Renal Tubular Permeability during Increased Intrarenal Pressure

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ABSTRACT Renal tubular permeability was studied by microinjection techniques during increased intrarenal pressure in anesthetized diuretic rats. Intrarenal pressure, as evidenced by intratubular pressure (ITP), was increased by elevation of ureteral pressure, partial renal venous constriction, or massive saline diuresis. Various combinations of radioactive inulin, creatinine, mannitol, sucrose, and iothalamate in isotonic saline were microinjected into superficial proximal and distal convolutions, and recovery of the isotopes was measured in the urine.

Inulin was completely recovered in the urine from the injected kidney at both normal and elevated ITP. Creatinine, mannitol, sucrose, and iothalamate were also completely recovered at normal ITP, but recoveries were significantly lower, averaging 73, 85, 89, and 85%, respectively, after early proximal injection when proximal ITP was increased to 30 ±2 mm Hg by elevation of ureteral pressure. Since transit time is prolonged under these conditions, mannitol recovery was also studied during aortic constriction, which prolongs transit time but lowers ITP. Recovery was complete. A significant loss of mannitol was observed during massive saline diuresis, which shortens transit time but increases ITP. During renal venous constriction producing a proximal ITP of 30 ±2 mm Hg, mannitol recovery was significantly less than 100% even after microinjection into distal convolutions, but the loss was greater after injection at more proximal puncture sites. Mannitol recovery was complete during elevation of ureteral pressure in the contralateral kidney.

These studies demonstrate a change in the permeability characteristics of all major segments of the renal tubule during elevation of intrarenal pressure. This change is rapidly reversible and does not appear to be due to a humoral factor which gains access to the general circulation.

INTRODUCTION

It is generally accepted that in normal animals inulin is freely filtrable at the glomerulus and is neither reabsorbed nor secreted by the renal tubule (1–5). It is also usually tacitly assumed that the tubule remains impermeable to inulin under various conditions which alter the hydrostatic and hemodynamic relations in the kidney, but this has not been directly proven. Bank, Yarger, and Aynedjian (6), have recently suggested that the permeability of the proximal tubule changes during partial renal venous constriction, as evidenced by movement of sucrose from peritubular capillaries into the proximal tubular lumen. To evaluate the permeability of the rat tubule under various conditions of elevated intrarenal pressure, we have carried out experiments utilizing the microinjection technique (3). Intrarenal pressure, as evidenced by intratubular pressure (ITP),[1] was increased by elevating the ureteral pressure, by partially constricting the renal vein, or by producing a massive diuresis. Our studies indicate that under these conditions the tubular epithelium remains impermeable to inulin but becomes permeable to various smaller substances to which it is normally impermeable.

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[1] *Abbreviations used in this paper:* GFR, glomerular filtration rate; ITP, intratubular pressure.

METHODS

Male Wistar rats weighing 270–340 g were anesthetized with sodium pentobarbital 50 mg/kg body weight intraperitoneally, and the left kidney exposed for micropuncture as previously described (7). Both ureters were catheterized with P.E. 50 polyethylene catheters. To permit rapid serial urine collections the animals were made diuretic by continuous infusion of 5% mannitol in 0.85% sodium chloride solution at the rate of 100 μl/min. Intratubular microinjections were performed under these control conditions and during various experimental interventions. Ureteral pressure was increased by elevating the free end of the left ureteral catheter to the height required to raise intratubular pressure to the desired level. An adjustable metal clamp was used to partially constrict the left renal vein and elevate the renal venous pressure. Care was taken not to constrict the vein sufficiently to cause cyanosis of the kidney. The degree of constriction was adjusted to elevate the intratubular pressure to the desired level. To evaluate the effect of reduced velocity of tubular flow without elevation of intratubular pressure, the aorta was partially constricted with an adjustable metal clamp above the renal arteries. The degree of aortic compression was adjusted so that transit times to the distal convolution were suitably prolonged, but care was taken not to constrict the aorta sufficiently to produce collapse of the tubules. To study the effect of elevated intratubular pressure when tubular transit time was decreased, massive diuresis was induced by the intravenous infusion of 2.5% sodium chloride solution. The infusion was begun at 100 μl/min and subsequently increased to 200, and then 400 μl/min.

Intratubular pressures (ITP) were measured as previously described (7). Since ITP was relatively unstable during renal venous constriction, it was measured before and after each microinjection, and microinjection results were not accepted if the two pressure measurements differed by more than 4 mm Hg. Since ITP was quite stable during elevation of ureteral pressure, three or four microinjections were performed between pressure measurements.

