The Relationship between Peritubular Capillary Protein Concentration and Fluid Reabsorption by the Renal Proximal Tubule

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ABSTRACT The relationship between peritubular capillary protein concentration and rate of sodium reabsorption by the rat proximal tubule was examined using free-flow recollection micropuncture techniques. Tubule fluid–to-plasma inulin ratios were measured before, during, and at successive intervals after brief (15–25 sec) intra-aortic injections (at the level of the renal artery) of colloid-free, isoncotic, and hyperoncotic solutions. Arterial hematocrit and protein concentrations were measured simultaneously in these rats. In other rats, total protein concentration of peritubular capillary blood plasma was determined before, during, and after these same infusions with a newly described submicroliter fiber-optic colorimeter.

In the 15–25 sec interval necessary to infuse 2 ml of these test solutions, fractional and absolute sodium reabsorption varied directly with peritubular capillary colloid osmotic pressure, declining during infusion of colloid-free solutions, increasing during hyperoncotic infusions, and remaining unchanged during isoncotic infusions.

In the subsequent 20-min interval after intra-aortic injection of these test solutions, capillary protein concentration remained at (isoncotic infusions) or returned to (colloid-free and hyperoncotic fluids) control values. Whereas reabsorption after colloid-free solutions returned to base line levels in parallel with the return in capillary protein concentration, after colloid infusions (which resulted in continued expansion of extracellular fluid volume), a progressive decline in reabsorption was observed.

These results afford strong evidence that peritubular capillary colloid osmotic pressure is one important determinant of proximal sodium reabsorption. Nevertheless it is apparent that mechanisms other than or in addition to this must be invoked to explain the delayed inhibition of reabsorption that accompanies expansion of extracellular fluid volume by colloid solutions.

INTRODUCTION

There is considerable evidence to indicate that the transfer of sodium from tubule lumen to peritubular capillary blood is mediated by an active transport process. Many of the proposals concerning the regulation of proximal fluid reabsorption, therefore, have involved mechanisms that either control the availability of sodium at the active transport site, or that influence the sodium pump directly. Thus, the geometry of the tubule lumen and the velocity of fluid flow are examples of proposed factors thought to operate at the luminal membrane to regulate reabsorption by governing the passive rate of entry of sodium into the cell (1–5). Tests of these theories, however, have shown them to be inadequate to explain the observed changes in reabsorption (6–9). Humoral factors, acting via unspecified pathways, have also been proposed (10–12). Again, except for inferential and indirect evidence, critical tests of these proposals have yet to be confirmatory (13–15).

Another recent proposal considers net proximal transtubular fluid movement to be the result of a coupling between active transport processes at the level of the epithelial cell and purely passive (Starling) forces operating at the antiluminal region between peritubular capillary and tubule basement membrane. Thus, Earley and Friedler (16–18) and Lewy and Windhager (7)

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have suggested that depression of tubule sodium reabsorption after saline loading, and reduction in glomerular filtration rate, respectively, is the result, not of inhibition of some energy-dependent step per se, but rather of a diminution in the rate of peritubular capillary removal of reabsorbate brought about by alterations in the net balance of colloid osmotic and hydrostatic pressures operating across the capillary wall. Accordingly we undertook to examine the influence of graded changes in peritubular capillary protein concentration on the rate of fluid reabsorption by the rat proximal tubule. By use of free-flow recollection micropuncture techniques, fluid reabsorption was measured before, during, and at successive intervals after intra-aortic injections (at the level of the renal artery) of isotonic solutions of varying colloid osmotic pressures. The results indicate that absolute and fractional sodium reabsorption by the proximal tubule vary directly with the colloid osmotic pressure of peritubular capillary blood. These findings are consistent with, and extend the experimental support for, the view that changes in the balance of Starling forces operating across the peritubular capillary wall are of importance in the regulation of net sodium transport by the proximal tubule.

METHODS

General. Studies were performed on male Sprague-Dawley rats weighing 200–320 g and which were allowed free access to a rat pellet diet, except for 14–16 hr before study. The rats were anesthetized by intraperitoneal injection of Inactin (100 mg/kg), placed on a temperature-regulated micropuncture table, and a tracheostomy was performed. Indwelling polyethylene catheters were inserted into the left jugular vein for infusion of inulin, into the right jugular vein for injection of Lissamine green, and into the femoral artery for periodic blood collection and estimation of arterial pressure. In addition, a length of No. 50 polyethylene tubing was inserted into the left common carotid artery and its tip threaded down the thoracic and abdominal portions of the aorta for a distance of 7 cm. This distance was found to place the catheter tip within 1 cm of the orifice of the left renal artery. After a left subcostal incision, palpation of the abdominal aorta disclosed the exact location of the catheter tip. The catheter then was repositioned, if necessary, so that the final position of its tip was 1–2 mm above the orifice of the left renal artery. The left kidney, with its capsule intact, was gently separated from the adrenal gland and surrounding perirenal fat. In eight rats, the left ureter was cannulated near the renal pelvis with a 3 cm No. 10 polyethylene catheter. The left kidney was suspended on a Lucite holder and its surface illuminated with a quartz rod and bathed with mineral oil heated to 37°C. In these studies, rats were used for micropuncture only if operative blood and fluid losses were considered to be minimal. The fluid losses that were incurred were not replaced.

Experimental protocol. 30 min before micropuncture, each animal received 0.7 ml of a solution of 10% inulin in 0.5% NaCl as an intravenous priming dose. This was followed immediately by a sustaining infusion of the same solution given at the rate of 0.02 ml/min and was continued for the duration of the experiment. Late surface convolutions of two to three proximal tubules were located by observing the passage of Lissamine green which was injected rapidly (0.05 ml of a 10% solution) into the right jugular vein. The relative position of each late convolution was mapped so as to facilitate subsequent identification and relocation. After the 30-min inulin equilibration period, an exactly timed (1–2 min) sample of fluid was collected from the first tubule. The transit time, estimated with a stopwatch as the interval from the appearance of Lissamine green dye in the peritubular capillaries to the arrival of the color wave at the site of puncture, was determined during fluid collection. The rate of fluid collection was adjusted to maintain the position of a polymer oil block (Kel-F Polymer Oil, Minnesota Mining & Manufacturing Co., St. Paul, Minn.), four to six tubule diameters in length, placed just distal to the site of puncture. Sharpened micropipettes with tip diameters of 8–10 μ were used for sample collections. Immediately after this initial collection from the first tubule, base line measures of hematocrit, inulin concentration, and blood pressure were obtained from the femoral artery. In several but not all rats, base line measurements of arterial protein concentration and urine volume and inulin concentration also were made.

