The Influence of the Extracellular Fluid Volume on the Tubular Reabsorption of Uric Acid

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ABSTRACT Changes in tubular reabsorption of uric acid in response to alterations in the extracellular fluid volume (ECFV) were examined in rats by clearance studies and by direct intratubular microinjections. Contraction of the ECFV led to a rise in the serum uric acid concentration and a 47% decrease in the clearance of uric acid. The ratio of uric acid to inulin clearance also fell, indicating an increase in the net tubular reabsorption of urate. Volume expansion resulted in an increase in the urate clearance and a 37% decrease in the net tubular reabsorption of uric acid.

To localize the site in the nephron where these changes occur, microinjections of [2-^{14}C]urate were performed. The lack of conversion of radioactive urate to allantoin after microinjections was demonstrated by thin-layer chromatography. After contraction of the ECFV, urinary recoveries of uric acid were significantly decreased after microinjections into proximal tubular sites. In contrast, recoveries were increased from these proximal sites after volume expansion. No evidence for distal reabsorption was obtained in any group of animals. These studies demonstrate that net urate reabsorption is influenced by the state of hydration of the ECFV and that these alterations are mediated by changes in the rates of reabsorption in the proximal tubule.

INTRODUCTION

The state of hydration of the extracellular fluid compartment exerts an important influence on the tubular reabsorption of sodium as well as that of other filtered ions such as glucose, bicarbonate, and phosphate (1–3). Indirect evidence for a similar relationship between the renal handling of sodium and uric acid has also been advanced (4, 5). The more direct assessment of this interrelationship by micropuncture techniques, however, has been hampered by the methodologic problems associated with the measurement of uric acid in microsamples of tubular fluid. Furthermore, the fact that some animals, such as the rat, can convert uric acid to allantoin has precluded the use of tracer radioisotopes in the evaluation of uric acid reabsorption. Direct intratubular microinjections of [2-^{14}C]urate bypasses some of these problems, provided there is no intratubular conversion of the radioactive uric acid to allantoin. A thin-layer chromatographic method was, therefore, developed for the separation of urinary uric acid from allantoin. With this technique, conversion of radioactive uric acid to radioactive allantoin could readily be demonstrated after intravenous infusions of [2-^{14}C]urate. By contrast, after intratubular microinjection there was no demonstrable conversion. The use of the microinjection technique is, therefore, a valid method for the localization of the tubular sites of reabsorption of uric acid.

In the present study, the extracellular fluid volume (ECFV)[1] was varied over a wide range and its effect on the tubular reabsorption of uric acid was examined by clearance and microinjection techniques. The results indicate that reabsorption of uric acid occurs throughout the proximal tubule but principally in the early segment, and that alterations in the net tubular reabsorption of uric acid in response to changes in ECFV are also mediated at these same nephronal sites. No distal tubular reabsorption of uric acid was demonstrable.

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[1] *Abbreviations used in this paper:* C_{urate}/C_{inulin} ratio, the ratio of uric acid clearance to the simultaneously determined inulin clearance; ECFV, extracellular fluid volume; GFR, glomerular filtration rate.

METHODS

Male Sprague-Dawley rats weighing 200–400 g were used in all experiments. Anesthesia was induced with Inactin (Promonta, Hamburg) 100–130 mg/kg injected intraperitoneally. After a tracheostomy, the right and left jugular veins were cannulated and the urinary bladder catheterized. In the microinjection studies, the ureter of the experimental left kidney was catheterized with PE-50 tubing to permit separate urine collections from each kidney. In the clearance experiments, the left femoral artery was cannulated for collection of blood samples.

Special models

Clearance and microinjection studies were performed during control, chronic volume depletion, and acute volume expansion. Control rats had free access to regular Purina chow and tap water up to the time of the experiment.

Chronic volume depletion was accomplished by the intraperitoneal injection of furosemide (15 mg/kg) for 2 consecutive days during maintenance on a low-sodium diet (ICN Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio). Water was not restricted. Studies were performed 2-4 days after the last dose of the diuretic agent. Prior studies have shown that this regime produces a stable model of volume contraction in the absence of a direct effect of the diuretic (6).

