Urate-2-^{14}C Transport in the Rat Nephron

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ABSTRACT Intrarenal transport of urate-2-^{14}C was studied in anesthetized rats using the microinjection technic. During saline diuresis, small volumes of urate-2-^{14}C (0.24–0.48 mM) and inulin-^{3}H were injected into surface proximal and distal convoluted tubules, and ureteral urine was collected serially. Total (74–96%) and direct (57–84%) urate recovery increased significantly the more distal the puncture site. Delayed recovery (±20%) remained approximately the same regardless of localization of the microinjection. After proximal injections, total and direct recoveries of urate-2-^{14}C were significantly higher in rats treated with probenecid, pyrazinoate, or PAH than during saline diuresis alone, while the excretion rates were comparable after distal injection. Delayed recovery was not altered by drug administration. The decreased proximal reabsorption of urate is presumably due to an effect of the drugs on the luminal membrane of the nephron. For perfusion at high urate concentrations, nonradioactive urate was added to the injectate (0.89–1.78 mM). Urate-2-^{14}C recovery was almost complete and there was no delayed excretion, demonstrating saturation kinetics. These findings are compatible with a carrier-mediated mechanism for urate transport probably located at the luminal border of the proximal tubular epithelium. No definitive evidence for urate secretion was found in these studies.

INTRODUCTION

Although the renal excretion of urate has been extensively studied, the site(s) and mechanism(s) for urate reabsorption and/or secretion in the nephron require clarification. Very significant species differences in the tubular transport of urate have been identified (1), as have variations in urate excretion as a function of drug dosage (2, 3). In an effort to gain direct information about the localization and nature of tubular transport of urate, the microinjection technic described by Gottschalk, Morel, and Mylle (4) was employed in experiments on rats. Reabsorption of urate-2-^{14}C was found to occur primarily in the proximal convoluted tubule by a carrier-mediated mechanism exhibiting saturation kinetics. Under these circumstances, no definitive evidence for urate secretion was found in this species.

METHODS

Male Wistar rats weighing 250–420 g were anesthetized with sodium pentobarbital, 50 mg/kg body weight injected intraperitoneally, and the left kidney was exposed for micropuncture through an abdominal incision as previously described (5). The left and right ureters were catheterized with P.E. 50 polyethylene catheters, each of which had a dead-space of 48–58 μl. Except where indicated the animals were made diuretic by a continuous infusion into the jugular vein of 2.5% sodium chloride solution at 100–200 μl/min to permit rapid serial urine collections. The rate of urine flow from each kidney was 65–130 μl/min, and the urine pH was 6–6.5 (Hydrion pH paper). In some rats probenecid (100 mg/kg body weight) or pyrazinoic acid (10, 50, or 100 mg/kg body weight) was slowly infused (5–8 min) into the femoral vein 30–40 min before the abdominal surgery. In another group of rats a 1% solution of sodium *p*-aminohippurate (PAH) in saline was continuously infused during the experiment, after an appropriate priming dose (6).

A solution containing radioactive urate and inulin and stained with nigrosin was injected with a calibrated micropipette into superficial proximal and distal convolutions. 50 μCi of uric acid-2-^{14}C, shown by the supplier (Amersham/Searle, Des Plaines, Ill.) to be 98–99% pure by chromatography, were dissolved with gentle heating in 1 ml of a solution composed of NaCl (120 mEq/liter) and $NaHCO_3$ (30 mEq/liter) in distilled water. 200 μCi of inulin-^{3}H (New England Nuclear Corp., Boston, Mass.) were dissolved in 1 ml isotonic saline and mixed with an approximately equal volume of the urate solution. Except where otherwise stated the final urate concentration in the injectate was 0.24–0.48 mM (4–8 mg/100 ml). The pH of the mixture

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was 7–7.5. The volume of the injectate varied from 12 to 55 nl, and the mean of the rates of injection was 0.9 ±0.3 (SD) nl/sec (n = 175) during saline diuresis and 0.6 ±0.2 (SD) nl/sec (n = 20) in the nondiuretic animals. These rates of injection were chosen to approximate filtration rates observed in superficial nephrons of rats under similar states of hydration (reference 7 and unpublished observations). Injection rate was controlled to avoid retrograde flow of the injectate and gross dilatation of the tubular lumen. Localization of the micropuncture site was determined by measurement of the lissamine green transit time to the puncture site (8). The first or second, and the last convolutions of proximal tubules on the surface of the kidneys were termed "early" and "late," respectively.

Ureteral urine was collected from both kidneys into vials containing 10 ml of either a solution of 200 ml of Triton X-100 (Packard Instrument Co., Inc., Downers Grove, Ill.), 800 ml of toluene, and 4 g of Omnifluor (New England Nuclear Corp., Boston, Mass.) per liter or a modified Bray's solution (9). 0.5 g of silicon dioxide gel (Cab-O-Sil; Cabot Corp., Boston, Mass.) was added to each vial in order to prevent settling of the isotopes. Urine collections were started at the beginning of the microinjections and in diuretic animals included 10 30-sec, three 60-sec, and one 120-sec samples from the left (micropunctured) kidney, and five 120-sec samples from the right kidney. In nondiuretic rats, four to six 5-min collections were made from each kidney. Before each microinjection, urine was collected for control measurement of the level of radioactivity. Radioactivity was measured as previously described (9) in a three-channel liquid scintillation spectrometer with external standardization for quench correction. The per cent recovery of injected urate-2-^{14}C, hereafter also referred to as urate (nonradioactive urate is specifically identified), was calculated using the following equation:

$$\% \text{ recovery} = \frac{(\text{urate-2-}^{14}\text{C/inulin-}^{3}\text{H}) \text{ in urine}}{(\text{urate-2-}^{14}\text{C/inulin-}^{3}\text{H}) \text{ in injectate}} \times 100\%$$

To correct for the excretion of recirculated urate, an amount of radioactivity equal to that in the urine from the right kidney was subtracted from the values determined on the urine from the left kidney. These corrections were always small and were of the order of 1–3% after early proximal injections and were nonexistent after late proximal and distal injections.

The fraction of urate-2-^{14}C which was excreted with the same time course as the simultaneously injected inulin was termed direct recovery and was calculated as twice the fraction recovered simultaneously with recovery of the first 50% of the injected inulin. The per cent delayed recovery was calculated as equal to the per cent total recovery minus the per cent direct recovery and was equivalent to the fraction of urate excreted more slowly than inulin. The method and rationale for calculation of direct and delayed recoveries have been previously described in detail (4, 10). The per cent inulin leakage was calculated as twice the inulin excreted by the right kidney divided by the total inulin excreted from both kidneys times 100 (4).