With a new micropipette positioned directly over the previous puncture site, a rapid (square-wave) injection of 2 ml of one of several solutions of varying colloid composition was given rapidly via the intra-aortic catheter.[1] The duration of this injection ranged from 15 to 25 sec. The micropipette was inserted into the tubule during the initial 10 sec of injection. Tubule fluid recollection was begun as soon after this initial 10-sec interval as possible, was continued for an exactly timed period of 10–15 sec, and was concluded within 25 sec of the onset of intra-aortic fluid injection. In this manner, a portion of the initial 1 ml of test solution entered the renal arterial and peritubular capillary circulation before tubule fluid collection and continued to occupy the capillary bed during the entire period needed to complete the collection. Despite the rapidity of this collection, it nearly always was possible to control the position of the distal oil block. When control of the oil block was not possible the fluid sample was discarded. The volume of tubule fluid collected in this brief interval ranged from 3 to 8 nl. An aliquot of femoral arterial blood, collected at the midpoint of the intra-aortic injection, was used for measurement of hematocrit, and inulin and protein concentrations. In order to avoid dead-space errors in this blood collection, the length of the femoral artery catheter was made to be less than 3 cm and was allowed to remain unclamped from the onset of the intra-aortic injection so that blood dripped continuously from its free end.[2] Tubule fluid, femoral arterial blood, (and urine in several rats) were obtained at regular intervals over the next 20 min in order to assess the

[1] In initial experiments in this study, Lissamine green was added to each test solution so as to measure the time required for fluid, injected via the intra-aortic catheter, to reach the surface capillaries. In every instance, the peritubular capillaries were filled with green dye within 1 sec of the onset of fluid injection.

[2] The volume of blood that was lost at the time of these intra-aortic fluid injections was less than 0.2 ml. The total volume of arterial blood lost in the course of an experiment was estimated to be less than 1.5 ml.

time course response to the various test solutions.[3] These subsequent tubule fluid collections, lasting 1–2 min, were obtained from the previous puncture sites (recollection). The transit time was estimated during each recollection. This same protocol was repeated once or twice in most rats; occasionally the composition of the test solution for the second or third injection differed from that of the first injection. At least 30 and often 40 min were allowed to elapse between injections of test solutions. In addition, since changes in extracellular volume undoubtedly were occurring after these fluid injections, no more than 2 min elapsed from collection of the initial fluid sample to intra-aortic injection of the test solution.

In order to evaluate the effect of peritubular capillary oncotic pressure on proximal sodium reabsorption when glomerular filtration rate was kept relatively unchanged, we attempted to prevent the otherwise frequently observed increase in glomerular filtration rate by means of an aortic clamp. In eight rats, using a clamp identical with that described previously (6), we produced a very mild degree of aortic constriction (just above the level of the left renal artery and the tip of the intra-aortic catheter) as judged by the appearance of the kidney and measurement of arterial pressure. The latter was reduced by about 10–15 mm Hg. Blood pressure was monitored in the left femoral artery by means of a Statham strain gauge (Statham Instruments, Inc., Los Angeles, Calif.) connected to a Sanborn recorder (Sanborn Co., Cambridge, Mass.). In these experiments, immediately after the base line fluid collection the aorta was constricted for only the brief interval needed to inject the test solution and obtain the early recollection. A total of 12 tubules were studied in this way.

The six intra-aortic infusion solutions employed in this study were chosen to test the effect of changes in peritubular capillary colloid osmotic pressure on the rate of fluid reabsorption by the proximal tubule. Their compositions were as follows:

1. *Isotonic Ringer's solution.* 115 mM NaCl, 25 mM $NaHCO_3$, 4 mM KCl. This solution was bubbled for 2 hr before use with 5% CO_2. The pH was checked just before infusion and, if necessary, adjusted to 7.4.
2. *Isoncotic rat plasma.* Blood from the abdominal aorta was collected into lightly heparinized syringes from hydropenic donor rats just before study.
3. *Isoncotic albumin solution.* Crystalline bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) was dissolved in isotonic Ringer's solution to make final concentrations of 5.0–7.0 g/100 ml. These solutions were prepared just before use in each experiment.
4. *Polyvinylpyrrolidone (PVP).* A 6 g/100 ml solution of PVP (kindly supplied as a 45% aqueous solution by General Aniline & Film Corporation, New York) (mol wt ~100,000) in isotonic Ringer's solution was prepared just before use.
5. *Hyperoncotic albumin solutions.* Solutions of 9.0–10.0 g/100 ml and 15.0 g/100 ml crystalline bovine serum albumin in isotonic Ringer's solution were prepared on the morning of study.

Before the use of each test solution, a volume of 10% inulin in 0.5% NaCl was added to yield a final inulin concentration to approximate that is plasma. All solutions were warmed to body temperature immediately before use.

The total protein concentration of peritubular capillary blood was measured in 15 rats. Because micropuncture collections of tubule fluid and capillary blood could not both be obtained in the brief interval during intra-aortic fluid infusion, tubule fluid reabsorption was not measured in these rats. Only capillaries that coursed directly adjacent to proximal tubules were studied but were otherwise chosen randomly. Different capillaries were punctured before, during, and at successive intervals after intra-aortic injection of isotonic Ringer's solution, 6% albumin solution, and 15% albumin solution. A base line value for femoral arterial protein concentration also was obtained. Approximately 20 nl of capillary blood was collected into sharpened micropipettes (tip diameter 10–12 μ) by gentle aspiration. The internal surfaces of these micropipettes had previously been coated with a dilute silicone solution (Siliclad, Clay-Adams, Inc., New York) and heat dried. This maneuver was found to prevent clotting of the peritubular capillary blood. The tip of the micropipette was filled with clear mineral oil before and after capillary blood collection. Soon after blood collection the pipette tip was sealed with acrylate adhesive (methyl-2-cyanoacrylate, Eastman 910 Adhesive, Armstrong Cork Co., Lancaster, Pa.), inserted into a larger glass capillary (to insure against damage to the blood-filled tip), and spun in a hematocrit centrifuge for a period of 15 min. Excellent separation of red blood cells and plasma was readily obtained. The mineral oil served to cover the plasma layer and thereby prevent loss by evaporation.

Analytical. The volume of tubule fluid collected from individual nephrons was estimated from the length of the fluid column in a constant bore capillary tube of known internal diameter. The concentration of inulin in tubule fluid was measured by the fluorescence method of Vurek and Pegram (19). Inulin concentration in plasma and urine was determined by the method of Führ, Kaczmarczyk, and Krüttgen (20).

Protein concentration in arterial blood plasma was measured by the technique of Lowry, Rosebrough, Farr, and Randall (21), modified as follows: to 1 μl of protein standard or arterial blood plasma was added 3.0 ml of reagent A (1.0 ml of 2% Na tartrate, 1.0 ml of 1% $CuSO_4$, and 100 ml of 2% Na_2CO_3 in 0.1 N NaOH). After a 10 min waiting period, 0.3 ml of reagent B (a 50% aqueous solution of 2 N phenol reagent, Fisher Scientific Company, Fairlawn, N. J.) was added and the entire mixture shaken. Color development was observed to be maximal after 30 min, and per cent transmission then was read at 750 mμ in a Beckman DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.).

Protein concentration in peritubular capillary blood plasma and appropriate crystalline albumin and femoral arterial blood standards was measured by a further microadaptation of the method of Lowry et al. (21). 10 nl of plasma (or standard protein solution) was added to a glass capillary (50 μl capacity) containing 30 μl of reagent A. This was mixed, and after a 10 min incubation period, 3 μl of reagent B was added. The ends of the capillary then were heat sealed and the mixture centrifuged several times in each direction to insure optimal mixing of the reagents. 30 min later, one end of the capillary was broken and 200-nl aliquots were removed for measurement of per cent transmission at 750 mμ with a fiber-optic colorimeter designed specifically for submicroliter quantities of fluid (22). The cuvette for this device is a glass capillary with an internal

[3] A similar characterization of the time course of changes in sodium excretion was not performed in this study because of the relatively large urinary dead space imposed by the length of the urinary catheter that was required, and because of the hydropenic condition of these rats.

