Effect of Saline Infusions on Intrarenal Distribution

of Glomerular Filtrate and Proximal Reabsorption in the Dog

Henry Mandin, Arnold H. Israelit, Floyd C. Rector, Jr., and
Donald W. Seldin

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

ABSTRACT The effect of acute extracellular volume expansion with saline on the intrarenal distribution of glomerular filtrate, was studied in dogs utilizing micropuncture techniques in which samples were obtained by both recollection and from new tubules.

Recollection was examined in seven dogs during continuous hydropenia and in five dogs during continuous saline diuresis. Recollection was associated with an increase in nephron flow rate of 8% during hydropenia and 27% during saline diuresis. In addition, during continuous saline diuresis, shortened transit times and lowered intratubular pressures were recorded in previously punctured tubules. Despite increased tubular flow, fractional reabsorption was unchanged.

Nephron glomerular filtration rates (gfr) were measured during hydropenia and then after acute volume expansion in 10 dogs. In the repunctured tubules gfr rose 38% more than total glomerular filtration rate (GFR). In contrast, when new tubules were punctured during volume expansion, nephron gfr and total GFR changed proportionately. The disproportionate rise in nephron gfr after volume expansion noted with the recollection technique appears to be artifactual when contrasted to micropuncture of new tubules. With acute volume expansion, fractional reabsorption decreased 15% in recollected samples and 16% in newly sampled tubules. Increased nephron gfr cannot account for the fall in fractional reabsorption. It is concluded that in dogs, saline diuresis is not associated with redistribution of filtrate from deep to superficial nephrons, and that the fall in proximal fractional reabsorption is caused by diminished absolute reabsorption.

INTRODUCTION

Two recent studies on the effects of acute volume expansion with saline infusion in dogs (1, 2) indicated that the increase in superficial nephron filtration rate was much greater than the increase in total GFR,[1] thereby suggesting redistribution of filtrate to superficial nephrons. The increase in superficial nephron filtration rate in these two studies was of such magnitude (57 and 62%) that it accounted for a major portion of the fall in fractional reabsorption after saline, with only modest or no change in absolute reabsorption.

A critical weakness of our previous study (1) was the poor reproducibility of nephron filtration rates in recollected samples. If for any reason recollections during saline diuresis were excessively large, thereby falsely raising filtration rates, a systematic error would be introduced, which would suggest redistribution of filtrate with volume expansion. Although it has been shown that recollection technique does not influence TF/P inulin ratios during either continued hydropenia or continued saline diuresis (3), the effect of recollection on flow rates has been tested only during continued hydropenia.

The present study was therefore undertaken with the following aims: (*a*) to ascertain whether recollection technique artifactually alters measurements of tubular flow rates during hydropenia or volume expansion; (*b*) to determine the relationship of superficial nephron filtration rate to total filtration rate in volume expansion in order to clarify whether redistribution of filtrate occurs and, if so, to what extent; and (*c*) to define the

[1] Abbreviations used in this paper: GFR, total kidney nephron glomerular filtration rate; gfr, single nephron glomerular filtration rate.

relative contribution of elevated nephron filtration and suppressed absolute reabsorption in volume expansion to the increased delivery of fluid out of the proximal tubule.

METHODS

Studies were performed in 22 mongrel dogs weighing between 12 and 22 kg. All animals were deprived of food for 18–24 hr before the study, but were allowed free access to water. The dogs were anesthetized with pentobarbital (20 mg/kg) and were subsequently given small maintenance doses as necessary. An endotracheal tube was inserted and the animals were ventilated with a Harvard Respirator (Harvard Apparatus, Millis, Mass.). Cannulas were inserted in two leg veins for infusions and in the femoral artery for blood pressure measurements and blood collection. Both ureters were cannulated through a suprapubic incision. The left kidney was exposed through a flank incision and the renal pedicle was gently dissected free for a distance of 2–3 inches from the hilum. The dog was then placed on a marble table with the right side down and the kidney was placed in a Plexiglas cup mounted on a steel table situated directly above the dog. A 1 cm sq of capsule was removed and the surface was bathed with mineral oil at 37°C.

Micropuncture

A priming dose of 250 mg/kg of inulin was followed by a constant infusion at a rate of 4.8 mg/kg in 1 ml/min Ringer's solution. Samples of proximal tubular fluid were obtained with sharpened micropipettes containing colored mineral oil. Each tubule was blocked with a long column of oil and all fluid reaching the puncture site was collected in a precisely timed interval to permit the determination of tubular flow rate. Three types of recollection experiments were performed:

(a) In seven experiments the infusion rate was maintained at 1 ml/min throughout the entire experiment. The recollections were obtained less than 5 min after the initial sample was collected. In each experiment six to seven recollection pairs were obtained.