In order to test for transtubular influx of urate, 1–10 µl of the urate-2-^{14}C and inulin-^{3}H solution was injected rapidly into the left renal artery or the jugular vein, or a droplet (approximately 100 nl) was placed on the capsule of the left kidney, and urine was collected every 30 sec from both kidneys for 5 min. In those animals in which injections were made into the renal artery the rat was heparinized, and a 26 gauge hypodermic needle bent to 90° and sealed into a P.E. 10 polyethylene tubing was placed in the lumen of the dissected artery as close as possible to the junction with the abdominal aorta. Criteria for acceptable maintenance of renal function included the absence of a significant change in the macroscopic or microscopic appearance of the exposed kidney surface, in the rate of urine flow, and in late proximal and early distal transit times before and after puncture of the renal artery.

Clearance studies were performed in diuretic and nondiuretic rats. Diuresis was induced with 2.5% saline as in the animals studied by microinjection, and after a suitable prime they were infused with 80 µCi of inulin-^{3}H and 20 µCi of urate-2-^{14}C per hr. Urine was collected from both ureters for periods of 15 min. In nondiuretic rats the ureters and the jugular vein were catheterized with P.E. 10 tubing, and a volume of isotonic saline equivalent to 0.5% of the body weight was given i.v. as a replacement for fluid losses. A prime of 0.5 ml containing 40 µCi inulin-^{3}H and 7.5 µCi of urate-2-^{14}C was infused i.v. over 4 min and was followed by a sustaining infusion of 60 µCi of inulin-^{3}H and 10 µCi of urate-2-^{14}C in isotonic saline at 0.82 ml/hr. Urine was collected from both kidneys for periods of 40 min. Blood samples were withdrawn from the carotid artery at the midpoint of the urine collection periods in both groups. Radioactivity levels in blood and urine were determined as described above.

The dispersion of radioactive urate and inulin during flow through a P.E. 50 catheter (i.d. = 0.58 mm) was also studied. The mixture was injected rapidly through a 27 gauge needle into various lengths (20–60 cm) of tubing which were being perfused with saline solution at rates of 68 or 90 µl/min. The effluent was collected serially, and the radioactivity was measured.

The Student's *t* test was employed for evaluation of statistical significance, and regression equations were determined by the method of least squares. Segmental contributions to fractional reabsorption of labeled urate injected into the early proximal tubule close to the glomerulus were estimated as follows: if X = fractional recovery after early proximal injection, extrapolated to the glomerulus, Y = fractional recovery after late proximal injection, and Z = fractional recovery after early distal injection, then fractional reabsorption, proximal convolution = $F_p = 1 - X/Y$, fractional reabsorption, loop of Henle = $F_l = (1 - F_p)(1 - Y/Z)$, and fractional reabsorption, distal nephron = $F_d = (1 - F_p - F_l)(1 - Z)$.

RESULTS

A total of 195 technically satisfactory tubular microinjections were performed in 60 rats under various experimental conditions. A microinjection was not considered satisfactory if there was a visible leak at the puncture site, the calculated transtubular inulin leakage exceeded 1%, or total inulin recovery was less than 95% of the estimated amount injected.

Saline diuresis (14 rats). Typical excretion curves for inulin and urate after microinjection into an early proximal and into a distal convolution of a diuretic rat are shown in Fig. 1. In the upper panels the results are presented as the per cent of the injected isotopes recovered in each urine collection. In the lower panels, the same data are presented as cumulative recoveries. Urate

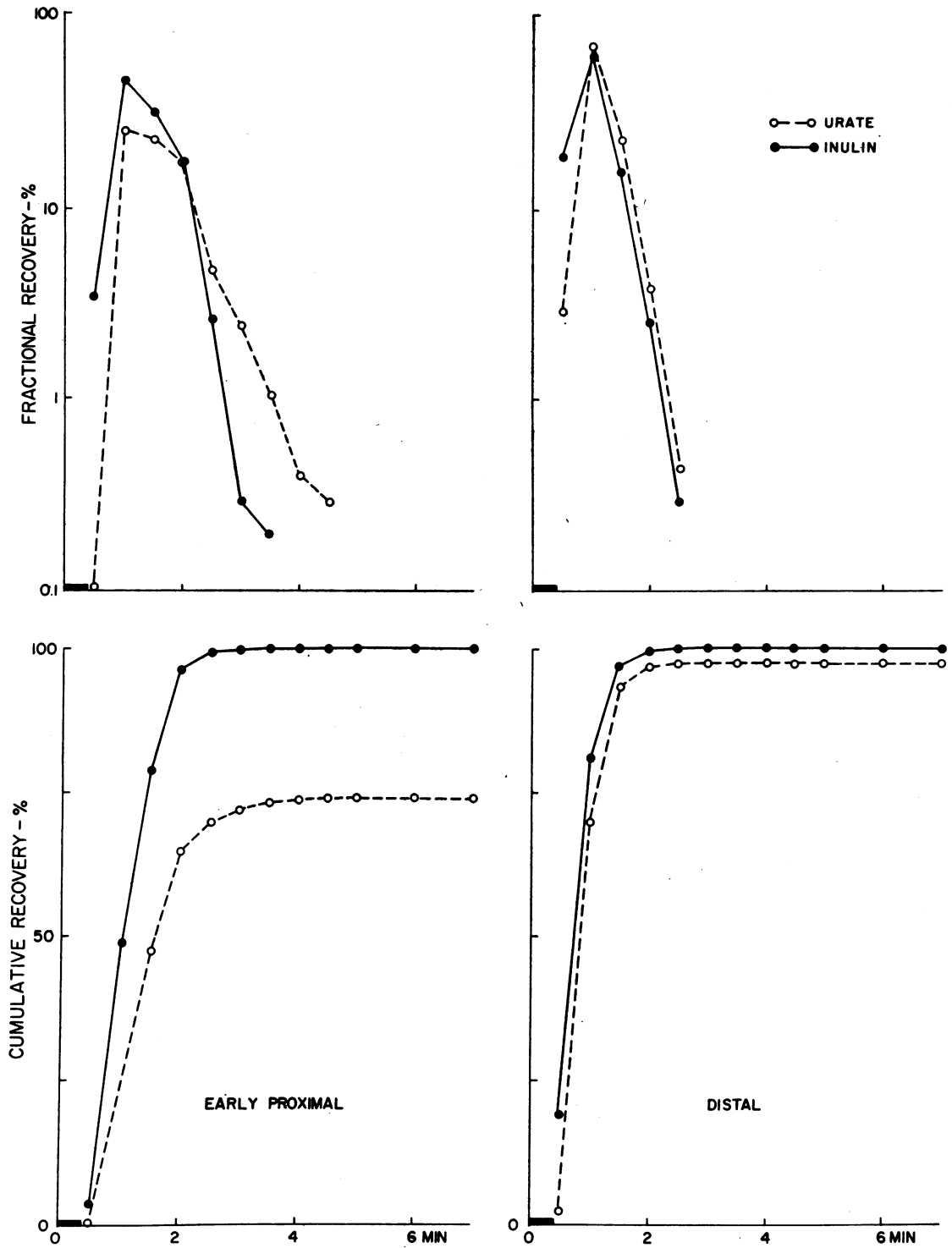

FIGURE 1 Urate and inulin recovery patterns following early proximal (left) and distal (right) microinjections during saline diuresis. Fractional (upper) and cumulative (lower) recovery curves are shown. Duration of injection indicated by black bar on time scale.