TABLE I

The Effect of Intra-Aortic Injections of Solutions of Varying Oncotic Pressures on Individual Nephron Function

Experimental condition	Preinjection control values			During injection		1–3 min postinjection		4–6 min postinjection			7–10 min postinjection			15–20 min postinjection		
	$(TF/P)_{In}$	Nephron GFR, V_0	Transit time, TT	$(TF/P)_{In}$	V_0	$(TF/P)_{In}$	V_0	$(TF/P)_{In}$	V_0	TT	$(TF/P)_{In}$	V_0	TT	$(TF/P)_{In}$	V_0	TT
		nl/min	*sec*		*nl/min*		*nl/min*		*nl/min*	*sec*		*nl/min*	*sec*		*nl/min*	*sec*
Isotonic Ringer's solution [19]*	2.32 ±0.10 (31)‡§	41.3 ±3.3 (31)	10.5 ±0.2 (31)	1.59 ±0.06 (26)	53.5 ±5.3 (26)	1.76 ±0.15 (5)	68.9 ±6.2 (5)	1.77 ±0.11 (15)	40.6 ±3.6 (15)	10.2 ±0.4 (12)	1.94 ±0.17 (8)	45.6 ±6.0 (8)	9.9 ±0.5 (8)	2.42 ±0.20 (7)	57.1 ±5.7 (7)	9.4 ±0.3 (7)
	Unclamp§			Unclamp												
	2.41 ±0.18 (19)	42.0 ±5.4 (19)	—	1.63 ±0.08 (14)	68.9 ±6.2 (14)											
	Clamp§			Clamp												
	2.18 ±0.11 (12)	40.3 ±4.2 (12)	—	1.54 ±0.09 (12)	35.5 ±4.5 (12)											
Isoncotic albumin, 5–7% [7]	2.11 ±0.09 (11)	45.2 ±6.3 (11)	10.2 ±0.6 (11)	2.20 ±0.18 (7)	43.0 ±5.9 (7)			1.78 ±0.12 (6)	59.5 ±4.9 (6)	10.1 ±0.5 (6)				1.58 ±0.11 (4)	44.7 ±9.2 (4)	8.6 ±0.9 (3)
Isoncotic plasma [5]	2.32 ±0.10 (10)	48.9 ±4.2 (10)	11.0 ±0.6 (10)	2.37 ±0.18 (9)	53.9 ±11.1 (9)			2.00 ±0.11 (7)	54.0 ±6.4 (7)	9.7 ±0.6 (7)				1.88 ±0.14 (4)	34.2 ±4.9 (4)	8.6 ±0.3 (4)
Isoncotic PVP, 6% [2]	2.17 ±0.09 (4)	39.4 ±3.8 (4)	9.9 ±0.9 (4)	2.17 ±0.04 (3)	36.0 ±0.7 (3)			2.09 ±0.06 (4)	37.5 ±6.9 (3)	9.4 ±0.4 (4)						
Hyperoncotic albumin, 9–10% [8]	1.66 ±0.09 (11)	43.0 ±3.5 (10)	8.9 ±0.3 (10)	1.88 ±0.11 (10)	53.1 ±5.7 (10)			1.56 ±0.08 (4)	52.9 ±8.4 (4)	8.4 ±0.5 (4)				1.43 ±0.18 (4)	28.1 ±2.9 (4)	8.3 ±0.3 (4)
Hyperoncotic albumin, 15% [6]	2.03 ±0.10 (10)	49.1 ±5.1 (10)	10.4 ±0.5 (9)	3.07 ±0.26 (6)	55.4 ±7.4 (5)			1.99 ±0.06 (6)	57.8 ±7.5 (6)	10.6 ±0.8 (6)				1.70 ±0.07 (7)	61.4 ±5.7 (7)	9.7 ±0.6 (7)

$(TF/P)_{In}$, tubule fluid-to-plasma inulin ratio; GFR, glomerular filtration rate; PVP, polyvinylpyrrolidone.

* Number in brackets refers to number of animals.

‡ Number in parenthesis refers to n tubules.

Values represent mean ±1 SE for n tubules.

§ Of the 31 tubules in this group, 12 were studied during aortic constriction ("clamp" subgroup). The remaining 19 are designated "unclamp."

diameter of 0.1 mm and a length of 12 mm. Fiber-optic rods channel light from a pinpoint source through the sample-filled glass capillary and out to a photomultiplier photometer. For each experiment, per cent transmission was determined first for standard solutions of crystalline albumin ranging in concentration from 2.0 to 12.0 g/100 ml. The protein concentrations of peritubular capillary and femoral arterial blood plasmas then were estimated from these standard curves.

RESULTS

A number of control observations were required to validate the experimental approach in these studies. For convenience these will be considered under the headings: Physiological, Procedural, and Methodological.

Physiological. The following observations were made in rats while the intra-aortic catheter was in place. Arterial blood pressure before intra-aortic fluid injection averaged 112 mm Hg ±3 SE (n = 32). During fluid injection this measure either remained unchanged or increased by less than 10 mm Hg. 5 min later this value was unchanged from control (mean = 117 mm Hg ±5 SE, n = 16). Experimental kidney glomerular filtration rates, measured in eight rats before intra-aortic injection, averaged 1.24 ml/min ±0.09 SE and was unchanged in the 20 min interval after injection (mean = 1.18 ml/min ±0.09 SE). Despite the presence of an indwelling aortic catheter, the base line control values for tubule fluid-to-plasma (TF/P) inulin ratios, nephron filtration rates, and transit time did not differ appreciably from values reported by us previously for similarly hydropenic rats during conventional micropouncture studies (Table I, preinjection control values) (23).

Procedural. In the experimental design of these studies, three assumptions regarding procedure were made.

(*a*) The extent to which the inulin concentration in tubule fluid rises relative to that in plasma is a measure of the volume of filtered water removed by the nephron to the site of puncture. Abrupt changes in plasma inulin concentration make it difficult to be certain of the exact inulin concentration in glomerular filtrate as well as plasma at any given time. Undoubtedly this would be the case if inulin-free fluid was injected rapidly into the vicinity of the experimental renal artery. In order to avoid this source of error, inulin was added to each intra-aortic injection fluid in quantities calculated to give a final concentration approximating that of plasma. The inulin concentrations of injection fluid and of the plasma sample obtained just before fluid injection were measured and compared. 41 such comparisons were made (at least one for each rat) and revealed a mean difference of 1.2% ±2.2 SE.

(*b*) It also was assumed that at the mid-point of the intra-aortic injection, blood collected from the left femoral artery accurately reflected the composition of blood actually entering the experimental kidney. This assumption was tested in four rats not included as part of the experimental group in these studies. In each rat small diameter polyethylene (PE 50) catheters were inserted into the left renal and left femoral arteries. At the midpoint of a 20 sec intra-aortic fluid injection, blood was collected simultaneously from each catheter, and the respective hematocrits and inulin concentrations were determined. For these four rats the mean differences between the two collection sites for each measure were 0.45% ±0.5 SE and 0.2% ±0.3 SE, respectively.