Constriction micropipettes of known volume were used for microinjection into superficial early and late proximal and distal convolutions, identified with the aid of lissamine green (8). A small volume (1–4.5 nl) of isotonic saline stained with nigrosin and containing various combinations of radioactive compounds was injected at an average rate of 0.23 ± 0.07 (SD) nl/sec. The injection rate was controlled to avoid visible retrograde flow of the dye-stained test solution and dilatation of the tubular lumen.

Inulin-^{3}H was obtained from the International Chemical & Nuclear Corporation, Burbank, Calif., mannitol-^{14}C and sucrose-^{14}C from the New England Nuclear Corp., Boston, Mass., creatinine-^{14}C from the Amersham/Searle Corp.. Arlington Heights, Ill., and ^{125}I-sodium iothalamate (sodium 5-acetamido-2,4,6 triiodo-*N*-methylisophthalamate) from Abbott Laboratories, Radiopharmaceutical Division, North Chicago, Ill. All compounds were certified by the manufacturer to be at least 99% pure by paper chromatography. Maximal concentrations in the test solution for mannitol, creatinine, sucrose, and iothalamate were 6.5 mM, 3.5 mM, 2.2 mM, and 0.8 mM, respectively.

Ureteral urine from both kidneys was collected into vials containing 10 ml of a solution containing 200 ml Triton X-100, 800 ml toluene, and 4 g Omnifluor (Pilot Chemicals, Inc., Watertown, Mass.) per liter. 0.5 g of silicon dioxide gel (Cab-O-Sil, Cabot Corporation, Boston, Mass.) was added to each vial to prevent settling of the isotopes. Urine collections were started at the beginning of each microinjection, and three consecutive 2-min collections were made after injection under control conditions and six to nine 3-min collections under experimental conditions. Before each microinjection urine was collected to measure residual radioactivity. Immediately preceding each microinjection, a timed urine collection of the same duration as each sample after microinjection was taken. A volume of test solution equal to that which was subsequently injected was deposited into this sample and used as a reference standard. On occasion, and without known cause, significant quenching of tritium counts was observed in the reference standard. Although there appeared to be little or no quench- of the ^{14}C counts, these results were discarded. Radioactivity was measured in a three channel liquid scintillation spectrometer as previously described (9). The per cent recovery of injected radioactive substances was calculated as follows:

$$\% \text{ recovery} = \frac{\text{isotope in urine}}{\text{isotope in injectate}} \times 100\%.$$

Clearance studies were performed in another group of rats made similarly diuretic. A priming injection containing 10 μCi mannitol-^{14}C and 40 μCi inulin-^{3}H was followed by the intravenous infusion at 15 μCi/hr of mannitol-^{14}C and 60 μCi/hr of inulin-^{3}H in 5% mannitol and 0.85% NaCl at 100 μl/min. After a 60 min equilibration period two 20-min control collections of urine were made, and the left ureteral catheter was then elevated 30–35 cm above the level of the kidney. After 5–10 min urine flow from the left kidney stabilized, and three consecutive 20-min urine collections were made. Blood samples were withdrawn from the carotid artery at the midpoint of each clearance period. Radioactivity levels in blood and urine were determined as described above.

Results are presented as means $\pm$SD. Student's t test was used for evaluation of statistical significance, and regression lines were determined by the method of least squares.

RESULTS

A total of 282 technically satisfactory microinjections were performed in 67 animals. A microinjection was not considered satisfactory if there was visible leakage at the puncture site or retrograde flow. These problems were occasionally encountered under conditions of elevated pressure if pipettes with large diameter tips (> 7.0 μ) were used, but the difficulties were minimized when pipettes with smaller diameter tips (4–6 μ) were employed. Unless explicitly stated to the contrary the results presented are recoveries measured in the urine from the injected kidney. Except where otherwise indicated, all test solutions contained both inulin and a second test substance.