(b) In five experiments the animals were first expanded with Ringers solution. An infusion of 40 ml/kg over 30 min was followed by a maintenance infusion set at 2 ml/min above urine flow rate. The initial collections were obtained after the diuresis was well established. Once again, recollections were obtained within 5 min after the initial samples. In each experiment eight to nine recollection pairs were obtained. In addition to flow rate and TF/P inulin ratios, transit times were determined in this set of experiments so that they might serve as an additional indicator of tubular flow dynamics. Transit times to individual tubules were measured with the aid of a curved needle inserted into the left renal artery through which lissamine green in 100 μl vol could be injected. The time interval between the green capillary flush and the appearance of the dye in the chosen tubule was measured with a stop watch. The initial transit time was measured before the tubule was punctured. The second transit time was measured after the tubule was punctured, but before the tubule was repunctured for the second collection. In five additional experiments, intratubular pressure was measured either by means of a Kulite microtransducer (Kulite Semiconductor Products Inc., Ridgefield, N. J.) (4) or by the Landis technique (5). The pressure in previously punctured tubules was compared to the pressures in surrounding tubules.

(c) In 10 experiments, after a hydropenic control period, the dogs were expanded with infusions of Ringers solution as described above. During the hydropenic control period, five to six tubules were punctured, and at the end of each collection an adjacent tubular segment was marked with nigrosine and a map was drawn of the area to facilitate identification during the experimental period. At the completion of the period, 1 hr was allowed to elapse before starting the experimental period. During the experimental period each of the previously punctured tubules was repunctured at the same site. In addition to recollection samples, a similar number of new tubules previously unpunctured were also sampled. The recollected and new samples were obtained in random order.

Clearances

Glofil ¹²⁵I (Abbott Laboratories, North Chicago, Ill.) loading doses were given and maintained. In the first two groups of experiments (17 dogs) three 15–20-min clearance periods were obtained. These periods were spaced to span the duration of the entire experiment.

In the last series of 10 experiments three 15–20 min clearance periods were obtained in both the control and experimental periods. GFR was determined from the clearance of ^{125}I Iodothalamate (Glofil) (6–11). Previous studies in our laboratories revealed close agreement between simultaneous Glofil and inulin clearances in the dog. ^{125}I activity was measured in a gamma spectrometer.

In the micropuncture experiments, plasma inulin concentrations were determined by the diphenylamine method (12). The concentration of inulin in tubular fluid was measured by the fluorometric method of Vurek and Pegram (13). Tubular fluid volume was measured in a constant bore capillary tube of known internal diameter. Sodium and potassium concentrations in urine and plasma were measured by an Instrumentation Laboratory flame photometer (Instrumentation Laboratory Inc., Lexington, Mass.).

RESULTS

Effects of recollection technique during continued hydropenia. The effect of recollection on tubular fluid flow rates during continued hydropenia is shown in Fig. 1. In 44 recollections, the ratio of the recollection to the initial flow rate was 1.08 ±0.02 SE. TF/P inulin ratios were measured in 28 of these recollection pairs (Fig. 2). The ratio of the recollection to the initial TF/P inulin ratio was 0.99 ±0.01 SE. Therefore, the repeated puncture of the tubules does not alter TF/Pinulin ratios, but is associated with a small rise in flow rate during hydropenia (P < 0.05).

Effect of recollection technique during continued saline diuresis. The effect of recollection on transit time during continued saline diuresis is shown in Fig. 3. In six tubules the transit time following puncture was prolonged. In the remaining 37 tubules there was a shortening of transit time. Since lissamine green dye could be seen emerging from the initial puncture site, it is presumed that the shortened transit time was the result of a tubular leak. The six tubules with prolonged transit time may represent tubules in which the oil block was trapped in the loops of Henle and the puncture site became totally or partially sealed.