Volume expansion was produced by the intravenous administration of a volume of isotonic saline equivalent to 10% of body wt over a 60-min period. The rate of infusion was then adjusted to match the urinary losses.

Clearance studies. Clearance studies were performed on nine control, five volume-contracted, and four volume-expanded rats.

A priming dose of 50 μCi of [*methoxy*-^{3}H]inulin was infused followed by a sustaining infusion of isotonic saline containing 25 μCi/ml of [*methoxy*-^{3}H]inulin at a rate of 1 ml/h. After a 30-min equilibration period, two 20-min urine collections were obtained. 1 ml of arterial blood was obtained at the midpoint of each clearance period and was replaced with the same volume of blood from a donor rat.

In four of the control rats the effect of volume expansion was evaluated. After completion of the control periods, volume expansion (10% of body wt) was accomplished and two additional 20-min clearance periods were obtained.

To examine the effects of mannitol used in the microinjection experiments, further clearance studies were performed. After completion of the control clearance periods in five of the control rats and in four of the dehydrated rats, the infusion was changed to 5% D-mannitol in isotonic saline containing 25 μCi/ml of [*methoxy*-^{3}H]inulin infused at a rate of 1 ml/h. After an additional 30-min equilibration period, two 20-min clearance periods were obtained.

At the conclusion of the experiment, a large blood sample was obtained from the abdominal aorta. The kidneys were then removed and the perirenal fat and capsule stripped and then weighed in a Mettler analytical balance (Mettler Instrument Corp., Princeton, N. J.).

Microinjection studies. Animals were prepared for micropuncture as previously described (7). The left kidney was exposed and its capsule removed. The kidney was then immobilized in 2% agar in a plastic cup to reduce respiratory and vascular motion. Surgical losses were replaced with isotonic saline, 0.5% of the body wt.

Intratubular microinjections were performed according to the method of Gottschalk, Morel, and Mylle (8). The injectate was the same for all groups of animals studied and contained [2-^{14}C]urate and [*methoxy*-^{3}H]inulin adjusted to a pH of 7.0–7.5 with a solution of $NaHCO_3$ (30 meq/liter). The final urate concentration was 4 mg/100 ml and was prepared in an identical fashion to that of Kramp, Lassiter, and Gottschalk (9). The purity of the [2-^{14}C]urate was 98% as determined by thin-layer chromatography.

Microinjections were performed with long-tipped siliconized micropipets with an outer diameter of 5–7 μm. The volume of the injectate was 12 to 21 nl. The rate of injection was controlled such that it was less than the tubular flow rate. After the microinjections, urine was collected from both kidneys for 30–35 min.

Direct observations were made during the course of the injections and during the collection periods to detect visible leaks, retrograde flow, or dilatation of the tubule. Small oil droplets were injected before and after completion of the injection to insure that flow remained unobstructed in the punctured tubule. Occasionally, injections of 0.05 ml of 5% lissamine green dye were used for the same purpose. The proximal tubular transit time of lissamine green dye was obtained at these times. In the presence of obstruction to flow, retrograde flow, a visible leak, or dilatation of the tubule, the samples were discarded.

Proximal tubules were localized by the injection of small oil droplets of Sudan black-stained mineral oil and were considered to be an early portion of the proximal tubule if the droplet reappeared on the surface of the kidney in two or three successive loops. The site was considered to be a late portion of the proximal tubule if the oil droplet immediately disappeared below the surface of the kidney and did not reappear. Distal tubules were localized after the injection of lissamine green dye.

Triplicate droplets of the stock solution containing uric acid and inulin were prepared from a calibrated micropipet and placed under mineral oil. Two of these droplets were counted directly in a modified Bray's solution, the third was used as the injectate. The error of counting duplicate droplets for both uric acid and inulin was less than 5%.

