Variations in Renal Stop Flow Concentration Patterns Produced with Mannitol Infusions[1] (35673)

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The stop flow method developed by Wilde and Malvin (9) has been utilized in qualitative investigations of renal function. The method would appear to be adequate in demonstrating and locating qualitative differences in function occurring within the nephron (9) but the application of the stop flow method in the detection of quantitative differences in function has led to the realization that the diuretic employed may alter the results obtained.

Mannitol infusion has been used in stop flow studies to create an adequate osmotic diuresis, that is, a urine rate of at least 4 ml/min/kidney prior to ureteral occlusion. This urine flow rate results in minimal influence of the distal nephron on proximal stop flow samples after release of ureteral occlusion. Concentrations of mannitol from 5.5 to 20% have been employed.

In some experiments designed to explore the possibilities of the stop flow method for quantitative studies relating to the absorption of sodium in the proximal tubule as suggested by Malvin *et al.* (7), it was observed that marked shifts of water from intracellular to extracellular fluid compartments were occurring during the course of the mannitol infusion. These were evidenced by progressive decreases in plasma protein concentration. These changes are undesirable variables if quantitative studies of sodium absorption are to be undertaken. For this reason, various concentrations of mannitol were infused to determine the concentration which produced the least change in plasma protein concentration.

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Mannitol was infused in concentrations ranging from 5.5% (isotonic) to 20%. It was observed that the nature of the resulting stop flow concentration patterns depended upon the concentration of mannitol infused. Of particular interest were the variations in stop flow concentration patterns of creatinine and para-aminohippuric acid (PAH). An attempt was made, therefore, to evaluate the effects of various concentrations of mannitol infusion on plasma protein concentration and osmolality, and on the nature of the concentration patterns developed during stop flow.

Methods. Female mongrel dogs, weighing 15–26 kg, were anesthetized with 30 mg/kg of pentobarbital sodium or 50–100 mg/kg of chloralose. In all animals, both ureters were exposed through flank incisions and cannulated with polyethylene tubing. The left kidney was used for all stop flow procedures in each animal. Each animal initially received 5.5% mannitol, followed by 10%, then 20% mannitol infused into a femoral vein with a Sigma peristaltic action pump at approximately 0.4 ml/kg/min. Each animal group consisted of six dogs and the infusion sequence was randomized in blocks of two. One animal received an initial infusion of 20% mannitol for 39 min, followed by a 129-min period of no infusion. Ethacrynic acid [(Edecrin) (2 mg/kg/hr)] without mannitol was then infused for 25 min. Mannitol and ethacrynic acid infusion solutions contained 0.12% creatinine, 0.9% sodium chloride, and 25 mg/100 ml of para-aminohippuric acid (PAH), except in one animal in which PAH was omitted from the infusion solution and injected 2 min prior to release of ureteral occlusion. Attempts were made to infuse each

TABLE I. Changes in Plasma Osmolality and Total Protein and Sodium Concentrations with Infusion of 5.5, 10, and 20% Mannitol.

Dog no.	Duration of infusion (min)			Plasma (mOsm/kg)			Plasma sodium (μEq/ml)			Plasma total protein (g/100 ml)		
% mannitol infused:	5.5	10	20	5.5	10	20	5.5	10	20	5.5	10	20
1	118	70	63	332	357	411	139.5	136.5	135.0	4.5	4.0	3.9
2	61	60	54	329	355	384	138.0	145.6	147.2	4.8	4.4	3.8
3	94	68	69	323	363	413	142.2	147.2	149.4	5.0	4.7	4.3

concentration of mannitol for approximately 60 min, but this was not possible in all cases due to the difficulty in obtaining an adequate diuresis within 60 min with 5.5% mannitol infusion. The stop flow procedure was done at the end of each infusion period.

Body fluid balance was maintained with Ringer's solution diluted 0.75:1 with distilled water. After urine flow had stabilized at a rate of 4 ml/min/kidney or more, a free flow urine sample was collected for 1 min prior to ureteral occlusion. The ureteral cannula was then clamped for 8 min during which time a heparinized sample of femoral arterial blood was collected. Upon release of the occlusion, serial urine samples of 0.5 to 1.0 ml were collected in tared vials until approximately 15 to 20 ml of urine had been collected. Sample volume was estimated by weight.

Creatinine concentration was determined according to the method of Bonsnes and Taussky (2), PAH concentration according to the method of Brun (3), and total protein according to the Dade modification of the biuret method (4). Sodium concentration was determined with an IL flame photometer; osmolality was determined with an Advanced Instruments, Inc. osmometer.

