Glomerular Filtration during Stop-Flow* (33755)

Z. L. PAPADOPOULOU,[1] L. M. SLOTKOFF,[2] G. M. EISNER, AND L. S. LILIENFIELD

Department of Physiology and Biophysics, Georgetown University Schools of Medicine and Dentistry, and Cardiovascular Research Division, Department of Medicine, Georgetown University School of Medicine, Washington, D. C. 20007

Since the introduction of stop-flow analysis by Malvin *et al.* in 1958 (1) many investigators have used this technique to study the localization of transport systems along the length of the nephron. During a brisk diuresis a ureter is occluded; the method demands that as ureteral pressure is increased intracapsular pressure will rise and reach the filtration pressure of the glomeruli, thus resulting in cessation of filtration. However, the effectiveness of this technique in producing a cessation of glomerular filtration in the presence of diuresis has long been questioned and some evidence has been presented that glomerular filtration does not cease completely (2–4). Differences in the methods utilized by various workers in establishing stop-flow may very well account for some of the conflicting opinions as to the effectiveness of this technique. Different diruetics have been used and the magnitude of the diuresis present prior to the onset of ureteral obstruction has varied.

In the present experiments, the extraction ratio of radioactive Hypaque (^{131}I sodium diatrizoate) (E_{Hyp}) was examined during control periods and during stop-flow following diuresis produced with mannitol or hypertonic saline. RadioHypaque was used in the experiments because it can be measured more accurately than inulin. It has been shown by several investigators (5–8) that Hypaque is similar to inulin in that it is filtered at the glomerulus and neither secreted nor reabsorbed in the remainder of the nephron. The extraction ratio of a substance, such as Hypaque, that is only filtered, is equal to the ratio of the GFR/RPF. Under conditions in which the RPF is not altered, changes in the extraction ratio can be used as an index to changes in the GFR. Previous reports indicated that during stop-flow the RPF is not significantly altered during stop-flow. The extraction ratio of radioHypaque (E_{Hyp}) then was used as an indirect measure of the GFR. By measuring E_{Hyp} at regular intervals during ureteral blockade it was possible to assess the extent to which various diuretics influence the success of the stop-flow technique.

Methods. Mongrel dogs of either sex ranging in weight from 14–27 kg were anesthetized with 25 mg/kg of sodium pentobarbital intravenously. After an appropriate priming dose of Hypaque ^{131}I, a sustaining infusion of Hypaque ^{131}I was delivered through the brachial vein at a constant rate via a Harvard infusion pump. Both kidneys were exposed through a midline abdominal incision and both ureters were cannulated with polyethylene tubing. After intravenous administration of heparin a polyethylene catheter was introduced into the right femoral vein and guided into the right renal vein to obtain renal venous blood for measurement of extraction ratios. A catheter inserted into the aorta through a femoral artery was used to record blood pressure and to collect arterial blood samples.

Diuresis was produced by infusion of a 12.5%mannitol solution in seven experiments, and 3% NaCl in five experiments. The 12.5% mannitol was made up in saline so that its osmolar concentration was equal to that of 3% NaCl. The solution was administered at a rate of 10–12 ml/min. After a diuresis of 4 ml/min per kidney or more was achieved

* Supported in part by USPHS Grants HE-02987 and HE-5527, and by a general research support grant to Georgetown University.

[1] Research Fellow, Cardiovascular Research Division, Department of Medicine, Georgetown University School of Medicine. Currently, Research Fellow, Pediatric Renal Department, Children's Hospital of D.C., and Georgetown University School of Medicine, Washington, D.C.

[2] Advanced Research Fellow of the American Heart Association.

TABLE I. Postocclusion Ureteral and Arterial Pressures during Mannitol and Sodium Diuresis.

	Free urine flow before occlusion (ml/min)	Ureteral pressure during occlusion (mm Hg)	Mean arterial pressure during occlusion (mm Hg)	A–U gradient (mm Hg)
Mannitol diuresis (7)	5.4	82	119	37 (69%)
3% Saline diuresis (5)	4.3	70	104	34 (67%)

and a sufficient equilibration period, control clearances, and extraction ratios of radioHypaque were obtained. Following the control periods, unilateral urinary stasis was produced by connecting the right ureteral catheter to a Sanborn pressure transducer. Starting from the moment of occlusion (zero time) blood samples were collected simultaneously from the aorta and the right renal vein at regular intervals up to the twentieth minute of ureteral blockade. Aortic and ureteral pressures were recorded continuously throughout the period of ureteral occlusion. Following release of the obstruction a recovery period of 15-min duration was allowed before repeat clearances and extraction ratios of radioHypaque were obtained from each kidney. In six separate experiments the same protocol was used as described above to produce stop-flow, and renal blood flow was measured by an electromagnetic flow meter—before, during and after ureteral occlusion.