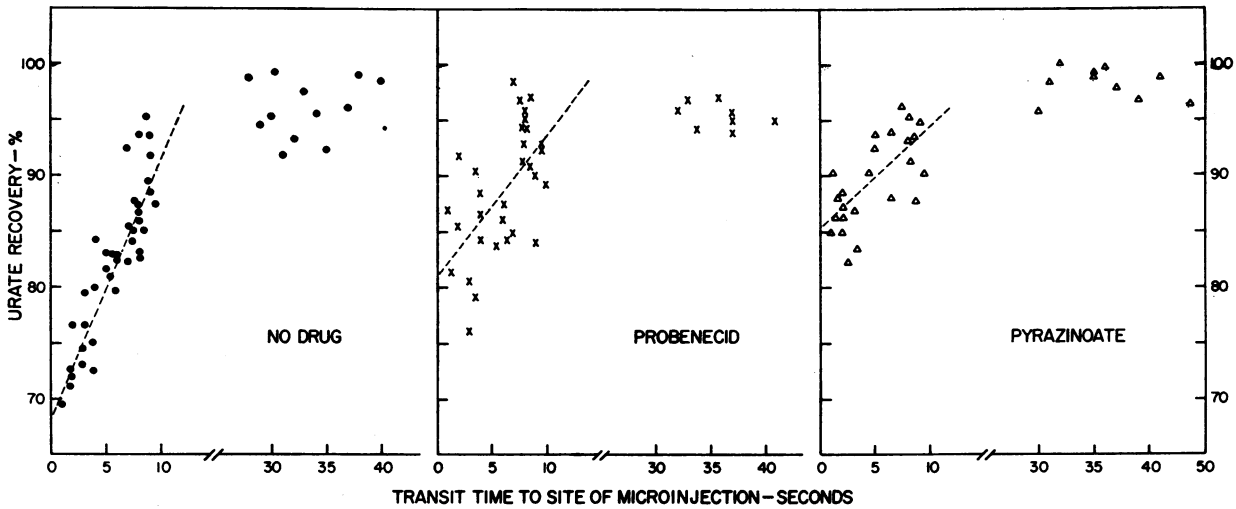

FIGURE 2 Per cent urate recovery as a function of tubular transit time to site of puncture during saline diuresis alone (left) and with probenecid (middle) or pyrazinoate (right) pretreatment. Regression lines were calculated from the relationship $Y = a + bX$, where Y represents the per cent urate recovered and X represents the transit time to the proximal puncture site. During saline diuresis alone $Y = 68.1 + 2.8X$. After probenecid, $Y = 81.1 + 1.5X$; and after pyrazinoate, $Y = 85.3 + 1.1X$.

recovery accompanied inulin recovery more closely after distal injection. There was some delayed recovery of urate after each microinjection, indicating a greater volume of distribution for urate than inulin. In Fig. 2 (left panel) total urate recovery is plotted as a function of the tubular transit time to the site of injection, and in Table I average values are presented for total, direct, and delayed urate recoveries after injection into early and late proximal tubules and distal convolutions. Total and direct recoveries were larger the more distal the site of injection, but delayed recovery did not vary significantly with the site of injection. The regression line for total urate recovery after proximal injection extrapolated to 68% excretion for injection into Bowman's capsule.

Probenecid. Results in 11 diuretic rats after pretreatment with probenecid are presented in Fig. 2 (middle panel) and in Table II. There was less urate recovery after early than late proximal injection; the difference after late proximal and distal injections was significant at the 5% level. The extrapolated value for total urate recovery after injection into Bowman's capsule was 81%. Compared with the values found in the control animals during saline diuresis alone, both total and direct recoveries after proximal injection were significantly increased. Recovery following distal injection was not significantly different.

Pyrazinoate. Results in eight diuretic rats after pretreatment with pyrazinoic acid are presented in Fig. 2 (right panel) and in Table III. Again, total urate recovery was a function of the site of microinjection, and the proximal results extrapolate to 85% excretion after injection into Bowman's capsule. No difference was found with the several drug dosages employed (10 to 100 mg/kg i.v.). When compared with results during

TABLE I

*Per Cent Urate-2-^{14}C Recovery after Intratubular Microinjection in Rats during Saline Diuresis**

Recovery	Early proximal (n = 11)		Late proximal (n = 11)		Distal (n = 12)
Total	74 ±3		89 ±4		96 ±3
Probability		<0.001		<0.001	
Direct	57 ±12		69 ±5		84 ±14
Probability		<0.01		<0.005	
Delayed	17 ±13		20 ±7		12 ±14
Probability		NS		NS	

* Mean values ±SD are presented.

TABLE II

*Per Cent Urate-2-^{14}C Recovery in Rats Pretreated with Probenecid after Intratubular Microinjection during Saline Diuresis**

Recovery	Early proximal (n = 9)		Late proximal (n = 12)		Distal (n = 8)
Total	84 ±5		92 ±4		95 ±1
Probability		<0.001		<0.05	
Direct	70 ±10		81 ±9		89 ±13
Probability		<0.02		NS	
Delayed	14 +6		11 ±8		6 ±12
Probability		NS		NS	

* Mean values ±SD are presented.

saline diuresis alone there was no statistically significant difference following distal injection, but both total and direct recoveries were increased after proximal injection.

The significance of differences in mean urate recovery between rats with and without pretreatment with probenecid or pyrazinoate are presented in Table IV. As is shown in Table V urine flow and early and late proximal and distal transit times were unaffected by drug pretreatment.

Effect of PAH loading. The per cent of urate excreted after microinjection into different tubular segments in three diuretic rats loaded with 1% PAH is shown in Table VI. In comparison with results obtained during saline diuresis alone, total recovery was greater ($P < 0.001$) after injection into early, but not late, proximal tubules; direct recovery was greater after both early ($P < 0.005$) and late ($P < 0.001$) injection. Recovery after three distal injections was unchanged by PAH loading.

Effect of varying urate load on recovery. Fractional urate recovery in three diuretic rats was unchanged when the urate concentration in the injectate was reduced from the control level of 0.24–0.48 mM (4–8 mg/100 ml) to 0.15 mM (2.5 mg/100 ml), the lowest value allowing accurate measurement of radioactivity in the urine. Total fractional recovery was 75% ±2 (n = 4) and 97% ±2 (n = 4) after early proximal and distal injections, respectively.