Inulin. The tubular epithelium was impermeable to inulin under all experimental conditions. The recovery of inulin after early proximal injection at control and elevated intratubular pressures is shown in the upper part of Fig. 1. At control pressures recovery averaged $100.2 \pm 2.4\%$ ($n = 18$). When proximal ITP was increased by partial renal venous constriction or by eleva-

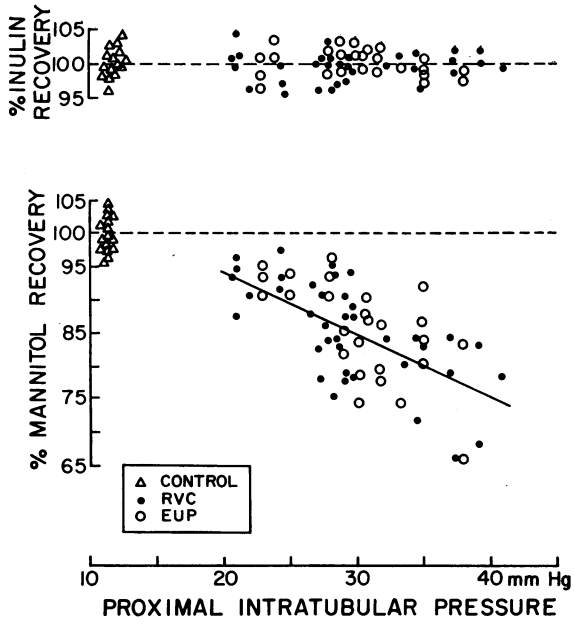

FIGURE 1 Fractional inulin and mannitol recoveries as a function of proximal intratubular pressure after simultaneous early proximal injection during control conditions, renal venous constriction (RVC) and elevation of ureteral pressure (EUP). The broken horizontal lines represent 100% recovery of injected substances. The regression line for mannitol recovery at elevated pressure was $y = 112 - 0.93x$.

tion of the ureteral catheter recovery was unchanged, averaging 99.9 ±2.2% (n = 67). Inulin recovery was 98.9 ± 4.4% (n = 20) when proximal ITP was decreased on the average by 2 mm Hg during aortic constriction (Fig. 2). During 2.5% NaCl diuresis inulin recovery was 99.6 ±2.1% (n = 20).

Mannitol. The recovery of mannitol simultaneously injected with inulin into early proximal convolutions at control and elevated intratubular pressures is shown in Fig. 1. Recovery of mannitol under control conditions was 99.8 ±2.9% (n = 18). However, there was an increasing loss of mannitol when the intratubular pressure was elevated by either renal venous constriction or elevation of ureteral pressure. At intratubular pressures of 30 ±2 mm Hg produced by renal venous constriction, mannitol recovery was 83.1 ±7.7%, and after elevation of ureteral pressure, recovery was 85.1 ±6.4%. Both are different from control values ($P < 0.001$) but not from each other. The correlation between mannitol recovery and elevated intratubular pressure was highly significant ($r = -0.60$, $P < 0.01$).

Recovery of mannitol from the contralateral kidney was measured after all early proximal injections during both renal venous constriction and elevation of ureteral pressure. During the period of collection 57.7 ±11.2% of the mannitol not recovered from the experimental kidney was excreted by the contralateral kidney during renal venous constriction and 59.9 ±8.5% during ele-