The effect of recollection on nephron flow rates during saline diuresis is shown in Fig. 4. The tubules in which transit time became prolonged following puncture tended to have lower recollected flow rates. In the tubules with shortened transit times the ratio of the recollection to the initial
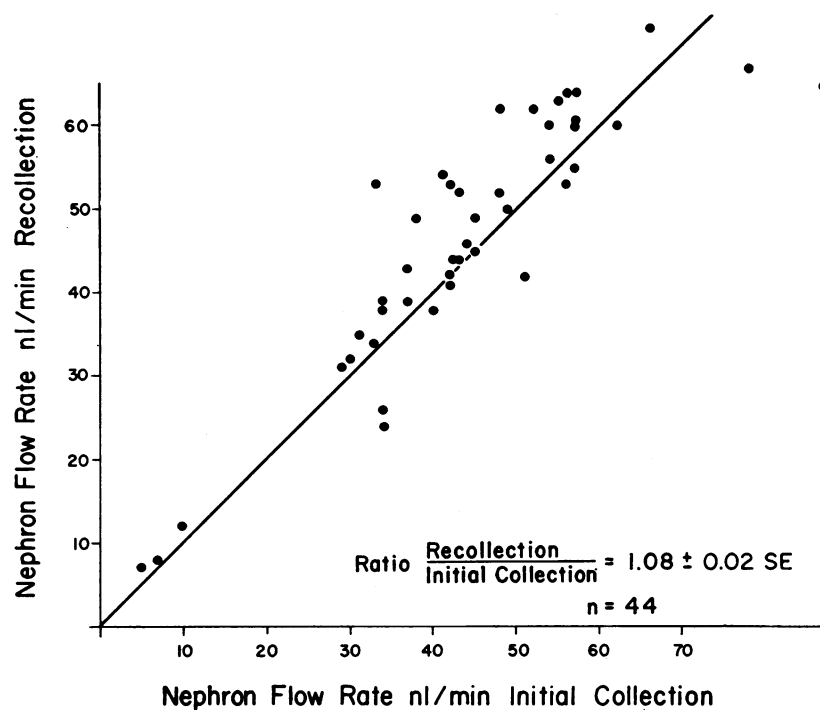
FIGURE 1   Effect of recollection on nephron flow rate during continued hydropenia.
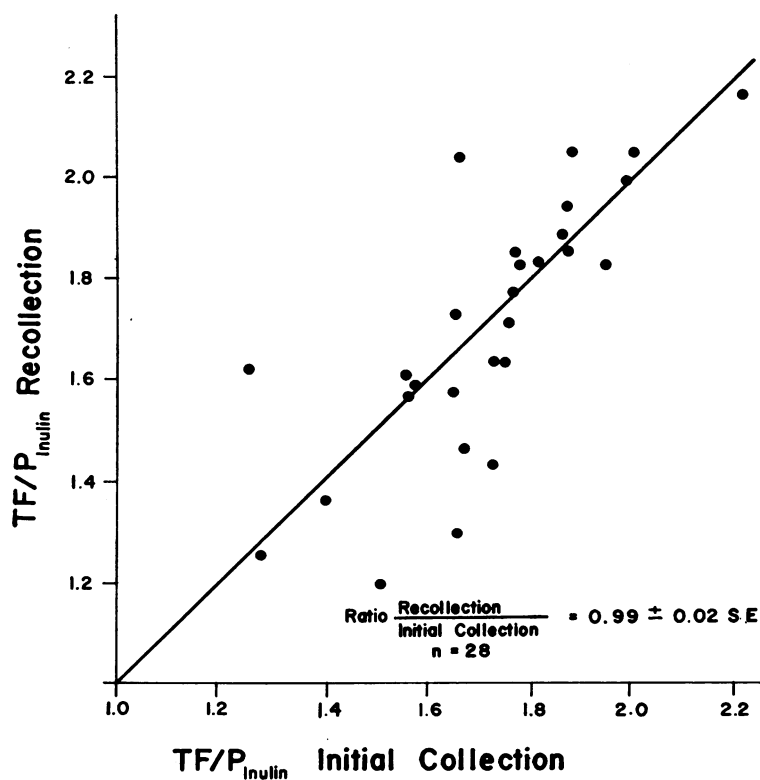

FIGURE 2   Effect of recollection on TF/P inulin ratio during continued hydropenia.

flow rate was 1.27 ±0.04 SE. In contrast to the mild effect during hydropenia, repeated puncture of the tubules resulted in an increase in the flow rates of those nephrons with shortened transit times ($P < 0.001$).

There appeared to be no significant change in the TF/P inulin ratio when all 43 recollection pairs were examined; the ratio of recollected to initial TF/P inulin was 0.98 ±0.01 SE (Fig. 5). However, when only those recollection pairs (n = 27) in which tubular flow increased greater than 10% are included, the ratio of the recollected to initial TF/P inulin was 0.94 ±0.01 SE ($P < 0.001$).

The average intratubular pressure during saline diuresis was 44 cm H₂O. In a total of 25 tubules that had been previously punctured the pressure was lower by an average of 5 cm H₂O when compared to surrounding unpunctured tubules. Only 4 out of 25 previously punctured tubules had higher pressures (Table I).

Effect of volume expansion. A protocol of one experiment is shown in Table II. A summary of all 10 micropuncture studies following volume expansion is shown in Table III. In these studies sodium excretion increased from an average of 23 μEq/min during hydropenia to an average of 276 μEq/min after saline, while fractional excretion of sodium rose from 0.5 to 5.7%. A total of 56 tubules were punctured before volume expansion and 47 of these could be repunctured after volume expansion. There was a mean fall of 15% in TF/P inulin after saline (Fig. 6).

Total GFR increased in 5 of 10 studies, but the mean increase was only 2% (Table III). Nephron gfr in 47 new tubules punctured during volume expansion increased 1% (Table III). These are not significant changes. On the other hand, nephron gfr, when samples were recollected during volume expansion, increased 40% (Table III).

An analysis of the individual experiments revealed that average nephron gfr, when new tubules were samples during volume expansion, changed to the same extent as total filtration rate. On the other hand, when samples were recollected during volume expansion, nephron gfr increased to a greater extent than total GFR in each of the 10 studies (Table III, Fig. 7).