Recovery was also studied after microinjection of solutions with a urate concentration in the range of 0.89–1.78 mM (15–30 mg/100 ml) produced by addition of nonradioactive urate to the injectate. Curves of fractional and cumulative recovery after proximal (left panel) and distal (right panel) injection are shown in Fig. 3. In contrast to the control results, the injected urate-2-^{14}C was recovered with the same time course as inulin. Total and direct recovery increased markedly after proximal and distal injections, and there was no delayed recovery (Table VII). These results differ significantly from those obtained in animals in which the concentration of urate in the injectate was 0.24–0.48 mM (i.e., no added nonradioactive urate).

As is shown in Fig. 4, reabsorption of injected urate varied with load when the injected urate load was 0.75 pmole/sec or lower. The reabsorptive rate was variable when the load was increased by addition of nonradioactive urate to the injectate, but it is evident that reabsorption did not continue to increase in proportion to load, and the data suggest the existence of a Tm for

TABLE III

*Per Cent Urate-2-^{14}C Recovery in Rats Pretreated with Pyrazinoate after Intratubular Microinjection during Saline Diuresis**

Recovery	Early proximal (n = 11)		Late proximal (n = 7)		Distal (n = 10)
Total	86 ±2		92 ±3		98 ±1
Probability		<0.001		<0.001	
Direct	77 ±7		85 ±7		86 ±13
Probability		<0.05		NS	
Delayed	10 ±7		7 ±7		12 ±12
Probability		NS		NS	

* Mean values ±SD are presented.

TABLE IV

Significance of Differences in Mean Urate-2-^{14}C Recovery between Rats with and without Pretreatment with Probenecid or Pyrazinoate

Recovery	Early proximal microinjection	Late proximal microinjection	Distal microinjection
Total	$P < 0.001$	$P < 0.01$	NS*
Direct	$P < 0.001$	$P < 0.001$	NS
Delayed	NS	$P < 0.01$	NS

* The significance of differences in total recovery after distal microinjection in the three groups was as follows: probenecid vs. no drug, NS; pyrazionate vs. no drug, $P < 0.05$; probenecid vs. pyrazinoate pretreatment, $P < 0.001$.

urate reabsorption at increased loads. The contribution to urate load of continued filtration of endogenous urate during microinjection was neglected in these calculations; hence both urate load and reabsorption in the injected nephron may have been slightly underestimated. If glomerular filtration continued unaltered in the nephron during microinjection, the error in estimation of urate load would be approximately 0.05 pmoles/sec, and in reabsorption, 0.015 pmoles/sec at low urate loads and 0.005 pmoles/sec at loads above 0.75 pmoles/sec. Actual errors introduced by neglecting endogenous urate filtration are undoubtedly smaller than this and almost certainly insignificant since glomerular filtration in injected nephrons was either temporarily reduced or abolished during microinjection.

Efforts to detect urate secretion. The following experiments were performed in diuretic rats to test for the possibility of transtubular influx of urate. When on 10 occasions a droplet of the urate-inulin mixture was placed on the capsule of the left kidney, inulin appeared simultaneously in the urine from the left and right kidneys when the urine flow rates in the two kidneys were the same. Urate appeared simultaneously with inulin in the urine from the left kidney in eight experiments and in the urine from the right kidney in three experiments. In the other experiments, urate appeared in the first or second succeeding urine sample. In no instance did the urate : inulin ratio of the urine from either kidney exceed that of the injectate during the 5 min of collection. In five experiments after pretreatment with probenecid, urate and inulin always appeared simultaneously in the urine, but the urate : inulin urinary ratio never exceeded that of the injectate.

In 10 experiments the excretion patterns were studied after rapid intrajugular administration of the urate-inulin mixture. The results were similar to those obtained after placement of a droplet of the injectate on the surface of the kidney except that urate and inulin always appeared simultaneously on both sides. The results were not different in four experiments after pretreatment with probenecid.

After injection into the left renal artery, urate and inulin were also excreted simultaneously without ($n = 6$) and after pretreatment with probenecid ($n = 6$) or with pyrazinoate, 10 mg/kg ($n = 4$), and 100 mg/kg ($n = 2$). In no instance was the urate : inulin ratio of the urine higher than that of the injectate.

Nondiuretic rats. Urate recovery following intratubular injection was studied in 10 nondiuretic rats. Total recovery averaged 64 ±3% ($n = 7$) after early proximal and 74 + 4% ($n = 7$) after late proximal injection. These values are different from each other ($P < 0.001$), and each is also different from recovery after injection in the same location in diuretic rats ($P < 0.001$). Total recovery after distal injection in nondiuretic rats (96 ±3%; $n = 6$) was similar to that observed in diuretic animals. Tubular transit time from glomerulus to the site of injection in early proximal tubules averaged 2.8 ±1.6 sec ($n = 7$), to the late proximal tubules, 11.0 ±1.1 sec ($n = 7$), and to the early distal tubules, 36.9 ±3.0 sec ($n = 6$). The rate of urine flow from the left kidneys averaged 4.4 ±1.4 µl/min ($n = 20$).

TABLE V

*Comparison of Rates of Urine Flow and of Tubular Transit Times in Rats during Saline Diuresis with and without Drug Pretreatment**

	No drug	Probenecid	Pyrazinoate
Urine flow, *µl/min per kidney*	93 ±16 (n = 52)	98 ±13 (n = 40)	97 ±14 (n = 35)
Transit time, *sec*			
Early proximal tubules	2.6 ±0.9 (n = 11)	2.7 ±1 (n = 9)	2.0 ±0.7 (n = 11)
Late proximal tubules	8.5 ±0.6 (n = 11)	8.6 ±0.7 (n = 12)	8.6 ±0.5 (n = 7)
Distal convolutions	33.1 ±3.8 (n = 12)	35.8 ±2.9 (n = 8)	36.0 ±4.4 (n = 10)

* Results after drug treatment were not significantly different. Mean values ±SD are presented.

TABLE VI

*Per Cent Urate-2-^{14}C Recovery after Intratubular Microinjection in Rats Loaded with PAH and Undergoing a Saline Diuresis**

Recovery	Early proximal (n = 6)	Late proximal (n = 6)	Distal (n = 3)
Total	85 ±5	89 ±4	96 ±1
Direct	72 ±11	79 ±5	86 ±9
Delayed	14 ±9	10 ±5	9 ±10

* Mean values ±SD are presented.