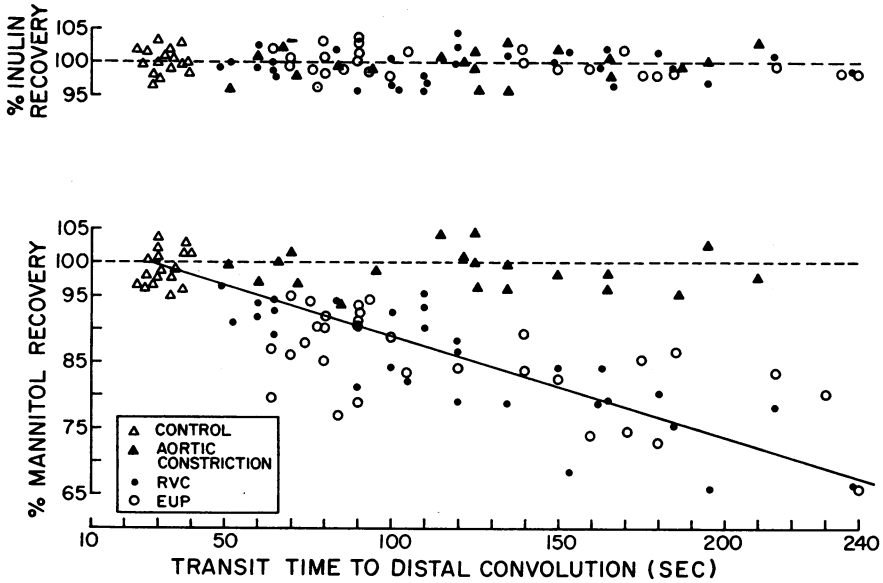

FIGURE 2 Fractional inulin and mannitol recoveries after simultaneous early proximal microinjection as a function of transit time of the test solution to the distal convolution during control conditions, aortic constriction, renal venous constriction (RVC), and elevation of ureteral pressure (EUP). The broken horizontal lines represent 100% recovery of the injected substances. The solid diagonal line, $y = 105 - 0.15x$, describes mannitol recovery during RVC and EUP.

vation of ureteral pressure. This demonstrates that the mannitol not recovered after microinjection traversed the tubular epithelium and gained access to the general circulation, and was not simply sequestered in the kidney.

To ascertain the location of mannitol loss from the tubule, a series of microinjections was performed in early and late proximal and distal convolutions during renal venous constriction. The results are shown in Table I. Loss occurred after injection at all sites during elevated ITP and was different ($P < 0.001$) at each of the three sites, increasing progressively after injection into more proximal parts of the tubule.

A series of microinjections into distal convolutions was also performed during elevation of ureteral pressure. Mannitol recovery was 92.7 ±2.7%, significantly less than control ($P < 0.001$), and less than observed after distal injection during renal venous constriction ($P < 0.05$). However, even though proximal intratubular pressures were 30 ±2 mm Hg in both series, the distal intratubular pressures were higher during elevation of ureteral pressure, and this may account for the difference in mannitol recovery. The decrease in pressure from proximal to distal convolutions was 4–8 mm Hg during renal venous constriction but only 0–2 mm Hg during ureteral pressure elevation.

Renal venous constriction and elevation of ureteral pressure both prolong transit time through the nephron and increase ITP. To determine which of these factors was primarily associated with the mannitol loss, a series of microinjections into early proximal convolutions was performed during aortic constriction. Aortic constriction prolongs transit time but decreases ITP. In Fig. 2 the recovery of mannitol is shown as a function of transit time under the three experimental conditions. The transit time for each microinjection is defined as the time from beginning of the injection into the early proximal convolution until the first appearance of the dye-stained test solution in the distal convolution of the nephron under study. Transit times were prolonged to a similar degree under all three experimental conditions, but ITP was decreased during aortic constriction, averaging 9.4 ±0.5 mm Hg, significantly lower than control (11.5 ±0.4 mm Hg, $P < 0.001$). Mannitol recovery during aortic constriction was 98.9 ±4.4%, not different from control. Even though the loss of mannitol was not due to prolongation of transit time, there was nevertheless a significant correlation between transit time and mannitol loss under conditions of elevated ITP ($r = -0.85$, $P < 0.01$).

Microinjections were performed during and after the release of elevation of ureteral pressure to determine the time course of recovery of normal tubular permeability. Paired injections were made at the same puncture site during, and at varying time intervals after release of elevated pressure. The results are shown in Fig. 3. Mannitol recovery was 86.4 ±5.5% at elevated pressure, and 98.1 ±3.3% ($P < 0.001$) after release of pressure. A return to normal permeability was evident as early as 5–6 min after release of pressure.

TABLE I

Per Cent Recovery of Mannitol after Microinjection at Various Tubular Locations under Control Conditions and during Renal Venous Constriction

Injection site	ITP	Mannitol recovery	P*
	mm Hg	%	
Control			
Early proximal, n = 18	10.5–14.5	99.8 ±2.9	
Renal venous constriction			
Early proximal, n = 19	28–32	83.1 ±7.2	<0.001
Late proximal, n = 16	28–32	90.6 ±3.5	<0.001
Distal, n = 18	22–28‡	96.2 ±3.9	<0.01

* Experimental versus control.
‡ Proximal ITP 28–32 mm Hg.

To test for the appearance in the circulating blood of a humoral factor affecting tubular permeability, in two animals ITP was increased in one kidney while microinjections were performed in the other. The right ureteral catheter was elevated to a height of 30–35 cm above the level of the kidney, a maneuver previously shown to produce proximal ITP in the range of 30 ±2 mm Hg. Mannitol recovery after early proximal injection in the left kidney was complete during and after release of right elevated ureteral pressure, averaging 98.1 ±2.2% and 98.9 ±2.8% (n = 11), respectively.