DISCUSSION

Fractional reabsorption of sodium in the proximal tubule is decreased by expansion of extracellular fluid volume with isotonic saline (3, 14, 15). This might be due to an inhibition of tubular reabsorption, an increase in filtration rate, or a combination of both factors. Two lines of evidence have been used to suggest that an inhibition of tubular reabsorption is involved in the fall in fractional reabsorption. Dirks, Cirksena, and Berliner (3) found in the dog that fractional reabsorption was suppressed after volume expansion, and that this reduction persisted when filtration rate was lowered below hydropenic levels by arterial clamping. Other investigators, however, have found that fractional reabsorption during saline diuresis tends to return toward hydropenic levels after aortic constriction in the rat (16–18). Second, the
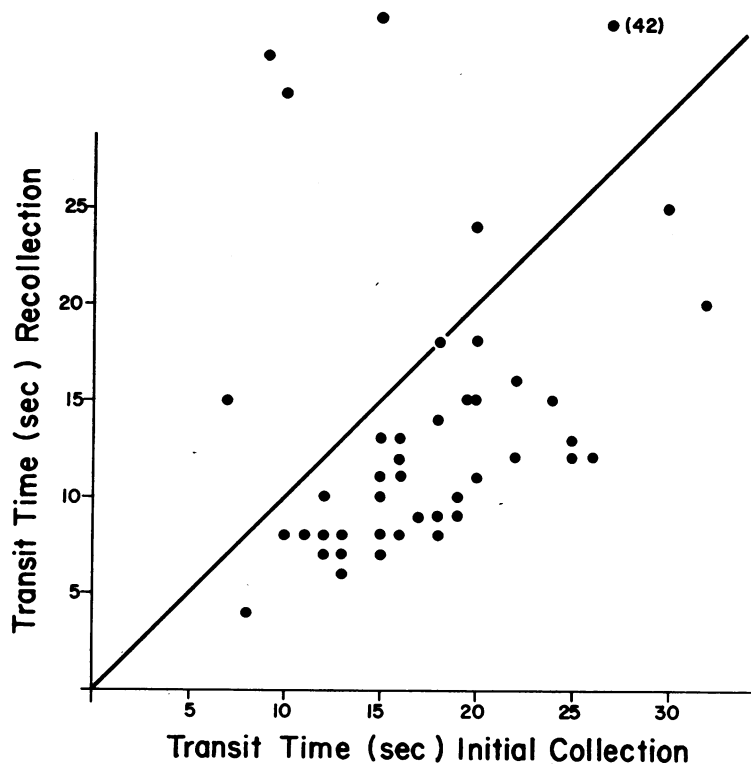

FIGURE 3 Effect of recollection on transit times during continued saline diuresis.
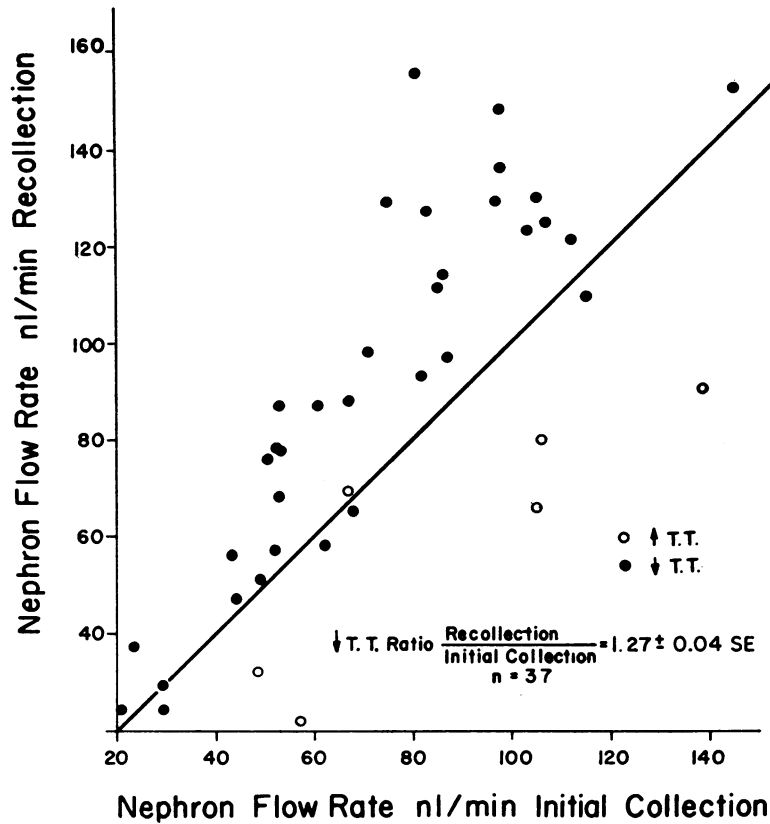
FIGURE 4 Effect of recollection on nephron flow rates during continued saline diuresis. Recollection samples with decreased transit times are shown by the solid circles and those with prolonged transit times by the open circles.

change in total GFR is not of a sufficient magnitude to explain the change in fractional reabsorption (3, 15). Therefore, it has been assumed that absolute reabsorption falls after volume expansion, and increased filtration rate is only a minor contributor to the increased delivery of fluid out of the proximal tubule.