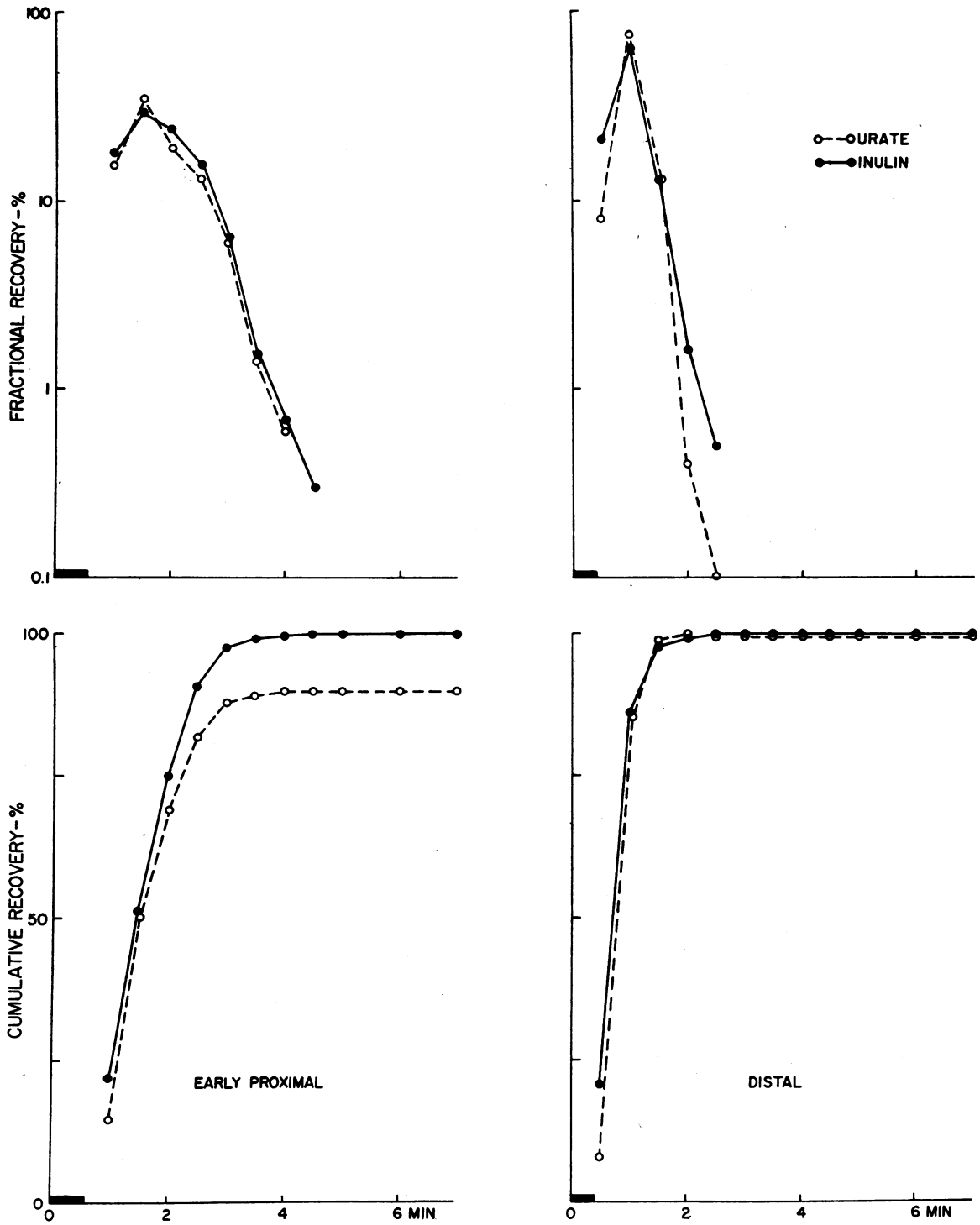

FIGURE 3 Urate and inulin recovery following early proximal (left) and distal (right) microinjections of mixtures with added nonradioactive urate (concentration = 0.98 − 1.78 mM). Fractional (upper) and cumulative (lower) recovery curves are shown. Duration of injection indicated by black bar on time scale.

TABLE VII

*Per Cent Urate-2-^{14}C Recovery after Intratubular Microinjection of Solutions with Added Nonradioactive Urate**

Recovery	Early proximal (n = 4)		Late proximal (n = 6)		Distal (n = 6)
Total	88 ±6		96 ±1		100 ±1
Probability		<0.02		<0.001	
Direct	91 ±5		98 ±9		101 ±11
Probability		NS		NS	
Delayed	−3 ±7		−2 ±9		−1 ±12
Probability		NS		NS	

* Mean values ±SD are presented.

Experiments with polyethylene tubing. The recovery pattern of urate and inulin after simultaneous injection into a section of polyethylene tubing through which saline was flowing is shown in Fig. 5. The mean transit times for urate and inulin were the same, but the urate curves were narrower and had higher peaks than the inulin curves (n = 10). These differences could be accentuated by increasing the length of the tubing or by reducing the velocity of flow.

Clearance of urate-2-^{14}C. The clearances of urate-2-^{14}C and of inulin were measured in 17 clearance periods in four rats undergoing a saline diuresis. The clearances of right and left kidneys were measured separately and were similar (Table VIII). The urate-2-^{14}C clearance for the four rats averaged 80% of the simultaneous inulin clearance; in individual periods it varied from 68–89% of the inulin clearance. The rate of urine flow was the same as in the diuretic rats studied by microinjection.

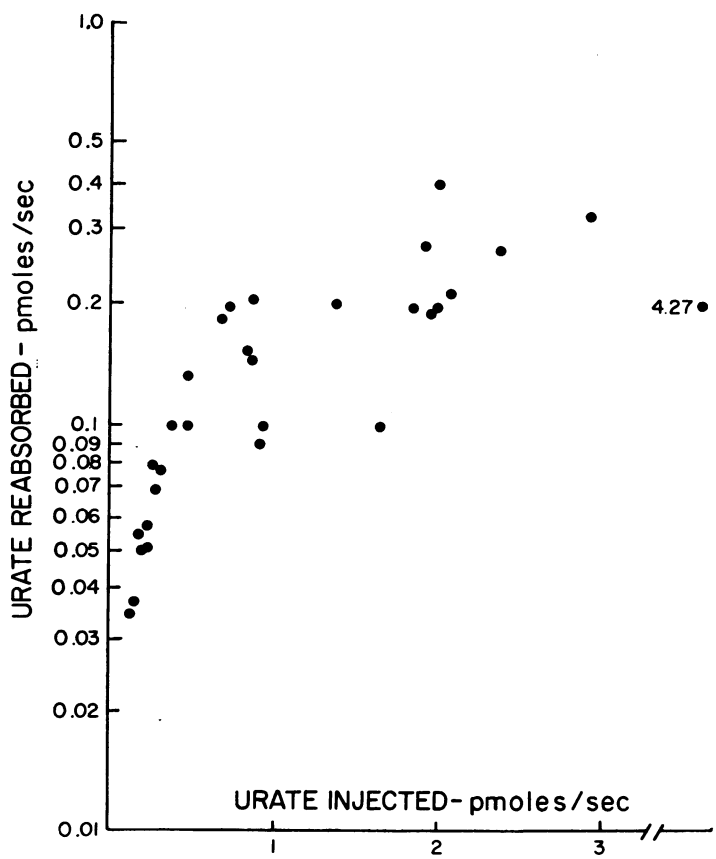

FIGURE 4 Relationship between rate of urate reabsorption and injected load after early proximal microinjection.