Profuse diuresis was induced in five animals by infusion of 2.5% NaCl. Proximal intratubular pressures ranged from 11–28 mm Hg. Mannitol recovery after early proximal injection was essentially complete in the four instances in which ITP was less than 20 mm Hg. At ITP between 20 and 28 mm Hg mannitol recovery was 86.7 ±6.9% (n = 15), significantly lower than control ($P < 0.001$). The transit time from point of injection to distal convolution was usually less than control and varied inversely with ITP. Nevertheless, mannitol loss varied directly with transit time.

The differing recoveries of mannitol and inulin after microinjection at elevated ITP suggested that differences in their clearances should be detectable at elevated pressures. Simultaneous mannitol and inulin clearances were performed in seven animals, with two clearance periods at control ITP followed by three during left ureteral pressure elevation. The results are shown in Fig. 4. $C_{mannitol}/C_{inulin}$ in the left kidney was 0.98

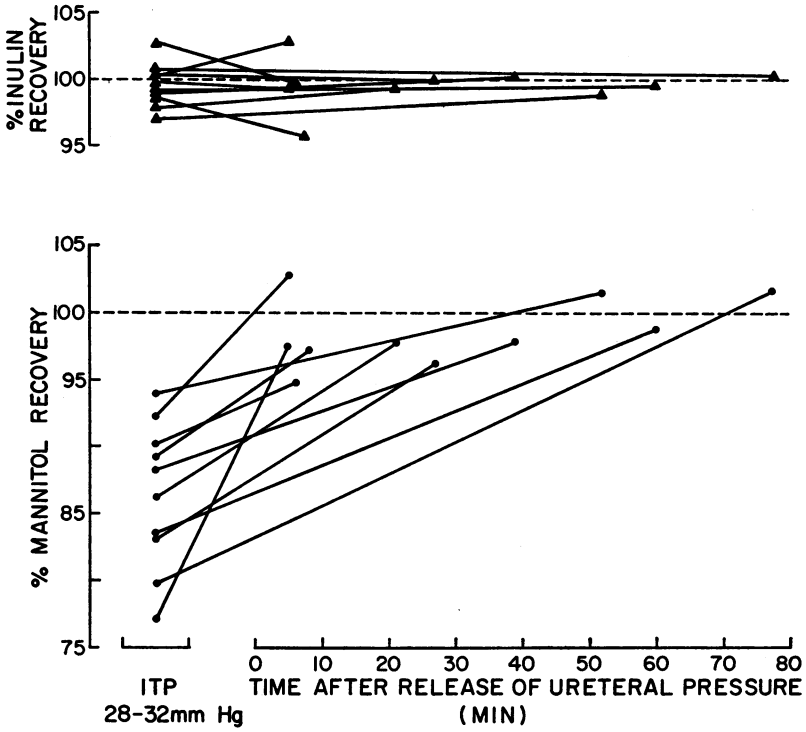

FIGURE 3 Fractional inulin and mannitol recoveries after simultaneous proximal microinjection during and after release of elevated ureteral pressure. Solid lines connect paired injections of the same puncture site. The broken horizontal lines represent 100% recovery of the injected substances.

±0.07 during control periods and 0.85 ±0.05 ($P < 0.01$) after elevation of left ureteral pressure to produce proximal ITP of 30 ±2 mm Hg. The clearance ratios in the right kidney were unchanged, averaging 0.97 ±0.04 during control and 0.97 ±0.01 during experimental periods. Interestingly, as C_{Inulin} and urine flow decreased in the left kidney during the experimental periods, compensatory increases of similar magnitude occurred in the right kidney, and plasma inulin concentration remained unchanged.

Sucrose. Sucrose recovery was studied under control conditions and during elevation of ureteral pressure to produce proximal ITP of 30 ±2 mm Hg. Recovery under control conditions was 98.7 ±3.3% (n = 15). In one series of animals either sucrose and inulin or mannitol and inulin were microinjected into different early proximal convolutions at elevated ITP. Sucrose recovery was 88.8 ±3.2% and mannitol recovery 83.9 ±4.4% ($P < 0.05$). Inulin recovery was complete in both groups. To further evaluate this small but significant difference in the two recoveries at elevated pressures, mannitol-^{3}H and sucrose-^{14}C were injected simultaneously into early proximal convolutions in additional experiments. The ratio of mannitol to sucrose recovery was 0.91 ±0.06 (n = 12), significantly different from 1.0 ($P < 0.001$).