Urine and plasma specimens from all studies were analyzed for Hypaque ^{131}I concentration. Radioactivity was measured in an automatic gamma well counter (Nuclear-Chicago).

Results. The rate of free urine flow, ureteral pressure during occlusion, and mean arterial pressure did not differ significantly between the two series of experiments (Table I). The final ureteral pressure averaged 82 mm Hg (range 60–105 mm Hg) when 12.5% mannitol was infused, and 70 mm Hg (range 55–100 mm Hg) when 3% NaCl was infused. This difference in ureteral pressure appeared, for the most part, to be a reflection of the average mean arterial pressure. This, in general, was higher under mannitol diuresis (119 mm Hg) than under hypertonic saline diuresis (104 mm Hg). The final A–U gradient, *i.e.*, the pressure difference between the mean arterial pressure and the ureteral pressure during occlusion, did not differ significantly between the two sets of experiments. The latter averaged 37 mm Hg with mannitol diuresis, and 34 mm Hg with hypertonic saline diuresis, the difference between those two values not being statistically significant.

Although the experimental conditions between the two sets of experiments were quite comparable, during ureteral blockade there was a striking and significant difference in the E_{Hyp} during stop-flow. Tables II and III show the values for E_{Hyp} obtained in each experimental animal during control periods and during stop-flow for saline and mannitol diuresis, respectively. With mannitol diuresis there was a rapid fall of the E_{Hyp} starting almost immediately after the onset of ureteral blockade and progressing from an average control value of 0.14–0.02 by the fifth minute of occlusion, and approaching zero thereafter. With 3% saline diuresis, the E_{Hyp} remained relatively high during the entire period of occlusion, decreasing only from a control average of 0.18 to an average of 0.14 on the twentieth minute of stop-flow (Fig. 1).

Direct measurements of the renal blood flow utilizing an electromagnetic flow meter were performed in six additional animals. Table IV shows the values obtained before and during ureteral occlusion in four dogs undergoing saline diuresis. During saline diuresis,

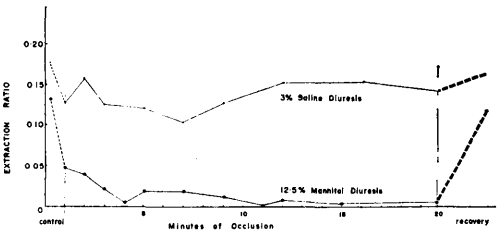

FIG. 1. Average values of E_{Hyp} obtained during control periods, during ureteral occlusion and during recovery under diuresis produced with mannitol (7 expts.) and hypertonic saline diuresis (5 expts.).

TABLE II. Effect of Ureteral Occlusion during Saline Diuresis on the Extraction Ratio of Radio-Hypaque.

Expt. no.	Wt. of dog (kg)	Urine flow prior to occlusion (ml/min)	E_{Hyp} following ureteral occlusion									C_{Hyp}		RPF (calculated)	
			Control	1 min	2 min	5 min	7 min	9 min	12 min	15 min	20 min	Control	Recovery	Control	Recovery
1	13.6	5.0	0.27	0.17	0.24	0.11	—	0.23	0.25	0.23	0.23	0.21	31.0	25.3	115	120
2	25.0	4.3	0.15	0.09	0.13	0.07	—	0.08	—	—	—	—	39.0	—	260	—
3	14.1	3.6	0.16	0.16	0.16	0.17	0.13	0.16	0.18	0.17	0.16	0.14	32.2	27.5	201	196
4	25.0	4.4	—[b]	—[b]	—[b]	0.15	0.11	0.10	0.11	0.12	0.10	0.15	34.5	31.8	—	—
5	27.3	4.0	0.13	0.11	0.11	0.11	0.07	0.07	0.08	0.10	0.08	0.15	41.6	31.7	320	211
Mean		4.3	0.18	0.13	0.16	0.12	0.10	0.13	0.16	0.16	0.14	0.16				
± SD			±0.06	±0.04	±0.06	±0.04	±0.03	±0.07	±0.08	±0.06	±0.07	±0.03				
p value[a]				>0.2	>0.6	>0.1	>0.1	=0.3	>0.6	=0.6	>0.4	>0.6				

[a] As compared to the mean control value.