(*c*) A third assumption concerns the validity of comparing values obtained during 10–15 sec tubule fluid collections with those obtained for fluid collections performed over a greater period of time (i.e., 1–2 min). To test this assumption TF/P inulin ratios and nephron filtration rates were measured for 1-min fluid collections from a single tubule in each of 11 rats during control hydropenic conditions. A 10 sec recollection then was

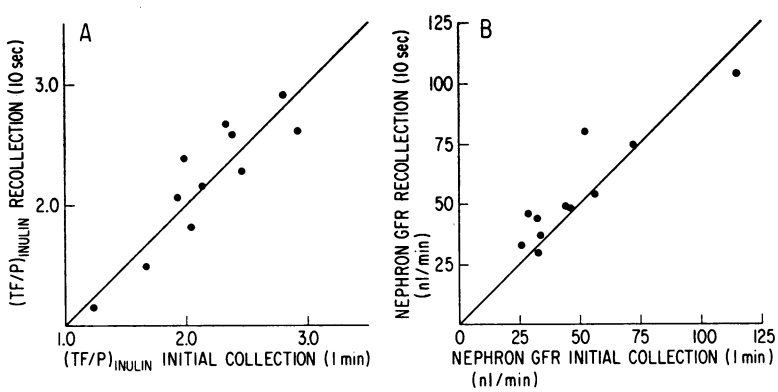

FIGURE 1 Comparison of initial and recollection (TF/P) inulin ratios (panel A) and nephron filtration rates (panel B) for 11 control tubules. The initial collection was obtained in 1 min and the recollection in 10 sec. No experimental intervention took place between collections.

made from each tubule immediately after the longer control collection (hence in exactly the same way as was done during actual experiments). No experimental maneuver intervened between collections in these control studies. As can be seen in Fig. 1 (panel A), no systematic difference in the recollection: initial collection TF/P inulin ratios was found (mean = 1.00 ±0.03 SE). The initial and recollection values for nephron filtration rates (V_0),[4] also were computed and are shown in Fig. 1 (panel B). The mean recollection: initial collection ratio for V_0 for these 11 tubules averaged 1.15 ±0.07 SE.[5]

Methodological. Two assumptions regarding analytical techniques required validation:

(*a*) Since, on the average, 5 nl of tubule fluid was collected in the 10–15 sec period during intra-aortic fluid injection, the sensitivity of the microfluorescence method (19) (used to measure tubule fluid inulin concentration) needed to be enhanced and the resulting accuracy measured. The sensitivity was increased by reducing the volume of the phosphoric acid–dimedone reagent mixture from 2.4 to 1.8 μl. Using a 2.3 nl volumetric pipette (rather than the usual 8–12 nl), the relationship between inulin concentration (of standard solutions as well as experimental tubule fluid samples) and per cent of transmission was found to be linear. The inulin concentration of a standard solution determined simultaneously by the microfluorescence and macroanthrone methods (the latter utilizes 200 μl fluid samples) revealed an average difference for 38 paired measurements of 0.5% ±2.2 SE.

(*b*) A new ultramicromethod for measurement of total protein concentration in capillary blood plasma is described. To validate this method, we compared the values for 10 nl samples of a standard 4 g/100 ml solution measured with the ultramicrotechnique to values obtained simultaneously on 1 μl samples of the same known solution measured with a Beckman DU spectrophotometer. The mean of 13 measurements with the ultramicromethod was 4.0 g/100 ml ±0.10 SE compared with an average value of 4.1 g/100 ml ±0.05 SE for 11 macrodeterminations.

Experimental observations. During control conditions before intra-aortic fluid infusions, peritubular capillary plasma protein concentration (mean = 8.6 g/100 ml ±0.3 SE) for 11 rats always was higher (mean difference = + 38.8% ±4.7 SE, range: + 15.5 to + 68.4%) than that of plasma collected simultaneously from the femoral artery and measured by the same ultramicrotechnique (mean = 6.3 g/100 ml ±0.2 SE).[6] This higher protein concentration in the postglomerular efferent

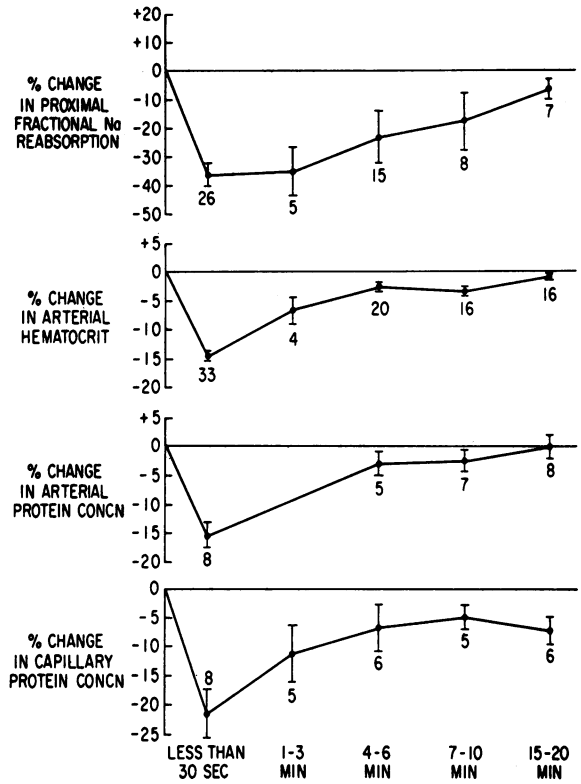

FIGURE 2 The effect of rapid intra-aortic injections (15–25 sec duration) of 2 ml of isotonic Ringer's solution on proximal fractional reabsorption, arterial hematocrit, and arterial and peritubular capillary plasma protein concentrations. With this and other test solutions, fractional reabsorption, arterial hematocrit, and arterial protein concentration always were measured simultaneously in the same rat, whereas peritubular capillary protein concentration was measured in a separate group of animals. The data in this and subsequent figures are expressed as percentage changes in each measure relative to control values and are grouped into arbitrarily selected time intervals after the onset of fluid injection. Values represent the mean ±1 SE. The number of tubules studied in each interval is also given.

[4] Calculated with the expression: $V_0 = (TF/P)$ inulin × tubule fluid flow rate, where V_0 and flow rate are in units of nanoliters per minute.

[5] The slightly higher values for V_0 during the 10 sec recollection period result, very likely, from the rapid and not readily avoidable inrush of fluid into the collecting pipette when the latter initially enters the fluid-filled tubule. For prolonged collections (i.e. those obtained in 1 or more min) this error may be neglected, whereas for very brief collections the 1–2 nl that initially enters the collecting pipette may be expected to raise slightly the calculated value for V_0.