Results. Table I indicates the increase in plasma osmolality as well as the simultaneous decrease in plasma protein concentration occurring with increased concentration of mannitol infusion. Although the duration of infusion of animal 1 with 5.5% mannitol was almost twice that of animal 2, the osmolalities of the two animals are similar. Plasma osmolality was increased in spite of little or no change in plasma sodium concentration.

Table II indicates the increase in urine flow rate occurring with the higher concentrations of mannitol infused at the same rate as 5.5% mannitol. Changes in creatinine clearance are variable with changes in mannitol infusion. Table II also indicates the decrease in urine:plasma concentration ratio for creatinine (U/P_{Cr}) occurring with increased concentration of mannitol infusion in the best distal stop flow sample, that is, the sample of lowest sodium concentration or highest urine flow rate.

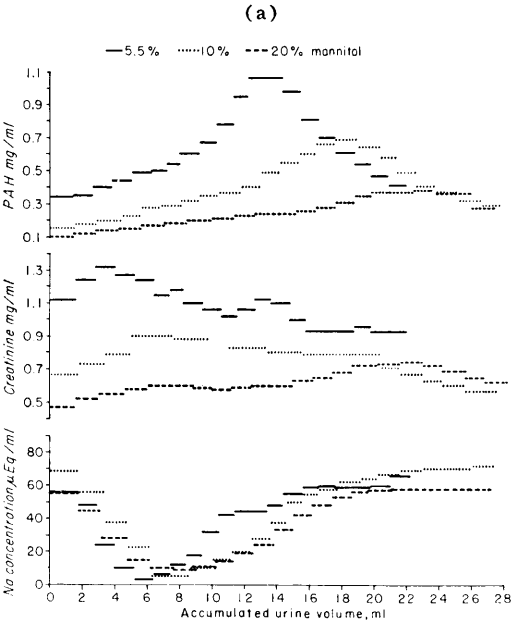
TABLE II. Changes in Urine Rate, Distal U/P_{Cr}, and Creatinine Clearance with Infusion of of 5.5, 10, and 20% Mannitol.

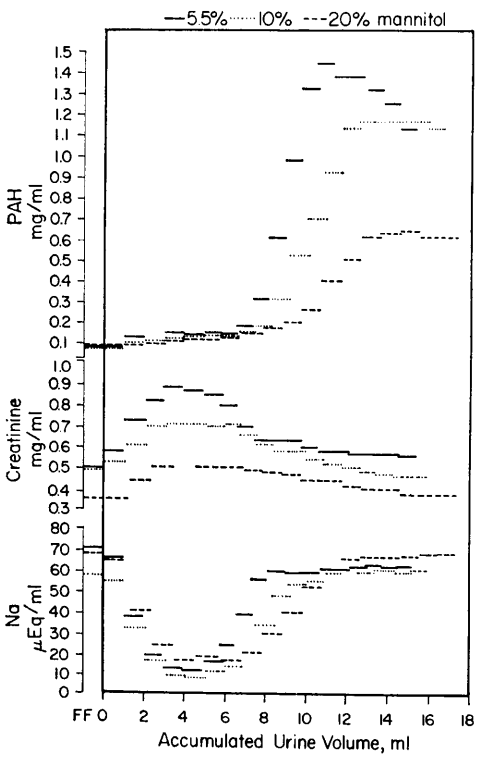
Dog no.	Urine rate (ml/min/kidney)			Distal U/P_{Cr}[a]			Creatinine clearance (ml/min/kg/kidney)		
% Mannitol infused:	5.5	10	20	5.5	10	20	5.5	10	20
1	4.2	8.9	12.8	12.4	7.5	4.3	1.53	1.71	1.62
2	4.0	5.4	9.8	18.5	10.9	5.9	1.99	2.06	1.98
3	4.3	5.0	7.2	12.4	7.9	4.5	1.91	1.69	1.32

[a] *Distal* U/P_{Cr} = urine to plasma creatinine concentration ratio of best distal stop flow sample.

Figure 1a, b, and c illustrates the variation in stop flow concentration patterns developed with the various concentrations of mannitol or ethacrynic acid infusion. In Fig 1a, PAH was infused continuously but as shown, the PAH peak occurs more distally with the lower concentrations of mannitol infusion, although there is little qualitative difference in the stop flow patterns for sodium concentration. More prominent distal creatinine peaks occur in both animals with lower concentrations of mannitol infusion as was noted by distal U/P_{Cr} in Table II.