More recently, it has been proposed that the principal factor responsible for the decrease in fractional reabsorption during volume expansion might be a redistribution of renal blood flow resulting in a disproportionate rise in the filtration rate of superficial nephrons. Barger (19) has shown, using the inert gas technique for measuring regional blood flow, that superficial cortical flow is disproportionately increased in conditions in which natriuresis occurs. Horster and Thurau (20) found that chronic salt-loading in rats produces a disproportionate increase in filtration rate of the superficial nephrons. A previous study from our laboratory (1) using recollection micropuncture technique in dogs indicated that the increased delivery of filtrate out of the proximal tubule

after volume expansion was due to both a fall in absolute reabsorption and a disproportionately large increase in nephron gfr. Moreover, the major portion of the increment in distal sodium delivery was a result of the increased filtration rate. These findings have been recently confirmed (2).

The present study demonstrates that the disproportionate rise in superficial nephron filtration rate following volume expansion previously noted (1) is an artifact of recollection technique and does not occur in nephrons which have not been previously punctured. The artifact is the result of a systematic error which leads to excessively large recollections during saline diuresis.

Two different experimental models suggest the presence of such a systematic error. First, during continued hydropenia and continued saline diuresis, in a time span short enough to obviate changes in the physiological condition of the animal, recollected samples consistently reveal higher nephron flow rates than do initial samples.
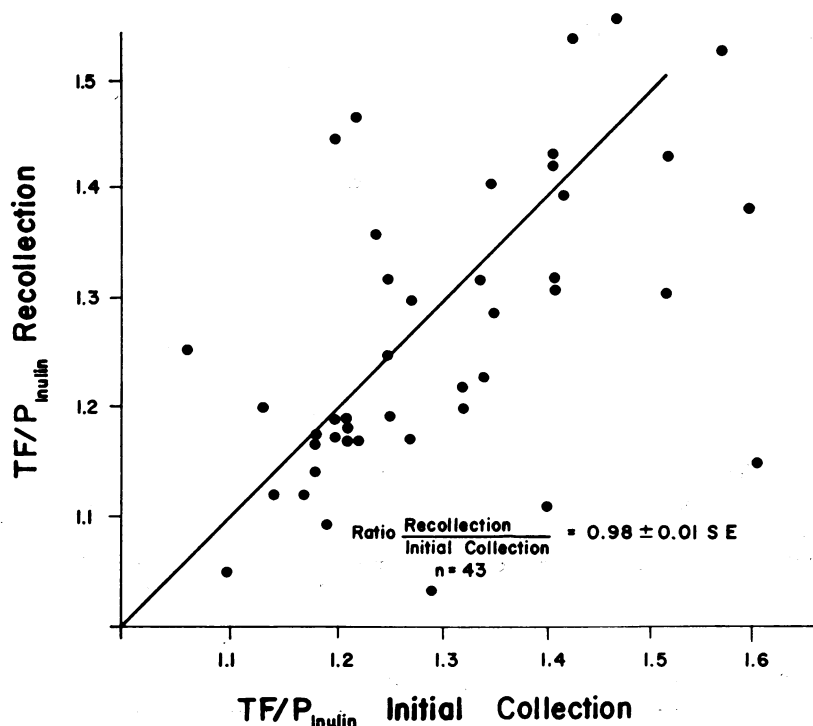
FIGURE 5 Effect of recollection on TF/P inulin ratio during continued saline diuresis.

This error is only 8% in hydropenia, but 27% in saline diuresis (Figs. 1 and 4). Second, nephron gfr in samples recollected following volume expansion are 40% greater than nephron gfr in samples collected during the hydropenic control period (Table III). Yet, if previously unpunctured tubules in the same dog are sampled following volume expansion, the nephron gfrs are altered to only a minor extent when compared to hydropenic nephron gfrs (Table III).

We can only speculate on the nature of the systematic error, but at least two possibilities exist: (a) a collection error; and (b) a rise in nephron gfr secondary to reduced intratubular pressure. By injecting lissamine green into the renal artery following the initial puncture, it could be demonstrated that the dye emerged from the puncture site. This clearly indicates that the puncture site frequently did not seal completely. Moreover, if oil flow onto the surface of the kidney was momentarily stopped and the surface wiped dry, tubular fluid could be seen emerging from the puncture site. It is conceivable that some of this tubular fluid accumulated about the site of puncture and was aspirated along with fluid from within the tubular lumen. However, in some tubules it was possible to recollect from a surface convolution proximal to the original site and prevent the aspiration of fluid accumulated around the original puncture with the oil block; in these collections the 20–30%

increment in flow rate and nephron gfr was still observed. This suggests that a collection error involving the aspiration of extruded tubular fluid is an unlikely explanation for the increase in flow rate.