[b] Initial values not obtained because catheter had slipped out of the renal vein. Following the 2 min postocclusion it was repositioned.

TABLE III. Effect of Ureteral Occlusion during Mannitol Diuresis on the Extraction Ratio of Radio-Hypaque.

Expt. no.	Wt. of dog (kg)	Urine flow prior to occlusion (ml/min)	E_{Hyp} following ureteral occlusion during mannitol diuresis								C_{Hyp}		RPF (calculated)	
			Control	1 min	2 min	5 min	7 min	9 min	15 min	20 min	Recovery	Control	Recovery	Control	Recovery
1	20.0	7.3	0.22	0	—	0	0	—	—	—	—	16.9	—	76.8	—
2	18.2	4.3	0.10	0.02	0.05	0.04	0.04	0.03	—	—	0.16	21.4	9.0	214	56.4
3	14.1	4.0	0.08	0.02	0	0.02	0	0.003	0.006	—	—	16.6	—	208	—
4	21.8	7.0	0.11	0.07	0.006	0	0.02	0	0	—	0.14	30.0	23.7	273	169
5	16.8	4.0	0.09	—	—	0	0.01	0.02	0.01	0.02	0.06	16.9	15.5	188	258
6	22.7	6.6	0.18	0.08	0.06	0.04	0.03	0.02	0.03	0.002	0.09	34.7	19.0	193	211
7	27.3	4.7	0.18	0.10	0.06	0.03	0.03	0.04	0.003	0.002	0.15	37.6	33.9	209	226
Mean		5.4	0.14	0.05	0.04	0.02	0.02	0.02	0.01	0.008	0.12				
± SD			±0.05	±0.04	±0.029	±0.018	±0.016	±0.015	±0.012	±0.010	±0.04				
p value[a]				<0.001	<0.001	>0.001	>0.001	>0.001	>0.001	<0.001	<0.001				

[a] As compared to the mean control value.

TABLE IV. Effect of Ureteral Occlusion on Renal Blood Flow during Saline Diuresis (measured with electromagnetic flow-meter).

Expt. no.	Control (ml/min)	Ureteral occlusion (ml/min)			Recovery (ml/min)
		3 min	7 min	15 min	
1	264	200	120	120	ND[a]
2	180	200	190	200	180
3	106	92	92	92	106
4	120	130	132	126	120

[a] Not done.

the effect of ureteral occlusion on the renal blood flow was a maximum change of 13% in 3 of the 4 cases. In the fourth dog, the renal blood flow fell progressively throughout the experiment indicating that the animal's condition was deteriorating. During mannitol diuresis in the two dogs there was a drop in renal blood flow of 30% or more in each case. However, the case showing the largest fall was not technically satisfactory, making interpretation difficult.

Discussion. The effectiveness of the stop-flow technique in the localization of the reabsorption and secretory processes along the length of the nephron is based on the assumption that, as a result of increase in the ureteral pressure during occlusion, glomerular filtration is completely interrupted. Omachi and Macey (2) and Taylor and Ullman (3) showed that substances which are believed to enter the nephrons only by glomerular filtration (inulin or creatinine) continued to do so during ureteral obstruction, not only in the initial stages but also after the ureteral pressure had attained very high levels. However, as pointed out by the latter authors, the concentration of the recovered marker is no direct guide to the volume of filtrate in which it was contained. In the absence of any precise information on the exact time course of the decline in filtration rate, several assumptions were made in order to calculate the volume of filtrate formed during a given interval, based chiefly on the mean plasma clearances of the injected substances. Thus, the values presented cannot be considered as precise measurements. Carrasquer and Baldwin (4) showed that when creatinine-^{14}C was injected in three diuretic dogs after the ureter was obstructed, a significant amount of it was found in the renal pelvis of the stop-flow kidney. However, in these studies back pressure was achieved by retroureteral injections of urine, thus introducing another variable in the method of the stop-flow technique. The present experiments showed that the use of a nonreabsorbable solute as a diuretic is necessary for successful stop-flow.