In eight nondiuretic rats C_{urate}/C_{in} averaged 77% which is not different from the value found during diuresis. C_{urate} and C_{in} were both significantly less ($P < 0.01$) than during diuresis. There was also greater variability in the clearances between right and left kidneys, but C_{urate}/C_{in} was no different in the two kidneys of individual nondiuretic rats even though differences in absolute rates of transport were observed in some.

DISCUSSION

Interpretation of results of tracer microinjection experiments. As with most applications of radioisotopes, we assume that the basic mechanisms of transport of urate are not altered by carbon-14 labeling. We also assume that all of the ^{14}C radioactivity measured in the injectate and in the urine was urate-2-^{14}C. Chromatography of the injectate showed a purity of 98% or greater, and no change in behavior of the several urate-2-^{14}C preparations was observed with time. Further, Podevin, Ardaillou, Paillard, Fontanelle, and Richet (11), using urate-2-^{14}C obtained from the same source, observed that 98% of ^{14}C radioactivity in the urine of humans after intravenous injection was due to urate-2-^{14}C. Inferences made in respect to net excretion of nonradioactive urate from experiments with urate-2-^{14}C, however, require consideration of specific activities, route of administration, etc. The average of 0.80 for the ratio of the simultaneous urate-2-^{14}C and inulin clearances during saline diuresis compares reasonably well with the average of 0.65 found by Mudge for nonradioactive urate in rats during mannitol diuresis.[1] The relationship between the urinary recovery of radioactive (or nonradioactive) materials after intratubular microinjection and their usual excretion is more complicated, especially if the excretory mechanisms involve bidirectional transtubular fluxes. If only filtration and reabsorption are involved and if the reabsorptive process does not have a transfer maximum, fractional excretion after injection of varying amounts into the first part of the proximal tubule should approximate that following filtration. If fractional reabsorption is not constant with changes in filtered load, fractional excretion after microinjection into the first part of the proximal tubule would equal C_x/C_{in} only if the amount injected equals the amount filtered. Similarly, fractional excretion after injection into more distal parts of the nephron will equal net fractional excretion only if the amount injected equals the amount normally arriving at that part of the tubule. Microinjection into various portions of the nephron permits the determination of relative permeabilities, effects of drugs, etc., and addition of nonradioactive material

[1] Mudge, G. H. Personal communication.

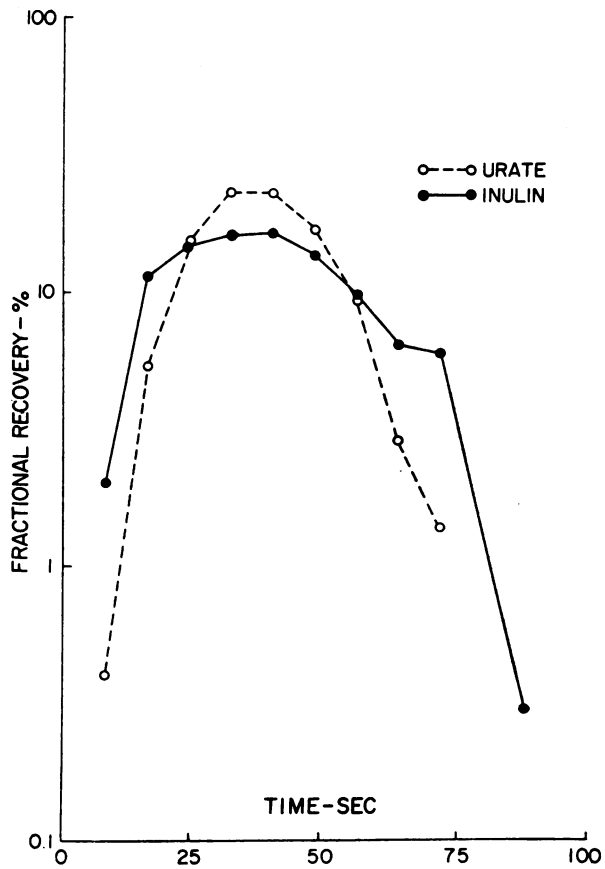

FIGURE 5 Urate and inulin excretion after rapid injection into polyethylene tubing (30 cm in length) through which saline was flowing (98 μl/min).

to tracer amounts of isotopes allows measurement of rates of transport at various loads. The presence of transtubular influx, whether carrier-mediated or by simple diffusion, may or may not be detected by intratubular microinjection. Delayed recovery, which is that portion of microinjected tracer excreted after gaining access to a greater volume of distribution than that of inulin, may or may not represent a process of transtubular influx. As computed, delayed recovery was not due to systemic recirculation and excretion of urate, since a correction was made for this when present. Recirculation was always small, and measurement of urate excretion by the right kidney permitted the correction. If it is assumed that the microinjected inulin remains in the tubular lumen, isotope excreted more slowly than inulin may have penetrated into the epithelial cells only before returning to the lumen or may have been translocated across the tubular epithelium and then been transported back into the tubular lumen and measured as delayed