A more likely possibility is a true increase in tubular flow rate secondary to lowered intratubular pressure. Two lines of evidence support this possibility. First, a shortened transit time was recorded in 37 out of 43 tubules (Fig. 3). Second, the intratubular pressure was lower in previously punctured tubules than in nonpunctured tubules (Table I). These findings suggest that the initial puncture site serves as a low resistance pathway for

TABLE I
Effect of Recollection on Intratubular Pressure during Continued Saline Diuresis

Exp. no.	No. of tubules	Pressure in unpunctured tubules	Pressure in punctured tubules	Pressure change
		cm H₂O	cm H₂O	cm H₂O
1	10	39	30	−9
2	2	37	31	−6
3	3	61	53	−8
4	3	41	47	+6
5	7	44	35	−9
Mean		44	39	−5

Mechanism of Saline Diuresis in the Dog 519

loss of fluid which ultimately leads to lower intratubular pressure. Studies by Schnermann, Horster, and Levine (21) indicate that relatively small changes in intratubular pressure have a significant effect on nephron gfr measurements. Preliminary studies in our laboratory in dogs during saline diuresis lend support to the possibility that intratubular pressure influences nephron flow rates. In these studies the pressure was measured with a Kulite microtransducer before, during, and after collections and recollections. In 9 out of 10 recollection pairs, the increase in recollection flow rates correlated with the fall in intratubular pressure. A reduction in tubular pressure and an alteration in tubular flow rate, therefore, appear to be the likeliest cause of the systematic error introduced by the recollection technique. It should be emphasized that the major fall in intratubular pressure does not occur during the initial sampling, but immediately following the withdrawal of the pipette and that repuncture does not bring the intratubular pressure back to control levels.

This type of error is more likely to occur under conditions in which initial tubular pressures and flow rates are high and, therefore, is consistent with the observations that recollection flow rate increased only 8% in hydropenia in contrast to the 27% increment during saline diuresis. Similarly, Schnermann et al. (21) observed that tubules with higher intratubular pressures appeared to be more likely to develop a pressure drop during sampling and thus have erroneously high determinations of nephron filtration rates. It is, therefore, apparent that superficial nephron gfr measured initially during hydropenia and by recollection during saline diuresis will increase proportionately more than total GFR, an observation which could be interpreted to mean a redistribution of filtrate from deep to superficial nephrons during saline diuresis. In contrast, when superficial nephron gfr is measured in previously unpunctured nephrons in both hydropenia and saline diuresis, the change in nephron filtration rate is closely related to the change in total GFR (Fig. 7). These findings, therefore, suggest that

TABLE II

Protocol of a Micropuncture Experiment during Volume Expansion

Time	Total GFR	$U_{Na}V$	C_{Na}/C_{In}	Tubular fluid sample	TF/P_{In}	Nephron GFR	Nephron GFR
	ml/min	*μEq/ min*	*%*			*nl/min*	*% change*
8:30	31.6	23	0.6				
8:34				1A	1.19	88	
8:40				2A	1.31	118	
8:50	32.3	26	0.6				
9:13				3A	1.31	78	
9:16				4A	1.01	80	
9:25				5A	1.60	77	
9:30	37.5	19	0.3				
Mean	33.8	23	0.5			88	
9:40	Ringer's solution infused—25 ml/min for 30 min						
					R*	N‡	R*
10:40	38.6	238	4.2				
10:45				1B	1.01	151	+71
10:53				2B	1.22	184	+56
10:57				6A	1.21	100	
11:03	39.6	207	3.2				
11:06				7A	1.06	103	
11:16				3B	1.10	70	− 9
11:21				8A	1.12	98	
11:25				4B	1.06	69	−16
11:33				5B	1.00	119	+64
11:45	40.3	236	4.0				
11:52				9A	1.13	107	
Mean	39.5	227	3.8			119	102
Average % change	+17					+16	+33

* R, recollected tubules.
‡ N, new tubules.

H. Mandin, A. H. Israelit, F. C. Rector, Jr., and D. W. Seldin

Table III

Effect of Volume Expansion on Proximal Reabsorption and Nephron Filtration Rate

Exp. no.	No. of tubules	Fractional reabsorption change	Nephron GFR H*	Nephron GFR N‡ S§	Nephron GFR N‡ change	Nephron GFR R‖ S§	Nephron GFR R‖ change	Total GFR H*	Total GFR S§	Total GFR Change	UNaV H*	UNaV S§	CNa/CIn H*	CNa/CIn S§	Mean BP H*	Mean BP S§
		%	nl/min	nl/min	%	nl/min	%	ml/min	ml/min	%	µEq/min		%		mm Hg	
1	6	−21	66 ±4	60 ±4	− 9	83 ±17	+29	18.5	19.1	+ 3	19	174	0.7	6.0	132	132
2	5	−17	68 ±4	79 ±10	+16	113 ±26	+62	22.6	24.6	+ 9	3	161	0.1	2.4	140	140
3	6	+ 3	139 ±8	113 ±5	−19	200 ±56	+60	51.2	50.3	− 2	58	257	0.8	3.5	130	130
4	6	− 5	182 ±13	180 ±5	− 1	255 ±21	+42	50.0	45.9	− 8	4	234	0.1	3.1	149	140
5	5	−14	88 ±8	102 ±2	+16	119 ±23	+33	33.8	39.5	+17	23	227	0.5	3.8	132	167
6	6	−19	71 ±6	72 ±9	+ 1	102 ±29	+36	24.9	20.2	−19	9	68	0.3	2.3	135	140
7	5	−14	85 ±5	67 ±3	−21	96 ±19	+20	23.9	22.9	− 5	20	151	0.6	4.4	105	121
8	6	−14	96 ±3	118 ±3	+23	129 ±11	+33	28.3	34.1	+20	61	640	1.5	12.6	127	13
9	6	−19	90 ±5	72 ±4	−20	116 ±4	+22	26.3	22.9	−13	20	306	0.5	8.7	125	120
10	5	−20	111 ±8	135 ±6	+22	204 ±15	+60	31.8	37.7	+19	9	544	0.2	10.1	127	135
Mean		−14	100	100	+ 1	142	+40	31.1	31.7	+ 2	23	276	0.5	5.7	130	136