Except for the stop flow PAH concentration patterns, the patterns developed in the animal (Fig. 1b) in which PAH was injected 2 min prior to release of ureteral occlusion are qualitatively similar to those in which





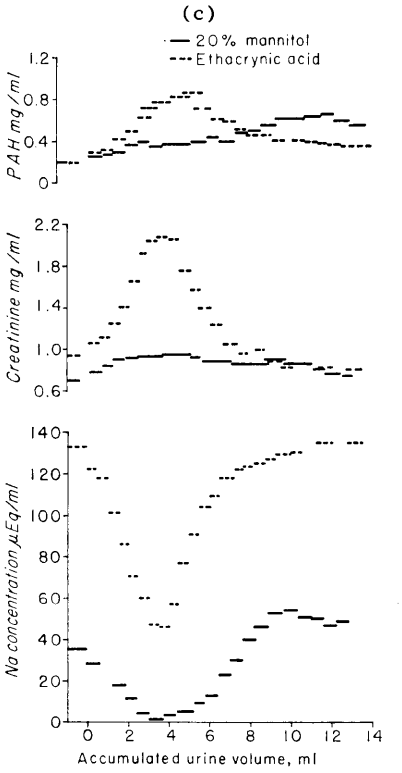


FIG. 1a. Constant PAH infusion: each mannitol infusion solution contained 0.12% creatinine, 20 mg/100 ml of PAH, and 0.9% NaCl infused at 6 ml/min (dog 1, Tables I and II). (b) 100 mg of PAH injected 2 min prior to release of ureteral occlusion: each mannitol infusion solution contained 0.12% creatinine and 0.9% NaCl infused at 6 ml/min (dog 2, Tables I and II). (c) Initial infusion of 20% mannitol (39 min) followed by infusion of ethacrynic acid (2 mg/kg/hr) without mannitol (25 min): each infusion solution contained 0.20% creatinine, 30 mg/100 ml of PAH, and 0.9% NaCl.

PAH was infused continuously. As shown, the PAH peak occurs more distally with lower concentrations of mannitol infusion.

Figure 1c illustrates that the stop flow concentration patterns developed during the initial infusion of 20% mannitol are qualitatively similar to those developed in the other animals with 20% mannitol infusion. However, the patterns developed during the subsequent ethacrynic acid infusion without mannitol appear to be exaggerated responses to the infusion of lower concentrations of mannitol. The PAH peak occurs almost coincidentally with the best distal stop flow sample and the distal PAH concentration pattern displays a more normal distribution than that occurring in the distal shift observed with infusion of lower concentrations of mannitol. The distal U/P_{Cr} with 20% mannitol is 4.8, rising to 11.6 with ethacrynic acid infusion.

Discussion. It has been shown that the concentration of mannitol selected for infusion during stop flow studies will determine the nature of the concentration patterns developed during stop flow. Of particular importance is the development of the PAH concentration pattern since the stop flow sample with the highest PAH concentration is thought to be the sample most representative of proximal tubule function (9). In any given animal in which there is little qualitative change in the stop flow patterns for sodium concentration, the shifting of the PAH peak to a more distal position can indicate only that under those circumstances in which the shift occurs, the PAH peak is not a reliable indicator of the best proximal stop flow sample.

The distal shift can be explained in part on the basis of previous micropuncture studies, which have demonstrated that hypo-osmotic fluid entering the distal tubule becomes isosmotic upon reaching the end of the distal convolution if ADH is present (5). During mannitol diuresis the sodium concentration of the distal tubular fluid was one-half or less that in the nondiuretic animal. This indicates that the presence of nonreabsorbable solute (mannitol) in the distal tubule inhibits water reabsorption and results in decreased sodium concentration of the distal fluid. The various degrees of inhibition of distal water reabsorption with various concentrations of mannitol infusion are illustrated in Fig. 1. It can be assumed that ADH is present in these animals in response to the increased plasma osmolality (8). Figure 1a and b shows that the creatinine peaks with lower concentration of mannitol infusion are more distal than the sodium minimum (9). The more distal location of the creatinine peaks suggests that the site of water reabsorption is probably in the collecting ducts. The higher distal water reabsorption associated with the lower mannitol concentrations probably is caused by a lesser inhibition of sodium reabsorption in the proximal tubule compared with that existing with the higher mannitol levels. The flow rate through the loop is, therefore, lower; washout of the renal osmotic gradient is less; and distal concentrating capability is, therefore, greater (6). The sodium concentrations of the distal samples are essentially identical and do not reflect the changes in water reabsorption. This may be due to the presence of nonreabsorbable solute in all distal samples, or may indicate that during the stop flow period there is adequate time for the establishment of a maximal concentration gradient for sodium.