Creatinine. Early proximal microinjections of a solution containing inulin and creatinine were performed during elevated ureteral pressure, and reinjections at the same puncture sites at varying time intervals after release of pressure. The results are shown in Fig. 5. Inulin recovery was complete under both conditions. Creatinine recovery was 73.3 ±9.4% during elevated pressure and 98.8 ±3.0% after release (n = 11, $P <$ 0.001). Within 5 min after release of elevated pressure the tubule was again impermeable to creatinine.

Iothalamate. ^{125}I-sodium iothalamate was injected into the same early proximal convolutions during and after release of elevated ureteral pressure. Iothalamate recovery was 85.0 ±5.2% during increased pressure, and 98.7 ±2.7% after release (n = 14, $P < 0.001$). The tubule appeared to regain its impermeability to iothalamate within 5–6 min after release of elevated pressure (Fig. 6). As shown in Fig. 7, there was a high degree of correlation between the fractional loss of iothalamate from the tubule and transit time ($r = -0.79$). Simultaneous injection of inulin-^{3}H with iothalamate was not feasible because the two isotopes could not be separated in our liquid scintillation system.

DISCUSSION

The results of this study clearly demonstrate that the tubular epithelium remains impermeable to inulin when the ITP is increased well above the physiologic range, thus extending the conditions for which there is direct evidence that inulin is a satisfactory indicator for measurement of glomerular filtration (1–5). Even small changes in permeability to inulin should have been apparent during renal venous constriction and elevation of ureteral pressure, since both conditions markedly prolong tubular transit time and increase the opportunity for diffusional loss.

The permeability characteristics of the tubular epithelium clearly change during elevation of ITP, as evidenced by transtubular loss of several substances to which the tubule is normally impermeable. The change in permeability involved the entire length of tubule. Significant transtubular loss occurred with distal microinjections, but the magnitude of the loss was greater at more proximal injection sites. The change in permeability was apparently nonspecific since molecules with a variety of configurations were similarly affected. On the other hand the loss did appear to be inversely related to molecular size, since of the three carbohydrate molecules tested the loss of mannitol was greatest, that of sucrose was somewhat less, and loss of inulin was undetectable (Table II). Of all substances tested the loss of the smallest molecule, creatinine, was greatest. Although the loss of iothalamate is inconsistent with its molecular weight, this is not unexpected since molecular dimensions are probably more critical in this regard than molecular weights and the relatively high molecular weight of iothalamate is due in large part to the presence of three iodine atoms.

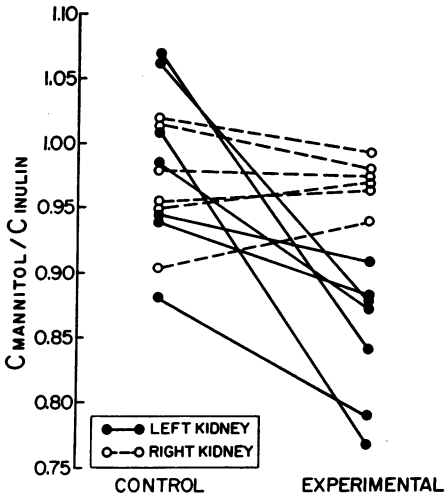

FIGURE 4 The ratio of $C_{mannitol}$ to C_{inulin} before (control) and during (experimental) elevation of left proximal ITP to 30 ±2 mm Hg. Each point in the control column is the mean of two clearance periods. Each point in the experimental column is the mean of three clearance periods. Solid and broken lines connect paired values in left and right kidneys, respectively.