Different investigators observed both increases and decreases in the renal blood flow (RBF) following ureteral obstruction. Malvin *et al.* (1) using a rotameter, showed that there was no significant change in the renal blood flow in six dogs during ureteral occlusion under mannitol diuresis. Kiil and Aukland (9), using an electromagnetic flow meter, showed that renal blood flow remained constant or increased slightly when ureteral pressure was increased up to 70–80 cm H_2O, but then decreased to 20–30% of control values with further increase of ureteral pressure. On the other hand, Thurau (10) showed that ureteral occlusion in dogs undergoing mannitol diuresis resulted in an increase in the renal blood flow (+27%). Although there was simultaneous increase in the interlobular venous resistance, Thurau (10) concluded that arteriolar dilation must be of such magnitude as to effect a net increase in the renal blood flow. Similarly, Selkurt (11), Gilmore (12), and Nash and Selkurt (13) showed that elevation of ureteral pressure in dogs undergoing either mannitol or hypertonic saline diuresis is accompanied by an increase in renal blood flow. Selkurt (11), by utilizing both the direct and indirect methods of measurement of the renal blood flow, showed that the average increase

in blood flow was 38% in six experiments in which 5% NaCl was used (absolute values were from a control average of 129–178 ml/min/kidney). However, in the present experiments, with the marked decrease in E_{Hyp} (approaching zero) shown during ureteral obstruction in dogs undergoing mannitol diuresis, it would be necessary to postulate an enormous increase in the renal blood flow in order to assume that the GFR remains constant or decreases only slightly. Such an extreme rise in RBF has never been shown to take place. In our preliminary experiments when the renal blood flow was measured during mannitol diuresis in two dogs, decreases were noted. During ureteral occlusion following saline diuresis, 3 of the 4 cases showed changes ranging from a reduction of 13% to an increase of 11% in blood flow. In these experiments, since the average maximal decrease in E_{Hyp} was approximately 45%, GFR may have been compromised. However, the relatively small blood flow changes, plus the finding that even the maximal decrease in E_{Hyp} does not differ significantly from the control value, indicate that GFR is relatively well maintained under these circumstances.

Based on findings of Gottchalk and Mylle (14–16) that increased ureteral pressure results in increased peritubular capillary pressure, Kiil and Aukland (9) deduced that this increased pressure is transmitted backward to the glomerular capillaries, resulting in no net change in the pressure gradient across the glomerular membrane. The present study excludes this possibility since the E_{Hyp} differed significantly with the two types of diuretics. It thus appears that elevation of the peritubular capillary pressure does not necessarily result in an identical increase in glomerular capillary pressure. Similarly, if during ureteral obstruction the "nonreabsorbable residue" becomes reabsorbable because of a leak in the distal segment as suggested by Winton (17), or because of a leak in the proximal tubule as postulated by Taylor and Ullmann (3), one would expect a similar effect on the E_{Hyp} in both the mannitol— and saline-loaded animals. The observation that under comparable mean arterial and ureteral pressures, E_{Hyp} approached zero by the fifth minute of occlusion with mannitol diuresis, whereas it remained very high when hypertonic saline was the diuretic, is highly significant. During ureteral obstruction, the rising intratubular pressure can reduce the net filtration across the glomerular membrane only if a nonreabsorbable substance such as mannitol is utilized as a diuretic, thus inhibiting the reabsorption of fluid in the occluded tubules. However, if the principal solute in the occluded tubule can be reabsorbed, as in the case of saline diuresis, new filtrate will continue to enter the tubules. This hypothesis is further supported by the findings of Carrasquer and Diana (18) who, by utilizing various sodium salts of mono- and multivalent anions as diuretics, showed that as the reabsorbability of the sodium salt increases the stop-flow filtration increases.