[6] By use of the macromethod for measurement of femoral arterial protein concentration, the average control value was found to be 6.7 g/100 ml ±0.1 SE, n = 33. Although control values for peritubular capillary protein concentration varied appreciably from animal to animal (range 7.0–10.3 g/100 ml), in individual rats this variability was far less. In seven rats in which control protein measurements were obtained from more than one capillary, the differences in each rat ranged from 0.1–0.8 g/100 ml and averaged 0.3 g/100 ml.

vessels is the consequence of ultrafiltration of colloid-free fluid across the glomerular capillary wall. From these data, it was possible to calculate filtration fraction for these cortical vessels using the formula given by Bresler (24).[7] The values averaged 0.27 and ranged from 0.15 to 0.41, with 9 of 11 measurements between 0.21 and 0.41.

Isotonic Ringer's solution. The effects of rapid intra-aortic injections of 2 ml of isotonic Ringer's solution on the changes in TF/P inulin ratios, fractional sodium reabsorption, transit time, arterial hematocrit, and arterial and peritubular capillary plasma protein concentrations as a function of time are shown in Table I and Figs. 2 and 3. During the 20–25 sec interval required to inject this colloid-free solution, TF/P inulin recollection ratios were noted to fall in each of 26 tubules (mean recollection: initial collection TF/P inulin ratio = 0.71 ±0.03 SE), corresponding to an average decline in fractional reabsorption[8] of 36.2% ±3.8 SE.

The simultaneous change in nephron filtration rate (V_0), although variable, was an increase in 11 of 14 tubules. For all tubules, initial V_0 averaged 42.0 nl/min ±5.4 SE and rose to 68.9 ±6.2 SE. In an effort to exclude the influence of V_0, per se, on the observed changes in fractional sodium reabsorption, we attempted to prevent the expected rise in V_0 during fluid injection by means of aortic constriction. In 12 tubules studied in this way, control V_0 averaged 40.3 nl/min ±4.2 SE and declined slightly (mean = 35.5 nl/min ±4.5 SE) during intra-aortic injection of isotonic Ringer's solution. In these 12 tubules, despite no significant mean change in V_0, the recollection TF/P inulin ratio averaged 0.72 ±0.04 SE, and fractional sodium reabsorption fell an average of 38.8% ±6.3 SE, from a mean value during the control period of 52.9% ±2.5 SE to 33.1% ±3.8 SE during the colloid-free fluid injection. Since V_0 was relatively unchanged during aortic constriction, it was possible to assess the effect of Ringer's solution on Cd, the absolute rate of reabsorption.[9] This measure was observed to fall in 10 of these 12 tubules (from an average

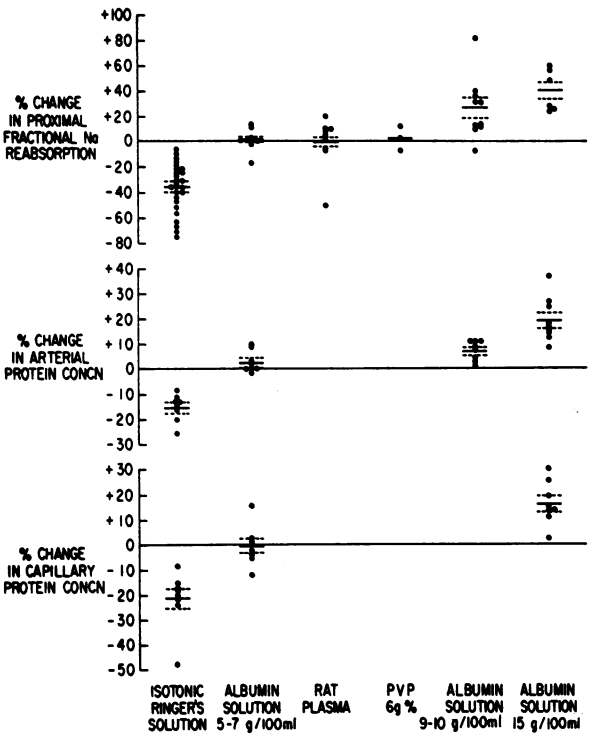

FIGURE 3 Comparison of the changes in fractional reabsorption, arterial, and peritubular capillary plasma protein concentrations observed in the 15–25 sec interval during intra-aortic infusion of each of the test solutions. Horizontal lines represent mean (solid bar) ±1 SE (dashed line). PVP, polyvinylpyrrolidone.

21.8 nl/min ±2.8 SE (n = 12) to 13.6 nl/min ±3.2 SE) for an over-all mean reduction in absolute reabsorption of 46.0% ±9.1 SE. In two tubules, despite aortic constriction V_0 increased by 25 and 28%. Nevertheless in these, Cd failed to increase proportionately (+ 3.7 and + 3.8%, respectively). The mean depression of absolute and fractional reabsorption observed in the period during injection of Ringer's solution was accompanied by uniform declines in arterial hematocrit (− 14.4% ±1.0 SE, n = 33) and arterial (− 15.5% ±2.1 SE, n = 8) and peritubular capillary plasma protein concentrations (− 21.6% ±4.1 SE, range − 8.5 to − 47.7%) (Figs. 2 and 3).

As can be seen in Fig. 2 and Table I, fractional reabsorption returned to or toward control levels over the period of the next 20 min and was accompanied by nearly parallel changes in hematocrit and arterial and peritubular capillary protein concentrations.

Isoncotic crystalline bovine serum albumin solution. The effects of injecting 2 ml of 5–7 g/100 ml of albumin solutions into the aortae of seven hydropenic rats on measures of individual nephron function are summarized in Table I and Figs. 3 and 4. Despite identical

[7] Filtration fraction $= 1 - \left(\frac{P_a}{P_e}\right)$, where P_a and P_e refer to the concentrations of total protein in arterial (afferent) and peritubular capillary (efferent) blood, respectively. Filtration fraction calculated in this way represents a minimum value, in that absorption of fluid by the efferent vessels would systematically tend to lower the total protein concentration below the higher value likely to be present immediately beyond the glomerular capillary bed.

[8] Calculated with the expression:

$$\text{Fractional reabsorption} = \left[1 - \left(\frac{\text{plasma}}{\text{tubule fluid}}\right)_{\text{inulin}}\right].$$

[9] Calculated with the expression: Cd = V_0 − tubule fluid flow rate. The units for each term are nanoliters per minute.

methods of fluid injection and tubule fluid collection, the addition of albumin in isoncotic proportions to Ringer's solution reversed the previously noted depression and was associated with an average recollection TF/P inulin ratio for seven tubules of 1.02 ±0.06 SE (mean change in fractional sodium reabsorption = + 1.1% ±3.8 SE). The simultaneous average change in V_0 also was small (Table I), but among individual tubules, the variability in V_0 was similar to that seen when isotonic Ringer's solution was given. Absolute fluid reabsorption was found to remain unchanged (mean preinjection value = 23.4 nl/min ±3.3 SE, n = 11, as compared with 22.6 nl/min ±3.6 SE n = 7, during fluid infusion). The average values for femoral arterial and peritubular capillary plasma protein concentrations did not differ significantly from control values measured before albumin injection (Figs. 3 and 4). The change in renal arterial hematocrit during isoncotic albumin injection was identical with that measured for the same period when Ringer's solution was given (Fig. 4).