When the permeability was changed during elevated

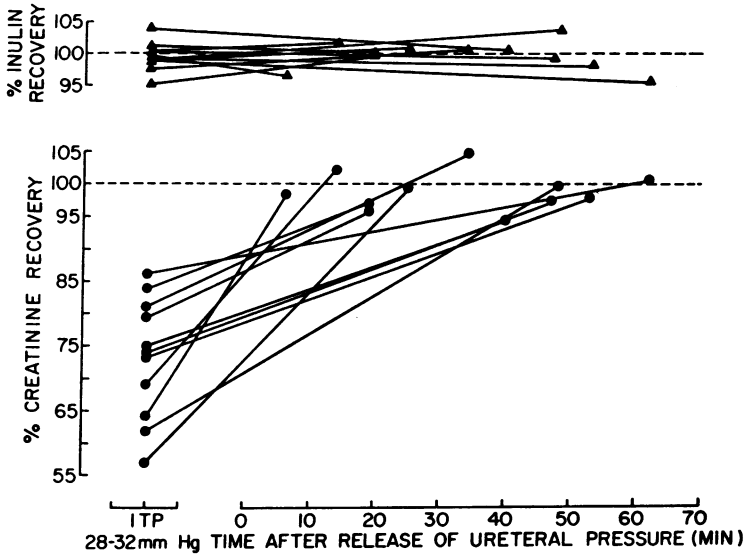

FIGURE 5 Fractional inulin and creatinine recoveries after simultaneous proximal microinjection during and after release of elevated ureteral pressure. Solid lines connect paired injections of the same puncture site. The broken horizontal lines represent 100% recovery of the injected substances.

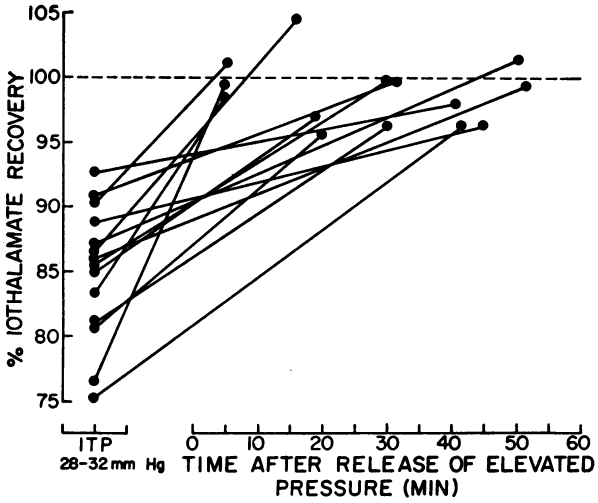

FIGURE 6 Fractional iothalamate recoveries after proximal microinjection during and after release of elevated ureteral pressure. Solid lines connect paired injections at the same puncture site. The broken horizontal line represents 100% recovery.

pressure there was a proportionality between fractional loss and transit time, which is also consistent with a passive mechanism such as diffusion through aqueous channels. On the other hand, prolongation of transit time was not a primary cause of transtubular loss, since no loss of mannitol was seen at similar prolongation of transit time due to aortic constriction, which lowered ITP. A similar loss was observed at elevated ITP induced by saline diuresis, despite normal or shortened transit times.

Although our experiments provide no direct evidence as to structural alterations which may lead to changes in permeability, an effect inversely related to molecular size suggests diffusion through aqueous channels of increased diameter. Our results provide, of course, no

TABLE II

Comparison of Molecular Weight and Per Cent Recovery of Various Test Substances after Early Proximal Injection at ITP of 30 ±2 mm Hg Produced by Elevation of Ureteral Catheter

	Molecular weight	Recovery*
		%
Creatinine	113	73
Mannitol	182	85
Sucrose	342	89
Iothalamate	607	85
Inulin	5500	100

* Average recovery.

information about the location of such channels. In view of the evidence which indicates the presence of low resistance intercellular shunts (10, 11), we think it reasonable to postulate that the pathway involves the tight junction and lateral intercellular spaces. During elevation of ureteral pressure and saline diuresis there is obvious dilatation of the tubular lumen. One could readily assume that this might lead to an increase in channel diameter. However, during renal venous constriction there is little or no change in tubular diameter in the steady state (12), and there is thus no obvious reason why channel size should change.

Increased intrarenal pressure was the common factor in the various conditions in which a change in tubular permeability was observed, but the precise locus at which pressure must be increased to produce this effect is unknown. Only ITP was measured, but the pressure in the peritubular vessels is also increased when ITP is increased (7). However Allison, working in this laboratory, finds that the pressure gradient from proximal tubule to small capillary is increased at elevated