* H, hydropenia.
‡ N, new tubules.
§ S, saline diuresis.
‖ R, recollected tubules.

saline diuresis in the dog does not cause redistribution of filtrate between deep and superficial nephrons.

Despite the systematic increase in flow rate introduced by recollection during sustained volume expansion, a change in TF/P inulin ratio was not detected when all 43 recollection pairs were examined (Fig. 5). A similar constancy of recollected TF/P inulin ratios during saline diuresis has been reported previously (3). However, in those tubules in which flow rate was increased during recollection, there was a slight drop in the TF/P inulin ratios (recollection to initial TF/P inulin ratio was 0.94 ±0.01 SE). This very slight change can be attributed to the fact that in conditions with low TF/P inulin ratios, such as saline diuresis, the ratio is insensitive to changes in flow rate. For example, when the TF/P inulin is 1.20, a 27% increase in flow rate would decrease the inulin ratio by only 5%. Considering the errors in the analytic techniques used for determination of TF/P inulin and the scatter of the recollection ratios, a change of this degree would be barely detectable.
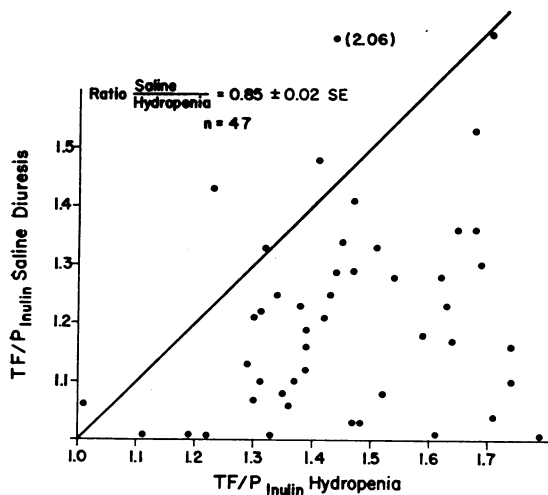

Figure 6 Effect of volume expansion with Ringer's solution on TF/P inulin ratios in recollection pairs.
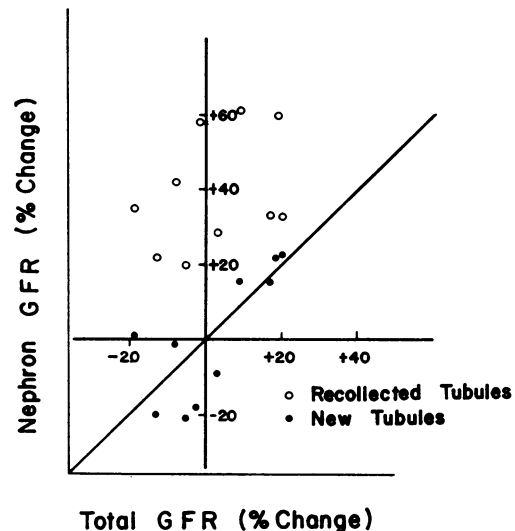

Total GFR (% Change)

Figure 7 Effect of volume expansion with Ringer's solution on alterations in total and nephron filtration rates in both new and recollected samples. The change in filtration rate in new tubules parallels the change in the total GFR, while the change in the repunctured tubules is consistently higher.

Mechanism of Saline Diuresis in the Dog   521

Following volume expansion, fractional reabsorption fell by 15% in the recollected tubules. This decrease in TF/P inulin ratio is not the result of falsely high flow rates since the recollection experiments during continuous saline diuresis showed little effect on the TF/P inulin ratio. Moreover, a 16% decrease in fractional reabsorption was observed when the mean of all newly sampled tubules during saline diuresis was compared to the mean of all the samples obtained in hydropenia. Since fractional reabsorption is equal to the absolute tubular reabsorption divided by the nephron filtration rate, a fall in fractional reabsorption could be due to a fall in absolute reabsorption, an increase in nephron filtration rate, or a combination of both factors. In the present studies nephron filtration rate in new tubules increased, decreased, or remained unchanged during saline diuresis (Table III). Consequently, the fall in fractional reabsorption must be due to a reduction in absolute reabsorption, rather than to a rise in nephron gfr.