The effect of the variations in distal water reabsorption on the location of the PAH peak is especially apparent in Fig. 1b. In this animal, 17.5 ml of water were reabsorbed (U/P_{Cr} — 1) to form 1 ml of distal stop flow urine with 5.5% mannitol infusion, while only 9.9 and 4.9 ml water were reabsorbed with 10 and 20% mannitol, respectively (9). PAH was injected during each stop flow period 2 min prior to release of ureteral occlusion. At the lower concentrations of mannitol infusion (high distal water reabsorption), the PAH peak was shifted distally. Since PAH secretion is known to occur in the proximal tubule (9), the presence of the PAH peak in a location more distal than that occurring with 20% mannitol infusion suggests that the increased distal water reabsorption with the lower concentration of mannitol is causing the PAH already present in the distal nephron to be concentrated during occlusion. This reabsorption of water permits filtration

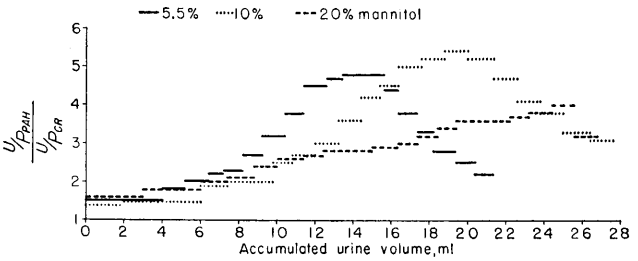


FIG. 2. Constant PAH infusion: There is no change in location of the PAH peak when results are corrected for water reabsorption. Compare with Fig. 1a.

during stop flow with subsequent replacement of the volume of water reabsorbed distally. The distal shift of the PAH peak in Fig. 1a (constant PAH infusion) is not as pronounced as that in Fig. 1b, but also indicates that the PAH peak is not a reliable indicator of the best proximal sample when insufficient amounts of nonreabsorbable solute are present distally. That these shifts are not caused by this method for presentation is shown by comparison of Fig. 1a with Fig. 2 and Fig. 1c with Fig. 3. Figures 1–3 show no difference in the location of the PAH peaks when the standard correction for water reabsorption is utilized.

Past investigations have shown that treatment with ethacrynic acid results in inhibition of proximal PAH secretion and sodium reabsorption in a region distal to the proximal tubule (1). Figure 1c illustrates that the more prominent distal creatinine peak (*i.e.*, increased water reabsorption) occurring with ethacrynic acid than with 20% mannitol infufusion is coincidental with the sodium minimum, indicating little water reabsorption in the collecting ducts, when compared with the

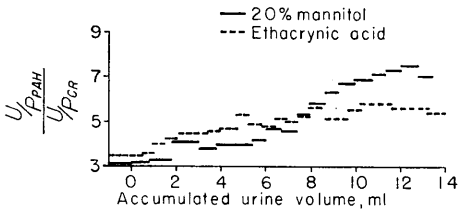


FIG. 3. Initial infusion of 20% mannitol followed by infusion of ethacrynic acid without mannitol. There is no change in location of the PAH peak when results are corrected for water reabsorption. Compare with Fig. 1c.

distal creatinine peaks for 5.5% mannitol infusion (Fig. 1a and b). Plasma osmolality fell from 388 mOsm/kg with 20% mannitol infusion to 367 mOsm/kg with ethacrynic acid infusion. The distal U/P_{Cr} increased from 4.8 with 20% mannitol infusion to 11.6 with ethacrynic acid infusion. Coincidentally, the PAH peak is shown to be shifted to the site of the sodium minimum with ethacrynic acid infusion. The proximal PAH concentration plateau may be the result of rapid distal movement of the PAH in new filtrate.

Summary. An attempt was made in this study to ascertain with what degree of validity and reproducibility the maximum PAH concentration during stop flow represents the best proximal sample. It has been shown that the diuretic chosen for stop flow studies will determine the location of the PAH peak by its effect on distal water reabsorption. With distal water reabsorption effectively inhibited with hypertonic mannitol infusion as indicated by decreased distal U/P_{Cr}, the stop flow patterns for PAH concentration in the proximal tubule is shown to approach a normal distribution. With increased distal water reabsorption, the stop flow patterns for proximal PAH concentration are shown to be skewed to the left, indicating a distal shift of the PAH peak from its proximal secretory site.

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