Note in Table I and Fig. 4 that in the 4–6 min and 15–20 min intervals after isoncotic albumin injection, the average TF/P inulin ratios and calculated values for fractional sodium reabsorption declined progressively. This decrease in fractional reabsorption could not be attributed to increased V_0, since in the 15–20 min interval the latter was largely unchanged. Indeed, Cd at

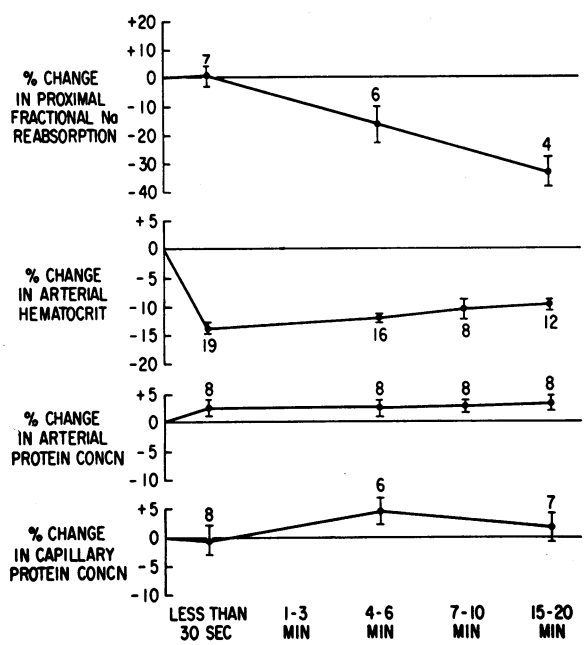

FIGURE 4 The effect of rapid intra-aortic injections of isoncotic (5–7 g/100 ml) of crystalline bovine serum albumin on the time course of changes in fractional reabsorption, arterial hematocrit, and arterial and capillary plasma protein concentrations.

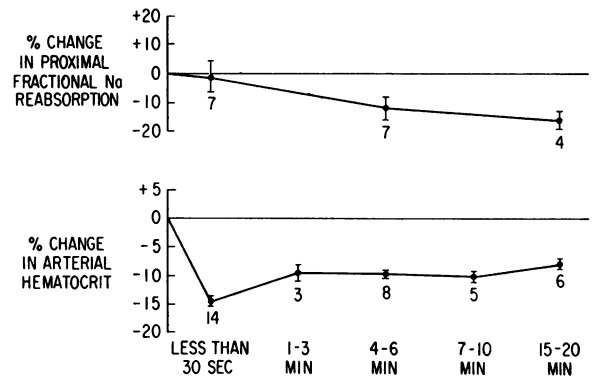

FIGURE 5 The effect of rapid intra-aortic injections of rat plasma on the time course of changes in fractional reabsorption and arterial hematocrit.

this time averaged 13.3 nl/min ±1.8 SE (n = 4), a finding indicating that absolute reabsorptive rate actually was inhibited. As can be seen in Fig. 4 plasma volume (as estimated from measurement of hematocrit) and peritubular capillary protein concentration during this later period remained relatively constant, whereas reabsorption (absolute as well as fractional) declined progressively.

Other isoncotic solutions. The above experiments were repeated using plasma from hydropenic rats and the synthetic colloid, 6% polyvinylpyrrolidone (PVP), as other forms of isoncotic solutions. The results with each are summarized in Table I and Figs. 3 and 5. It can be seen that for the interval during intra-aortic injection, TF/P inulin recollection ratios were nearly identical with values obtained during initial control collections (mean recollection: initial collection ratios = 1.03 ±0.06 SE, n = 9, for the plasma group and 1.02 ± 0.06 SE, n = 3, during PVP infusion). The time course for changes in fractional sodium reabsorption with each solution (measurements were carried out for 20 min with plasma but only for 5 min with PVP) paralleled almost exactly that seen with isoncotic (5–7 g/100 ml crystalline albumin solution. The change in arterial hematocrit during injection of plasma was identical with that already described for Ringer's and isoncotic albumin solutions (mean = − 14.4% ±1.0 SE, n = 19). Nevertheless, during injection of plasma as well as PVP, fractional reabsorption on the average again was unchanged (Fig. 3). Similarly, absolute reabsorptive rate averaged 27.4 nl/min ±2.5 SE and 29.5 nl/min ±6.4 SE before and during plasma injection respectively, and 21.2 nl/min (range 16.2–27.0) and 19.4 (range 19.2–19.8) for the tubules studied before and during 6% PVP injection. Measurements of arterial and peritubular capillary protein concentration were not made during these time course experiments.

Hyperoncotic albumin solutions. The values for TF/P inulin ratios, V_o, and fractional sodium reabsorption during intra-aortic injection of 9–10 and 15 g/100 ml of crystalline bovine serum albumin solutions are shown in Table I and Figs. 3, 6, and 7, along with the observed changes in arterial hematocrit and arterial and peritubular capillary plasma protein concentrations.

Although the mean TF/P inulin ratio during infusion of 9–10 g/100 ml of albumin solution was not significantly higher, statistically, than the mean value measured before this infusion (Table I), an increase in the recollection: initial collection TF/P inulin ratio was observed in 9 of the 10 tubules studied (mean = 1.17 ± 0.04 SE, $P < 0.005$). This increase corresponds to an average rise in fractional reabsorption of 26.6% ±7.6 SE. During infusion of 15 g/100 ml of albumin solution, TF/P inulin ratios increased in each tubule, the change at this colloid concentration being highly significant ($P < 0.001$). The recollection: initial collection TF/P inulin ratio for six tubules was 1.55 ±0.12 SE, corresponding to an average increase in fractional reabsorption of 41.5% ±6.3 SE. Note in Table I that average values for TF/P inulin ratios (and transit time) were slightly lower in the control periods for each group of rats given these hyperoncotic solutions than was seen in any of the other groups studied. In the 9–10 g/100 ml albumin group, of the 11 tubules studied in 8 the rat had already received one or two previous intra-aortic injections of either plasma or isoncotic albumin solution, thereby causing a modest fall in fractional reabsorption. In the 15 g/100 ml albumin group, 8 of 10

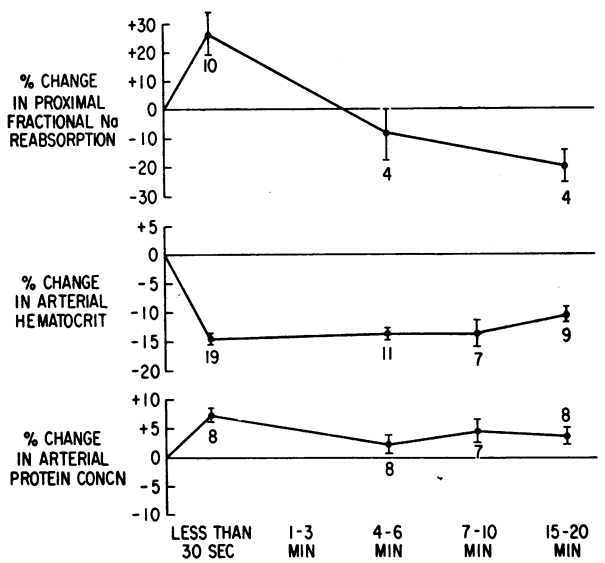

FIGURE 6 The effect of rapid intra-aortic injections of 9–10 g/100 ml of crystalline bovine serum albumin on the time course of changes in fractional reabsorption, arterial hematocrit, and arterial plasma protein concentration.