In the case of mannitol diuresis, ureteral occlusion resulted in a rapid reduction of the GFR until it ceased almost completely by the fifth minute of occlusion. The ureteral pressure increased steadily after the onset of occlusion and reached a plateau at about the same time. Malvin *et al.* (19) measured the glucose *Tm* during ureteral occlusion, reasoning that since the *Tm* is a function of the number of intact nephrons at any given moment, any decrease in the *Tm* glucose should be proportional to the number of nephrons that cease to filter. They showed that when ureteral pressure was increased to approximately 30% of the mean blood pressure, there was a very small decrease in *Tm* glucose. From that point on, however, *Tm*, glucose fell very rapidly as ureteral pressure was increased. Since not all of the glomeruli are perfused at the same pressure, at any given ureteral pressure above some minimum value some nephrons will be functioning while others will have ceased to filter. In their experiments, when the ureteral pressure was increased to approximately 50% of the mean blood pressure, most of the nephrons had ceased to filter. These findings correlate with those of the present study. Following ureteral occlusion during mannitol diuresis the GFR,

although it falls rapidly, does not cease completely until about the fifth minute of ureteral occlusion; this fact must be taken into account when one undertakes the study of localization of transport systems along the tubule. On the other hand, hypertonic saline diuresis should not be used in such experiments since glomerular filtration continues in spite of high ureteral pressure because of the continuing reabsorption of salt water.

Summary. The extraction ratio of radioactive Hypaque (E_{Hyp}) was examined during stop-flow under diuresis produced with mannitol or hypertonic saline. In seven dogs undergoing mannitol diuresis E_{Hyp} decreased from a control average of 0.14 to 0.02 by the fifth minute of ureteral occlusion and remained at or near zero thereafter. In five dogs undergoing hypertonic saline diuresis the E_{Hyp} decreased only slightly from the control average of 0.18 and generally ranged from 0.12 to 0.16 with a value of 0.14 at the twentieth minute of stop-flow. These experiments indicate that cessation of glomerular filtration during ureteral occlusion is dependent upon the diuretic agent used. With a nonreabsorbable solute such as mannitol, complete cessation of glomerular filtration occurs within a few minutes. With comparable diuresis produced by saline infusion glomerular filtration continues in spite of high ureteral pressure.

We wish to thank Dr. Charles McCauley for his advice in the statistical analysis of these data, and Mr. Thomas Doyle for his technical assistance.

1. Malvin, R. L., Wilde, W. S., and Sullivan, L. P., Am. J. Physiol. **194**, 135 (1958).

2. Omachi, A. and Macey, R. I., Proc. Soc. Exptl. Biol. Med. **101**, 386 (1959).

3. Taylor, M. G. and Ullmann, E., J. Physiol. (London) **157**, 38 (1961).

4. Carrasquer, G. and Baldwin., A. L., Am. J. Physiol. **209**, 1001 (1965).

5. Woodruff, M. W. and Malvin, R. L., J. Urol. **84**, 677 (1960).

6. Stokes, J. M., Conklin, J. W., and Huntley, H. C., J. Urol. **87**, 630 (1962).

7. Blaufox, M. S., Sanderson, D. R., Tauxe, W. N., Wakim, K. G., Orvis, A. L., and Owen, C. A., Am. J. Physiol. **204**, 536 (1963).

8. Tauxe, W. N., Burbank, M. K., and Maher, F. T., Mayo Clin. Proc. **39**, 761 (1964).

9. Kiil, F. and Aukland, K., Scand. J. Clin. Lab. Invest. **13**, 276 (1961).

10. Thurau, K., Am. J. Med. **36**, 698 (1964).

11. Selkurt, E. E., Am. J. Physiol. **205**, 286 (1963).

12. Gilmore, J. P., Circulation Res. (Suppl. 1) **15**, 148 (1964).

13. Nash, F. D. and Selkurt, E. E., Circulation Res. (Suppl. 1) **15**, 142 (1964).

14. Gottchalk, C. W. and Mylle, M., Am. J. Physiol. **185**, 430 (1956).

15. Gottchalk, C. W. and Mylle, M., Am. J. Physiol. **189**, 323 (1957).

16. Gottchalk, C. W., Circulation Res. (Suppl. 1) **15**, 110 (1964).

17. Winton, F. R., J. Physiol. (London) **83**, 38 (1935).

18. Carrasquer, G. and Diana, A. L., Proc. Soc. Exptl. Biol. Med. **124**, 784 (1967).

19. Malvin, R. L., Kutchai, H., and Ostermann, F., Am. J. Physiol. **207**, 835 (1964).

Received Oct. 17, 1968. P.S.E.B.M., 1960, Vol. 130.