The present studies indicate, therefore, that in the dog acute expansion of extracellular volume promotes natriuresis by suppressing absolute reabsorption in the proximal tubule and not by redistributing filtrate from deep to superficial nephrons. It would appear that in the dog redistribution of filtrate plays little, if any, role in saline diuresis since massive natriuresis can occur without significant redistribution. In this respect there appear to be important species differences. Studies in our laboratory[2] have revealed that acute volume expansion in rats suppressed absolute proximal reabsorption only slightly, but uniformly causes marked redistribution of filtrate from deep to superficial nephrons. The basis for this striking species difference is not clear at the present time.

ACKNOWLEDGMENTS

[2] Herrera, J., V. Andreucci, F. C. Rector, Jr., and D. W. Seldin. 1970. Effect of expansion of extracelluler volume on effective filtration pressure and single nephron filtration rates in the rat. Submitted to *J. Clin. Invest.*

REFERENCES

1. Stein, J. H., L. J. Barton, H. Mandin, L. H. Lackner, F. C. Rector, Jr., and D. W. Seldin. 1969. Effect of extra cellular volume expansion (VE) on proximal tubular sodium reabsorption and distribution of renal blood flow (RBF) and glomerular filtrate (GFR) in the dog. *Clin. Res.* 17: 449. (Abstr.)
2. Auld, R. B., E. A. Alexander, and N. G. Levinsky. 1970. Nephron filtration and proximal reabsorption during saline infusion, arterial clamping, and hemorrhage in the dog. *J. Clin. Invest.* 49: 5a (Abstr.)
3. 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.
4. Wunderlich, P., and J. Schnermann. 1969. Continuous recording of hydrostatic pressure in renal tubules and blood capillaries by use of a new pressure transducer. *Pfluegers Arch.* 313: 89.
5. Landis, E. M. 1926. The capillary pressure in frog mesentery as determined by micro-injection methods. *Amer. J. Physiol.* 75: 548.
6. Anderson, C. F., T. K. Sawyer, and R. E. Cutler. 1968. Iothalamate sodium I^{125} vs. cyanocobalamin Co$_{57}$ as a measure of glomerular filtration rate in man. *J. Amer. Med. Ass.* 203: 653.
7. Elwood, C. M., and E. M. Sigman. 1967. The measurement of glomerular filtration rate and effective renal plasma flow in man by Iothalamate ^{125}I and iodopyracet ^{131}I. *Circulation* 36: 441.
8. Griep, R. J., and W. B. Nelph. 1969. Mechanism of excretion of radioiodinated sodium iothalamate. *Radiology* 93: 807.
9. Maher, F. T., and W. N. Tauxe. 1969. Renal clearance in man of pharmaceuticals containing radioactive iodine. *J. Amer. Med. Ass.* 207: 97.
10. Sigman, E. M., C. M. Elwood, and F. Knox. 1966. The measurement of glomerular filtration rate in man with sodium iothalamate ^{131}I (Conray). *J. Nucl. Med.* 7: 60.
11. Sigman, E. M., C. Elwood, M. E. Reagan, A. M. Morris, and A. Cantanzaro. 1965. The renal clearance of I^{131} labeled sodium iothalamate in man. *Invest. Urol.* 2: 432.
12. Walser, M., D. G. Davidson, and J. Orloff. 1955. The renal clearance of alkali–stable inulin. *J. Clin. Invest.* 34: 1520.
13. Vurek, G. G., and S. E. Pegram. 1966. Fluorometric method for the determination of nanogram quantities of inulin. *Anal. Biochem.* 16: 409.
14. 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. *Amer. J. Physiol.* 209: 1199.
15. 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 infusion. *J. Clin. Invest.* 46: 47.
16. Bank, N., K. M. Koch, H. S. Aynedjian, and M. Aras. 1969. Effect of changes in renal perfusion pressure on the suppression of proximal tubular sodium reabsorption due to saline loading. *J. Clin. Invest.* 48: 271.
17. Brenner, B. M., C. M. Bennett, and R. W. Berliner. 1968. The relationship between glomerular filtration rate and sodium reabsorption by the proximal tubule of the rat nephron. *J. Clin. Invest.* 47: 1358.
18. Rodicio, J., J. Herrera-Acosta, J. C. Sellman, F. C. Rector, Jr., and D. W. Seldin. 1969. Studies on glomerulo–tubular balance during aortic constriction, ureteral obstruction, and venous occlusion in hydropenic and saline–loaded rats. *Nephron* 6: 437.
19. Barger, A. C. 1966. Renal hemodynamic factors in congestiv heart failure. *Ann. N. Y. Acad. Sci.* 139: 276.
20. Horster, M., and K. Thurau. 1968. Micropuncture studies on the filtration rate of single superficial and juxtamedullary glomeruli in the rat kidney. *Pfluegers Arch. Ges. Physiol.* 301: 162.
21. Schnermann, J., M. Horster, and D. Z. Levine. 1969. The influence of sampling technique on the micropuncture determination of glomerular filtration rate and reabsorptive characteristics of single rat proximal tubules. *Pfluegers Arch.* 309: 48.