Microinjection studies were performed in twelve control, seven volume-expanded, and nine volume depleted rats. Because of the difficulty in obtaining adequate urine flows in the volume-contracted rats, 5% D-mannitol was infused at a rate of 1 ml/h. To examine the effect of 5% D-mannitol in normal rats, seven of the control rats were given an infusion of isotonic saline and five received 5% D-mannitol in isotonic saline at a rate of 1 ml/h. There was no difference in the recoveries of uric acid or inulin from any microinjection site between these animals and the results are combined and designated as "controls" in the Results section. Corrections for recirculated urate in control and volume-expanded rats were 0–3% after injections into the early proximal tubule and 0–1% after late proximal tubular injections. No correction was required after distal tubular injections. The mean±SEM inulin recoveries were 95.0±2.1 and 94.9±2.5% in control and volume-expanded rats, respectively. In the volume-contracted rats, inulin recovery was 96.0±2.9%. Corrections for recirculation of urate in this group were as high as 8% after early proximal injections and 3–5% after late proximal tubular injections. No corrections were required after distal injections.

Separation of uric acid from allantoin. Since uric acid is converted into allantoin in the rat, a thin-layer chromatographic method for separating these compounds was de-

veloped. In preliminary experiments, rats were prepared for clearance experiments and [2-^{14}C]uric acid (10 μCi/ml) and [*methoxy*-^{3}H]inulin (25 μCi/ml) were infused intravenously at a rate of 1 ml/h. After 30–60 min of equilibration, blood and urine samples were collected. Varying volumes of urine ranging from 50 nl to 2 μl were spotted at the origin of thin-layer chromatographic plates (0.25 mm thickness, EM Laboratories, Inc., E. Merck, Darmstradt, Elmsford, N. Y.) Similar volumes of saturated solutions of unlabeled and labeled uric acid and unlabeled allantoin standards were spotted on the same plates. After drying, the plates were immersed in a tank containing 100 ml of ethyl alcohol, ammonium hydroxide, and water in a ratio of 94:1:5 and allowed to develop 7 cm from the origin. They were then dried again and sprayed with rhodamine to visualize the uric acid spots with ultraviolet light and with ninhydrin to delineate the allantoin spots. A clear separation of uric acid and allantoin was obtained. The plates were then scraped from the origin to the solvent front in 0.5-cm slots and counted in a modified Bray's solution. Virtually 100% of the counts were recovered from the plates. The R_f values were 0.84 and 0.60 for uric acid and allantoin, respectively. The radioactive labeled [2-^{14}C]urate standard was 98% pure. The sensitivity of the thin-layer assay system could detect a rate of conversion of uric acid to allontoin as low as 5%.

After the continuous intravenous infusion of [2-^{14}C]urate for 1 h, approximately 63% of the ^{14}C counts in the urine were associated with the allantoin spot on the thin-layer plates. Constant infusions of [2-^{14}C]urate for 1–2 h resulted in a constant urinary excretion of labeled allantoin and labeled uric acid, the latter constituting one-third of the counts in the urine (Fig. 1).

In four control rats, the simultaneous clearance measurements of [2-^{14}C]urate and the uric acid determined by the chemical method were compared. In each period the clearance calculated from excreted radioactivity exceeded the true uric acid clearance by a factor of 3–6. These results further support the demonstration that significant conversion of [2-^{14}C]urate to labeled allantoin must have occurred.

Since it is unknown if the rat kidney contains uricase activity, further preliminary studies were performed to determine if there was any intrarenal conversion of labeled uric acid to labeled allantoin. Microinjection studies were performed in three control rats and three dehydrated rats. After microinjections into early or late portions of the proximal tubule and into the distal tubule, urine from both kidneys was collected for two 15-min periods. The samples were analyzed as described above. In ten such microinjections, [2-^{14}C]urate was recovered and no labeled allantoin was recovered after any injections in any of the rats. During the time-course of the microinjection studies, therefore, there was no intratubular conversion of the injected uric acid to allantoin.