TABLE VIII

*Clearance Measurements in Diuretic and Nondiuretic Rats**

Body wt	Kidney	Number of periods	C_{urate}	C_{inulin}	$\frac{C_{urate}}{C_{inulin}} \times 100$	Urine flow
g			*ml/min*			*μl/min*
			Saline diuresis			
368	R	4	1.72 ±0.20	2.27 ±0.14	75.4 ±5.3	108 ±18
	L	4	1.62 ±0.14	2.18 ±0.15	74.3 ±3.8	98 ±17
380	R	4	2.19 ±0.11	2.96 ±0.14	74.3 ±6.3	80 ±14
	L	4	1.96 ±0.15	2.59 ±0.36	76.0 ±6.0	88 ±15
300	R	5	1.88 ±0.18	2.20 ±0.23	85.6 ±2.7	104 ±9
	L	5	1.76 ±0.15	2.05 ±0.17	86.0 ±3.7	101 ±11
325	R	4	1.65 ±0.20	1.99 ±0.28	82.8 ±4.4	87 ±17
	L	4	1.60 ±0.11	1.93 ±0.24	83.4 ±4.4	99 ±19
Mean	R	17	1.86 ±0.24	2.35 ±0.42	79.5 ±5.5	95 ±13
	L	17	1.73 ±0.16	2.19 ±0.29	79.9 ±5.7	96 ±6
			Nondiuretic rats			
360	R	5	1.28 ±0.28	1.46 ±0.26	87.2 ±9.2	3.0 ±0.7
	L	5	1.49 ±0.24	1.71 ±0.38	88.5 ±9.9	3.0 ±0.3
269	R	2	0.81 ±0.07	1.24 ±0.01	65.2 ±4.9	4.0 ±0.6
	L	2	1.46 ±0.05	1.44 ±0.71	75.5 ±2.1	4.4 ±0.1
320	R	3	0.93 ±0.38	1.20 ±0.42	77.1 ±5.7	3.2 ±1.2
	L	3	1.11 ±0.31	1.30 ±0.40	85.4 ±3.7	3.5 ±1.4
305	R	3	0.75 ±0.20	1.09 ±0.18	65.9 ±7.6	3.6 ±1.0
	L	3	0.89 ±0.25	1.36 ±0.51	67.7 ±8.4	3.3 ±0.1
320	R	3	1.07 ±0.08	1.25 ±0.12	85.6 ±8.4	2.2 ±0.3
	L	3	1.53 ±0.09	1.66 ±0.13	92.2 ±2.3	2.6 ±0.3
295	R	2	0.73 ±0.10	1.15 ±0.34	65.4 ±10.7	2.7 ±0.4
	L	2	1.10 ±0.07	1.80 ±0.34	62.1 ±8.1	4.2 ±0.2
315	R	3	0.82 ±0.41	1.10 ±0.61	75.1 ±6.7	2.0 ±0.7
	L	3	1.26 ±0.19	1.55 ±0.13	81.3 ±9.5	1.8 ±0.5
275	R	3	0.94 ±0.34	1.08 ±0.19	84.1 ±18.7	1.6 ±0.6
	L	3	0.97 ±0.18	1.22 ±0.07	79.7 ±17.1	1.5 ±0.4
Mean	R		0.91 ±0.18	1.20 ±0.13	75.7 ±9.4	2.8 ±0.8
	L		1.23 ±0.25	1.51 ±0.21	79.0 ±10.3	3.0 ±1.0

*The SD of the means was computed on the basis of the average kidney values.

recovery.[2] Since some of the material which gains access to the interstitium will be lost in the blood efferent from the kidney, total luminal efflux after microinjection equals the amount injected minus direct recovery. A special example of delayed excretion would be represented by transtubular influx into the collecting ducts or loops of Henle following initial loss into the medullary interstitium and trapping by countercurrent diffusion in the vasa recta.

The smaller dispersal of urate than inulin after the injection into plastic tubing through which saline was flowing was as predicted, and it is a consequence of the greater diffusivity of the smaller urate molecule. Presumably a similar mechanism was occurring in the catheter placed in the ureter in the animal experiments. As discussed earlier (6) this type of isotopic separation in a physical system cannot explain the delayed recovery in the urine, since mean transit time and, there-

[2] Measurements of total recovery are more accurate than attribution as either direct or delayed recovery since computation of the latter can be markedly affected by small changes in the ratio of isotopes in a single urine collection.

TABLE IX

*Percentage of Microinjected Urate-2-^{14}C Reabsorbed in Different Tubular Segments in Diuretic Rats with and without Drug Pretreatment**

	Proximal convolution	Loop of Henle	Distal convolution and collecting ducts
No drug	24	6	3
Probenecid	12	3	4
Pyrazinoate	8	6	2

* Values for the proximal convolution are extrapolated to the glomerulus. Values for remaining nephron segments are expressed as percent of urate injected proximally.

fore, direct recoveries of any two simultaneously injected isotopes are identical in the plastic tubing system.

As first demonstrated by Chinard and Enns (12), the process of transtubular influx of a substance may be detected by its earlier appearance in the urine after simultaneous intravascular injection than one which enters the urine only by filtration, e.g., inulin. The ease with which transtubular influx may be detected in this manner is a function of tubular and vascular transit times. Influx is easier to detect after placement on the kidney surface than it is after injection into the renal artery because of the longer circulation time from the peritubular capillaries to the glomerulus. The difference between the tubular transit time and the vascular transit time to the site of tubular secretion determines the maximum possible precession of the secreted substance in the urine compared with that of a glomerular marker. The time separation in the urine will be greater, of course, if the secretory process is located in the distal convolution rather than in the proximal convolution.

Urate reabsorption. The recovery pattern of urate microinjected into various portions of the nephron indicates that its reabsorption occurs primarily in the proximal convoluted tubule. Calculation of segmental contributions to reabsorption after extrapolation of the proximal recovery curve (Fig. 2) to the glomerulus indicates that 24% of filtered urate is reabsorbed by the end of the proximal pars convoluta under these conditions, 6% in the loop of Henle, presumably largely or entirely in the pars recta of the proximal tubule, and only 3% in the distal convolution and collecting ducts (Table IX). The very low rate of reabsorption in the distal portions of the nephron is as predicted from the microperfusion studies of Oelert, Baumann, and Gekle, who determined a permeability coefficient for urate in the distal convolution of rats which was not significantly different from zero (13). The values in Table IX also clearly demonstrate that the major action of probenecid and pyrazinoate is to diminish reabsorption in the proximal convolution.

The studies with added nonradioactive urate in the injectate suggest saturation kinetics for urate reabsorption in the proximal tubule. Reabsorption appeared to be rate limited, compatible with the existence of a transfer maximum, as has been shown in man (14) and in the Cebus monkey (15). The demonstration of saturation kinetics is consistent with a carrier-mediated process, presumably an active transport mechanism, although carrier-mediated exchange diffusion cannot be entirely excluded. The presumed carrier appears to be located at the luminal membrane since direct recovery was increased with urate loading. Systemic loading of rats with PAH, which is known to undergo bidirectional transport in the proximal tubule (6, 16, 17), demonstrated competition between urate and PAH in the proximal tubule. Urate recovery following proximal microinjection in PAH-loaded rats was increased, but recovery after distal injection was unchanged. The most marked effect was on direct recovery of urate which is also consistent with competition for a carrier localized at the luminal membrane.

Pretreatment of the rats with probenecid or pyrazinoate led to similar results. Total recovery was unchanged after distal microinjection but was increased after injection into the proximal convolution. The drug effect also seemed to occur at the luminal membrane since direct recovery was increased. Presumably these compounds interact with the carrier and compete with urate for transport sites. The effects of drug pretreatment cannot be attributed to change in urine flow or tubular transit time since these were unaltered (Table V).