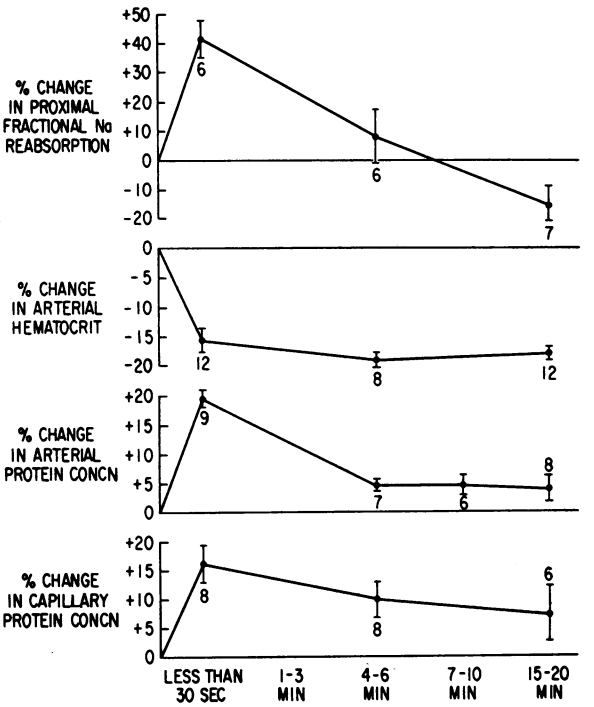

FIGURE 7 The effect of rapid intra-aortic injections of 15 g/100 ml of albumin solution on the time course of changes in fractional reabsorption, arterial hematocrit, and arterial and peritubular capillary plasma protein concentrations.

tubules were studied after previous fluid injections, but in this group Ringer's usually was the solution that was infused earlier. The increase in fractional reabsorption during 9–10 g/100 ml albumin injection was associated with an increase in Cd in 8 of 10 tubules (the average for all tubules increasing from 16.6 nl/min ±1.8 SE to 25.0 nl/min ±4.1 SE). Hence absolute as well as fractional reabsorption returned to but not above typical hydropenic levels. During 15 g/100 ml albumin injection, fractional and absolute reabsorption increased for all 6 tubules (on the average from 49.7% ±2.9 SE and 24.4 nl/min ±2.8 SE, respectively) to values well above those generally obtained from the middle third of the proximal tubule (mean = 65.5% ±4.3 SE and 36.8 nl/min ±6.7 SE). As can be seen in Figs. 3, 6, and 7, changes in arterial hematocrit during hyperoncotic albumin injection were identical with those observed when isoncotic and colloid-free solutions were injected, but despite this uniform effect on hematocrit reabsorption initially increased and then declined progressively. The changes in reabsorption during fluid injection once again were found to parallel the changes in arterial (Figs. 3, 6, and 7) and peritubular capillary protein concentrations (Figs. 3 and 7). Capillary protein concentration increased during seven of eight injections of 15 g/100 ml albumin

solution. The per cent increase ranged from + 10.7 to + 30.1%. In one no change occurred (+ 2.1%). Note that arterial and peritubular capillary protein concentrations (Figs. 6 and 7) subsequently fell toward control levels where they remained for the duration of the measured time course. Nevertheless, fractional reabsorption continued to decline so that by the 15–20 min postinjection interval, a mean net depression well below control values was observed. In the 9–10 g/100 ml albumin group, Cd was reduced on the average from 16.6 nl/min ±1.8 SE (n = 11) to 8.0 nl/min ±2.7 SE (n = 4), associated with an average decline in V_0 from 43.0 nl/min ±3.5 SE to 28.1 nl/min ±2.7 SE (Table I). By the 15–20 min period after intra-aortic injection of 15 g/100 ml albumin solution, Cd fell but only to control levels (24.8 nl/min ±2.6 SE, n = 7). Note however (Table I) that despite no net change in Cd (relative to control values) a substantial increase in V_0 was observed.

DISCUSSION

The experimental aim of this study has been to assess the effects of changes in the colloid osmotic pressure of peritubular capillary blood on rates of sodium and water reabsorption by the rat proximal tubule. The results indicate that in the period that corresponds to perfusion of the peritubular capillary bed with solutions of varying colloid osmotic pressures, changes in reabsorption take place simultaneously with and in the same direction as changes in peritubular capillary plasma protein concentration. In the 20 min period after the brief injection of isotonic Ringer's solution, values for fractional and absolute sodium reabsorption, arterial hematocrit, and arterial and peritubular capillary protein concentrations returned to or toward control levels (Fig. 2), a finding indicating that the rapid injection method exerts no untoward or detrimental effect on either the function of the whole animal or individual nephrons. In the same 20 min interval after colloid infusions, reabsorption was noted to decline not only to but well below control levels (Figs. 6 and 7). This delayed depression of reabsorption demonstrates that although the present approach to producing extracellular fluid volume expansion with colloid solutions differs from that used previously by us (15) and others (25), much the same inhibitory response is elicited.

The changes in femoral arterial (and therefore renal) hematocrit during the periods of intra-aortic fluid injection were observed to be identical in all experimental groups. Nevertheless, since reabsorption was found to increase (hyperoncotic solutions), decline (colloid-free Ringer's solution), and remain unchanged (isoncotic solutions), the role of packed cell volume (as well as any accompanying change in blood viscosity secondary to these changes in packed cell volume) as a determinant of reabsorption can be excluded.

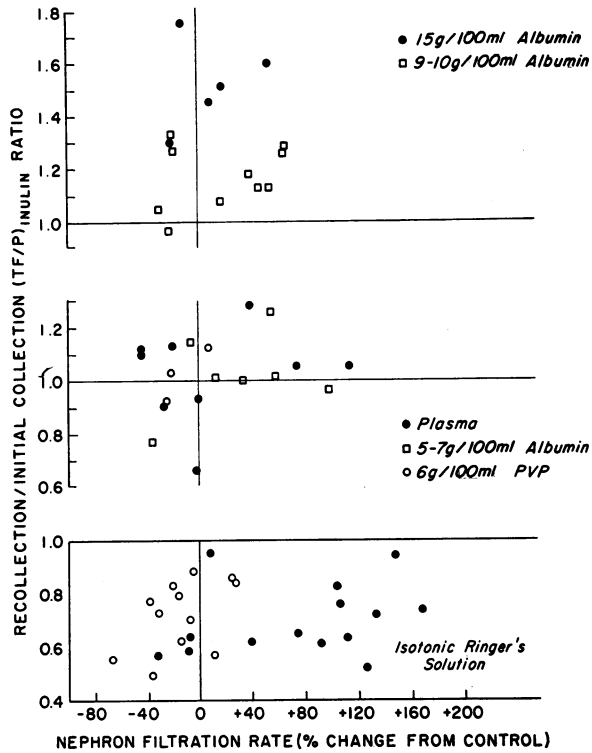

FIGURE 8 The relationship between recollection: initial collection (TF/P) inulin ratios and simultaneously measured changes in nephron filtration rate (V_0) for tubules studied in the 15–25 sec interval during infusion of colloid free (lower panel), isoncotic (middle), and hyperoncotic (upper) solutions. The open circles in the lower panel represent tubules studied during partial aortic construction, a maneuver aimed at preventing the expected increase in V_0.