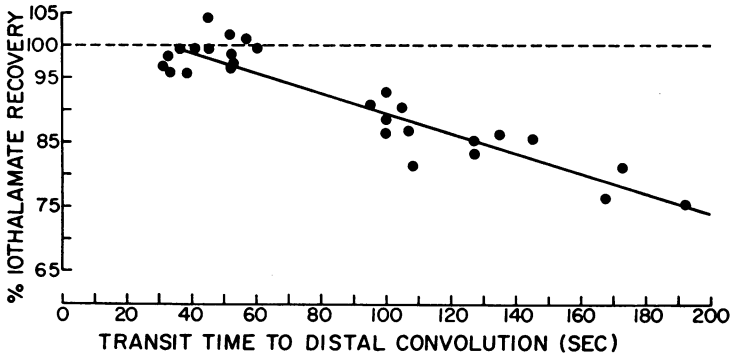

FIGURE 7 Fractional iothalamate recovery after early proximal microinjection as a function of transit time of the test solution to the distal convolution. The broken horizontal line represents 100% recovery. The equation of the regression line is $y = 105 - 0.15x$.

ureteral pressures but approaches zero with renal venous constriction (M. E. M. Allison, personal communication). Loss of mannitol was similar at comparable increases of ITP and transit time produced by differing means; thus the permeability change correlates with ITP and not with the tubule-capillary pressure gradient. It seems unlikely that ITP alone is the sole physical factor that influences tubular permeability. The unknown pressures in the lateral intercellular and interstitial spaces are probably also involved as well as the colloid osmotic pressure in the peritubular vessels. The latter may indirectly influence the former by regulating the volume of fluid in the intercellular and interstitial spaces. The intercellular and interstitial pressures undoubtedly bear a relationship to ITP, and this may be the reason for the observed correlation between ITP and tubular permeability.

It is unlikely that a humoral mediator which gains access to the general circulation is responsible for the change in tubular permeability, since our findings in both microinjection and clearance studies demonstrate that elevation of pressure in one kidney does not affect permeability in the other kidney. However, it is entirely possible that a humoral factor released in response to a change in pressure and which remains confined within the kidney is responsible for the change in permeability. Whatever the mechanism, it is rapidly reversible, since the tubule quickly regains its normal permeability upon release of the elevated pressure.

Recently Bank et al. (6) presented evidence suggesting a change in permeability of the proximal tubules during renal venous constriction. Under these conditions they consistently found movement of sucrose from peritubular capillaries into the proximal tubular lumen, an infrequent occurrence under control conditions. However, contamination of tubular fluid due to leakage around multiple puncture sites is quite possible in their experimental design, especially at elevated pressures. The low level of radioactivity in their collected perfusates made it extremely difficult to exclude the possibility of contamination. Also, ITP was apparently measured only at the beginning of renal venous constriction, and our experience indicates that frequent monitoring of ITP and adjustment of the clamp is necessary if a stable pressure is to be maintained. Since our experimental design is technically much less difficult, the likelihood of technical error is accordingly greatly reduced. In our studies gross leakage at the puncture site was excluded by the complete recovery in the urine from the microinjected kidney of inulin injected simultaneously with the smaller molecule. Nevertheless, our observations are in accord with those of Bank et al., since a change in tubular permeability should permit passive movement in either direction. Of particular significance in our study is the direct demonstration that tubular permeability was altered under a variety of conditions associated with increased intratubular and peritubular capillary pressures, and that the change in permeability was not limited to the proximal convolution but involved the entire tubule.

Changes in permeability obviously have potentially important implications with respect to sodium and water reabsorption, particularly to the extent that these may be influenced by such physical forces as hydrostatic and colloid osmotic pressure. Theoretically, a change in permeability to large molecules is not necessarily accompanied by a change in permeability to smaller molecules such as sodium and water, but we think it likely that such a change does occur in the renal tubule at elevated pressures, as Bank et al. (6) have also suggested. If this is the case, permeability coefficients are probably overestimated when determined by the Gertz split-oil droplet technique (13), since the pressure is presumably elevated in the oil-occluded tubule.

The various substances studied in this investigation have all been employed at one time or another to measure GFR (1, 14–18). Although all appear satisfactory at normal ITP, with the exception of inulin all leaked from the tubule when pressure was increased, indicating that they are unsatisfactory markers for GFR under these conditions, as we have demonstrated directly in the case of mannitol. Our results also have damaging implications in regard to the use of stop flow methodology in the analysis of renal function. In conventional stop flow experiments ITP reaches levels higher than those produced in the present study by elevating the ureteral catheter (19). Since creatinine escapes from the tubule under these conditions, its use as an indicator of water reabsorption is invalidated. Mannitol is generally used for the production of an osmotic diuresis in stop flow. Since it too leaks out of the tubule at the increased pressure, the amount of water reabsorption and replacement filtration which continues during ureteral occlusion may be of greater magnitude than previously appreciated.

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