Urate recovery after early and late proximal injections was less in nondiuretic than in diuretic rats, but there was no difference after distal injections. The ratio C_{urate}/C_{in} was the same in diuretic and nondiuretic rats despite marked changes in urine flow, consistent with the results of Mudge et al. in rabbits and mongrel dogs (18). Steele (19) and Steele and Oppenheimer (20) have presented evidence that the state of body hydration effects urate excretion in man. They found urate excretion to increase with expansion and to decrease with contraction of extracellular volume. Cannon, Svahn, and Demartini (21) found $C_{urate}/C_{in} \times 100$ to increase from 12.5 to 18.7% during infusion of 2.5% saline solution in normal and hypertensive man and fractional excretion of sodium to increase from 1.4% during control periods to 14.5% at peak natriuresis. There was also decreased fractional reabsorption of calcium, magnesium, potassium, and chloride. This suggested to these investigators "that depressed uptake of sodium and water by peritubular capillaries may retard the net reabsorption of a variety of other ions whose transport is electrogenically coupled to that of sodium or which are reabsorbed by

concentration dependent processes which would thus be influenced by alterations in the rate of tubular water reabsorption" as Walser had previously proposed (22). Although such a mechanism(s) could have been responsible for the increase in fractional excretion of urate after microinjection in our diuretic rats, it should be noted that 5% saline infusion in rats resulted in increased and not decreased proximal reabsorption of sodium with unchanged fractional reabsorption unless sodium excretion exceeded 12% of filtered load (23, 24). Whether proximal sodium reabsorption was increased in the rats loaded with 2.5% sodium chloride solution in the present experiments is not known. It probably was, however, since glomerular filtration rate (GFR) was increased and the plasma sodium concentration was undoubtedly increased also. In any event our microinjection experiments with varying tubular urate loads were accomplished independent of systemic fluid volume change and at unchanged urine flow rate. Our studies are confirmatory, therefore, of studies in mongrel dogs which suggest a proximal location of a carrier-mediated mechanism for urate reabsorption (25). The absolute magnitude of the Tm might be expected to vary with changes in the state of body hydration, as has been observed for glucose (26) and bicarbonate (27), and possibly other factors.

The possibility that urate may have been reabsorbed by a process of nonionic diffusion should also be considered. Weiner and Mudge (1) have concluded that nonionic diffusion must be assigned a very minor role in urate reabsorption. Large variations in urinary pH have little influence on urate excretion in the dog (18). Nonionic diffusion was tested for directly in the rat kidney by Sonnenberg, Oelert, and Baumann (28) and found to be absent, which they explained by lack of lipoid solubility of uric acid.

Urate secretion. No definitive evidence was found in these experiments for urate secretion despite the very large fractional excretion of urate after microinjection and filtration. The results after proximal microinjection extrapolate to 68% excretion of urate after injection into the initial part of the proximal tubules during diuresis and to 60% during antidiuresis. The $C_{urate}/C_{in} \times 100$ averaged 80% in four other similarly diuretic rats and 77% in eight nondiuretic rats. The difference of 12% in diuretic and 17% in nondiuretic rats between fractional excretion after microinjection and filtration under both conditions might well be due to secretion, but it could also represent differences in reabsorption in different animals or result from the fact that reabsorption after microinjection is not precisely the same quantitatively as after filtration. In any event, these results indicate that the large fractional excretion of urate in the rat under these conditions results largely if not entirely from a low rate of reabsorption. The general similarity of fractional excretion after microinjection and filtration also excludes any major component of exchange diffusion in the microinjection experiments.

In none of the experiments after application of a droplet of the injectate to the kidney surface or injection into the jugular vein or renal artery was there urinary precession of urate in comparison to inulin. In contrast, Podevin et al. found clear evidence of urinary precession of urate and of PAH in man after intravenous injection which they interpreted as indicative of a proximal site of entry into the tubule (11). Secretion of PAH by the rat proximal tubule under the conditions of the present experiments is readily demonstrable by urinary precession of PAH over inulin when a droplet containing these substances is placed on the kidney surface (6). Although our failure to find similar results with urate is strong evidence against a large component of urate secretion in the rat kidney under these circumstances, a secretory component of smaller magnitude might go undetected. In all tests for precession, influx into the medullary portions of the nephron may be masked by the slow medullary circulation and by countercurrent diffusion.

As discussed earlier, the delayed excretion of urate after microinjection is consistent with transtubular influx, but it is equally consistent with return (as by simple diffusion) of urate to the lumen after penetration into the cells. The latter implies a higher permeability to urate of the luminal than basal membrane since total recovery is almost 100% after distal injection. Delayed recovery was similar after both proximal and distal microinjections and suggests that this larger volume of distribution of urate is primarily accessible from the distal convolution and/or collecting ducts. It is possible that delayed excretion after intratubular injection represents a special case of transtubular movement, i.e. loss of urate into the medullary interstitium, presumably out of the collecting ducts, and reentry into the nephron in the thin loops of Henle or collecting ducts with almost complete recovery because of the efficient trapping of urate in the medulla by countercurrent diffusion, as has been demonstrated for urea (29). This would represent a mechanism for limiting reabsorptive loss and would not be a true (net) secretory mechanism since influx could not exceed efflux in the steady state. The relatively short transit time for that component of urate excreted with delay argues against recirculation to the collecting ducts through the loops of Henle and is more consistent with penetration into or through the collecting duct cells with direct reflux into the collecting duct lumen. The slice experiments of Epstein and Pigeon (30) and of Cannon, Symchych, and Demartini (31), in which a gradient of urate from cortex to papilla was demonstrated in dog, man, and monkey, with high papillary concentration un-

der some circumstances, suggests penetration of urate through the collecting duct cells with trapping by countercurrent diffusion under those conditions. Precipitation of urate would presumably occur at times under these circumstances.

Comparison of urate transport in the rat and other species. Urate transport varies remarkably in the nephrons of various species. Zins and Weiner (25) have divided animals into four groups on the basis of their renal handling of urate. In one group, composed of birds (32) and reptiles (33), urate is filtered and is also secreted by the tubules. A second group consisting of the Dalmatian coach hound (34, 35) and guinea pig (36) generally secrete urate, but secretion can be converted to net reabsorption by probenecid and PAH. In a third group, exemplified by the rabbit (37), it is possible to find individuals which exhibit either net secretion or net reabsorption of urate. The fourth group is composed of man, some other primates, and non-Dalmatian dogs, and in these species only net urate reabsorption is evident under most experimental conditions. Urate transport in the rat appears to be most similar to its transport in the last group. It remains to be determined, however, whether urate secretion can be demonstrated under the appropriate experimental conditions in the rat as it has been in man (38, 39) and non-Dalmatian dogs (18, 25, 35). The effect of pyrazinoate on urate transport in the rat nephron is strikingly different from its effect in man. We found urate excretion to increase after administration of pyrazinoate to rats, whereas in man it declines markedly (40). The latter finding has been interpreted to indicate that in the human nephron most filtered urate is reabsorbed proximally and that the excreted urate gains access to the urine through a secretory process located in a more distal portion of the nephron (41).

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