In the 15–25 sec interval during intra-aortic injection of the various test solutions, fractional as well as absolute sodium reabsorption by the proximal tubule varied directly with the colloid osmotic pressure of the injected fluid. Since glomerular filtration rate (V_0) also changed considerably, it remains necessary to determine to what extent these variations in reabsorption were influenced by simultaneous changes in V_0. This relationship is illustrated in Fig. 8, in which the recollection TF/P inulin ratios during colloid free (lower), isoncotic (middle), and hyperoncotic (upper) infusions are plotted against the simultaneously measured changes in V_0. It can be seen that despite the same relatively wide range of changes in V_0 for each infusion group, reabsorption was found to increase (hyperoncotic group), decline (colloid free), or remain, on the average, unchanged (isoncotic), and that within each group these changes in reabsorption were independent of the mag-

nitude or direction of the change in V_o.[10] Based on these observations, we believe it reasonable to exclude the influence of glomerular filtration rate, per se, as the stimulus for the changes in reabsorption observed during infusion of solutions of varying colloid osmotic pressure.

The design of the present experiments also permits a consideration of the possible role of humoral factors in bringing about the observed changes in reabsorption. Reabsorption was found to increase and decline in the 15–25 sec interval during hyperoncotic and colloid-free infusions, respectively. For an extrarenal hormone to have mediated these changes, a latent period of at least two circulation times would have been required (i.e. one circulation time to carry the stimulus to a sensor or site of storage of a hormone, and another to allow the humoral factor to be conveyed from its site of release to the renal proximal tubule where it effects the appropriate change in reabsorption). A single circulation time in the rat (measured in two animals in this study using Lissamine green in very high concentrations) was found to average approximately 10 sec. Since changes in reabsorption often were detected in 20 or fewer sec of the onset of intra-aortic fluid injection, the rapidity of these adjustments are viewed as evidence against the role of an extrarenal humoral mechanism. An intrarenal hormone is also considered unlikely, not only because of the rapidity with which reabsorption was observed to change, but also because divergent changes in reabsorption would require a hormone, if its action is that of an inhibitor (i.e. to explain the response to colloid-free infusions), to disappear in the same brief interval in order for reabsorption to increase (as seen for example when hyperoncotic albumin is infused into already expanded rats).

In the later intervals after isoncotic and hyperoncotic infusions, we observed a progressive decline in sodium reabsorption. This occurred despite values for arterial and peritubular capillary protein concentration that did not differ significantly from values observed during control periods. In addition, although plasma volume was increased considerably (as estimated from the changes in arterial hematocrit), this measure remained relatively constant at the time reabsorption was noted to decline. Therefore, the depression of reabsorption must have been mediated by one or more factors in addition to colloid osmotic pressure. In this regard, the release (after a latent period of several minutes) of a humoral substance that acts to inhibit reabsorption would appear to be an attractive possibility. However, based on the available data, a sound appraisal of this and other potential factors is not possible. It may nevertheless be noted that, irrespective of the nature of this inhibitory factor, its effect on reabsorptive rate can be counteracted by changes in peritubular capillary colloid osmotic pressure, since it was shown that an abrupt increase in peritubular capillary protein concentration induced by injection of hyperoncotic fluid into an already expanded rat always led to augmentation of absolute and fractional sodium reabsorption.

In this study, during the period of intra-aortic fluid injection, absolute and fractional sodium reabsorption varied directly with changes in arterial and peritubular capillary protein concentration. As discussed above, the design of these experiments has allowed us to exclude a variety of mechanisms thought important in regulating the rate of sodium reabsorption by the proximal tubule. We are led therefore to conclude that the total protein concentration of peritubular capillary blood is one important determinant of this reabsorptive process.

The existence of a directly proportional, and indeed causal relationship between peritubular capillary oncotic pressure and rate of tubule fluid reabsorption has been both advocated and denied by a number of investigators for more than a century (26, 27). According to these earlier as well as more recent proponents of this view (28–33), fluid reabsorption has been considered to be a passive process, determined solely by the net balance of colloid osmotic and hydrostatic forces acting across the tubule epithelial cell. The subsequent and overwhelming evidence from several sources that fluid reabsorption by the proximal tubule occurs against a concentration gradient (34, 35) and even takes place when the transtubular oncotic gradient is abolished (34, 36) makes these arguments for a simple, passive process generally unsatisfactory. More recently, Earley and Windhager and their respective coworkers have attempted to incorporate the role of Starling forces into our current concepts of active transepithelial transport (7, 16–18, 37–40). In attempting to clarify the mechanism that leads to decreased proximal sodium reabsorption and natriuresis after saline loading, Earley and associates concluded on the basis of clearance studies in the dog that net fluid reabsorption is determined, at least in part, by the rate of removal of reabsorbate (the quantity of sodium and water transported actively from tubule lumen to the more basal portions of the cell) by the capillary circulation. The rate of removal of this reabsorbate is, in turn, considered to be dependent upon the net balance of colloid osmotic and hydrostatic pressures across the peritubular capillary wall. Lewy and Windhager reached similar conclusions from micropuncture studies of glo-

[10] We wish to emphasize that V_o measured during brief (10 sec) collections tends to be higher (see footnote 5) and to vary more than when less rapid collections are made. Nevertheless, although it is difficult to be certain of the exact extent to which these measurements of V_o reflect this systematic as well as other not inconsiderable random errors, it is felt that V_o did in fact vary appreciably during these infusions.

merulotubular balance in the rat (7), in which they observed a roughly proportional relationship between proximal fluid reabsorption and filtration fraction.

An attempt was made as part of the present study to evaluate the contribution of changes in capillary hydrostatic pressure to the observed alterations in reabsorptive rate. However, because of the non–steady-state conditions (especially in the interval during intra-aortic fluid infusion) and the marked, albeit random, variability in hydrostatic pressure measurements which we found using conventional micropuncture techniques, it was not possible for us to obtain a meaningful assessment of the importance of this variable in these experiments. It nevertheless seems reasonable to assume that capillary hydrostatic pressure may have changed in the same direction during intra-aortic injection of equal volumes of colloid-containing and colloid-free solutions. Consequently, the observed divergent changes in reabsorption would appear to oppose a regulatory role for hydrostatic pressure. Nevertheless until specific and more accurate measurements can be obtained, a definitive statement regarding this variable must be postponed.

Neither the findings in the present study, nor those of others, provide clear insights into the mechanisms whereby changes in peritubular capillary colloid osmotic pressure influence net transfer of salt and water from the proximal tubule. The schema proposed recently by Lewy and Wandhager (7), in which the functional as well as ultrastructural similarities between the proximal tubule and other transporting epithelia (e.g. gall bladder) are emphasized, is attractive, but until a number of fundamental validating observations are provided, its utility remains chiefly that of a conceptual analogue.

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