerular filtration rate and aldosterone activity. *J. Clin. Invest.* **43**: 341.
4. Levinsky, N. G. 1966. Nonaldosterone influences on renal sodium transport. *Ann. N. Y. Acad. Sci.* **139**: 295.
5. Dirks, J. H., W. J. Cirksena, and R. W. Berliner. 1965. The effect of saline infusion on sodium reabsorption by the proximal tubule of the dog. *J. Clin. Invest.* **44**: 1160.
6. Cortney, M. A., M. Mylle, W. E. Lassiter, and C. W. Gottschalk. 1965. Renal tubular transport of water, solute, and PAH in rats loaded with isotonic saline. *Am. J. Physiol.* **209**: 1199.
7. Rector, F. C., Jr., J. C. Sellman, M. Martinez-Maldonado, and D. W. Seldin. 1967. The mechanism of suppression of proximal tubular reabsorption by saline infusions. *J. Clin. Invest.* **46**: 47.
8. Landwehr, D. M., R. M. Klose, and G. Giebisch. 1967. Renal tubular sodium and water reabsorption in the isotonic sodium chloride-loaded rat. *Am. J. Physiol.* **212**: 1327.
9. Cort, J. H. 1965. Electrolytes, Fluid Dynamics and the Central Nervous System. Academic Press Inc., New York. 139.
10. Lichardus, B., and J. W. Pearse. 1966. Evidence for a humoral natriuretic factor released by blood volume expansion. *Nature.* **209**: 407.

11. Johnston, C. I., and J. O. Davis, 1966. Evidence from cross-circulation studies for a humoral mechanism in the natriuresis of saline loading. *Proc. Soc. Exptl. Biol. Med.* **121**: 1058.
12. Johnston, C. I., J. O. Davis, S. S. Howards, and F. S. Wright. 1967. Cross-circulation experiments on the mechanism of the natriuresis during saline loading in the dog. *Circulation Res.* **20**: 1.
13. Earley, L. E., and R. M. Friedler. 1965. Studies on the mechanism of natriuresis accompanying increased renal blood flow and its role in the renal response to extracellular volume expansion. *J. Clin. Invest.* **44**: 1857.
14. Earley, L. E., and R. M. Friedler. 1966. The effects of combined renal vasodilatation and pressor agents on renal hemodynamics and the tubular reabsorption of sodium. *J. Clin. Invest.* **45**: 542.
15. Earley, L. E., J. A Martino, and R M. Friedler 1966. Factors affecting sodium reabsorption by the proximal tubule as determined during blockade of distal sodium reabsorption. *J. Clin. Invest.* **45**: 1668.
16. Barger, A. C. 1966. Renal hemodynamic factors in congestive heart failure. *Ann. N. Y. Acad. Sci.* **139**: 276.
17. Starling, E. H., and E. B. Verney. 1925. The secretion of urine as studied on the isolated kidney. *Proc. Roy. Soc. (London) Ser. B.* **97**: 321.
18. Mills, I. H., G. N. Osbeldiston, G. M. Grain. and B. L. Wise. 1966. The effect of change in perfusion pressure and hematocrit in the perfused isolated dog kidney. *Proc. Phys. Soc. (London)* **44**.
19. Rector, F. C., Jr., F. P. Brunner, and D. W. Seldin. 1966. Mechanism of glomerulotubular balance. I. Effect of aortic constriction and elevated ureteropelvic pressure on glomerular filtration rate, fractional reabsorption, transit time, and tubular size in the proximal tubule of the rat. *J. Clin. Invest.* **45**: 590.
20. Gertz, K. H. 1963. Transtubulare Natriumchloridflüsse und Permeabilität für Nichtelektrolyte in proximalen und distalen Konvolut der Rattenniere. *Pfleugers Arch. Ges. Physiol.* **276**: 336.
21. Brunner, F. P., F. C. Rector, Jr., and D. W. Seldin. 1966. Mechanism of glomerulotubular balance II. Regulation of proximal tubular reabsorption by tubular volume, as studied by stopped-flow microperfusion. *J. Clin. Invest.* **45**: 603.
22. Gertz, K. H., J. A. Mangos, G. Braun, and H. D. Pagel. 1965. On the glomerular tubular balance in the rat kidney. *Pfleugers Arch. Ges. Physiol.* **285**: 360.
23. Vurek, G. G., and S. E. Pegram. 1966. Fluorometric method for the determination fo nanogram qualities of inulin. *Anal. Biochem.* **16**: 409.
24. Davidson, W. D., and M. A. Sackner. 1963. Simplification of the anthrone method for the determinations of inulin in clearance studies. *J. Lab. Clin. Med.* **62**: 351.
25. Ramsay, J. A., and R. H. J. Brown. 1955. Simplified apparatus and procedure for freezing point determination upon small volumes of fluid. *J. Sci. Instr.* **32**: 372.
26. Hayslett, J. P., M. Kashgarian, and F. H. Epstein. 1967. Changes in proximal and distal reabsorption produced by rapid expansion of extracellular fluid. *J. Clin. Invest.* **46**: 1254.
27. Edelman, I. S., R. Borgoroch, and G. A. Porter. 1963. On the mechanism of action of aldosterone on sodium transport. The role of protein synthesis. *Proc. Natl. Acad. Sci. U. S.* **50**: 1169.
28. Williamson, H. E. 1963. Mechanism of the antinatriuretic action of aldosterone. *Biochem. Pharmacol.* **12**: 1449.
29. Rector, F. C., Jr., F. P. Brunner, J. C. Sellman, and D. W. Seldin. 1966. Pitfalls in the use of micropuncture for the localization of diuretic action. *Ann. N. Y. Acad. Sci.* **139**: 400.
30. Suki, W., F. C. Rector, Jr., and D. W. Seldin. 1965. The site of action of furosemide and other sulfonamide diuretics in the dog. *J. Clin. Invest.* **44**: 1458.
31. Seldin, D. W., G. Eknoyan, W. N. Suki, and F. C. Rector, Jr. 1966. Localization of diuretic action from the pattern of water and electrolyte excretion *Ann. N. Y. Acad. Sci.* **139**: 328.
32. Eknoyan, G., W. N. Suki, F. C. Rector, Jr, and D. W. Seldin. 1967. Functional characteristics of the diluting segment of the dog nephron and the effect of extracellular volume expansion on its reabsorptive capacity. *J. Clin. Invest.* **46**: 1178.
33. Stein, R. M., R. G. Abramson, T. Kahn, and M. F. Levitt. 1967. Effects of hypotonic saline loading in hydrated dog: evidence for a saline-induced limit on distal tubular sodium transport. *J. Clin. Invest.* **46**: 1205.
34. Sellman, J. C., F. J. Oerther, F. C. Rector, Jr., and D. W. Seldin. 1967. Evidence for inhibition of distal tubular reabsorption during saline diuresis. *Clin. Res.* **15**: 370. (Abstr.)
35. Earley, L. E., and R. M. Friedler. 1964. Observations on the mechanism of decreased tubular reabsorption of sodium and water during saline loading. *J. Clin. Invest.* **43**: 1928.
36. Sellman, J. C., M. Martinez-Maldonado, F. C. Rector, Jr., and D. W. Seldin. 1967. Influence of saline infusion on renal cortical hemodynamics and proximal tubular reabsorption. *Clin. Res.* **15**: 78. (Abstr.)