Analytic methods

The percent inulin leakage was calculated as twice the inulin excreted from the right kidney divided by the total inulin excreted from both kidneys. If the transtubular inulin leakage was greater than 1%, the results were not included in the analysis. The percent recovery of [2-^{14}C]-urate was calculated as:

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

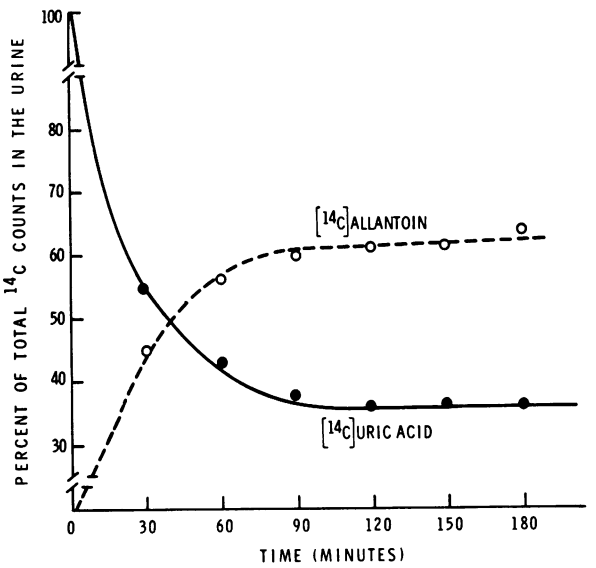

FIGURE 1 The fractionation of ^{14}C radioactive counts in the urine into [^{14}C]uric acid and [^{14}C]allantoin after a sustained intravenous infusion of [2-^{14}C]urate.

Total recovery was corrected for recirculated radioactivity by subtracting the amount of radioactivity recovered from the right kidney from that recovered from the left kidney. Delayed and direct recoveries were determined from the time-course of the excreted inulin and uric acid (8).

Radioactivity of blood, urine, and thin-layer chromatographic plate scrapings were determined in a modified Bray's solution in a Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.) with appropriate corrections made for ^{14}C counts appearing in the ^{3}H channel. At the settings employed, there was less than 1% of ^{3}H counts appearing in the ^{14}C channel. To calculate the crossover of ^{14}C counts appearing in the ^{3}H channel, standard samples were counted with each experiment. The average crossover was 80%. The efficiency of counting was 67.0% for ^{14}C and 27.7% for ^{3}H.

Urinary sodium concentration was determined by flame photometry. Hematocrits were measured in heparinized microhematocrit tubes. Uric acid in blood and urine was determined by the uricase method using the polarographic oxygen sensor in a Beckman glucose analyzer (Beckman Instruments, Inc., Fullerton, Calif.). The calibration standards were linear from concentrations of 0.5–10 mg/100 ml. The reproducibility for both plasma and urine samples was within ±1%. The clearances of inulin and uric acid are expressed in microliters per minute per gram kidney wt and are calculated from standard formulae. The tubular reabsorption of uric acid is expressed in micrograms per milliliter of glomerular filtration rate (GFR) and was calculated from the formula:

$$TR = (C_{inulin} \times P_{UA}) - V \times U_{UA}/C_{inulin}$$

where TR = net tubular reabsorption, P_{UA} = plasma concentration of uric acid, V = volume, U_{UA} = urine concentration of uric acid, and C_{inulin} = clearance of inulin in milliliters per minute. All data are expressed as the mean± SEM. Significance was determined by the Student *t* test or the Fisher *t* test.

TABLE I

*Clearance Measurements in Control and Volume-Contracted Rats**

	Controls	Volume contracted	*P*
Number of animals	9	5	
C_{inulin}, μl/min/g kidney wt	988.8±68.7	788.0±43.9	<0.05
C_{urate}, μl/min/g kidney wt	122.3±8.6	64.8±4.9	<0.001
C_{urate}/C_{inulin}	0.12±0.006	0.08±0.01	<0.01
Tubular reabsorption of uric acid, μg/ml GFR	11.3±1.04	17.9±1.12	<0.005
$U_{Na}V$, μeq/min	0.24±0.06	0.06±0.01	<0.005
Serum uric acid, mg/100 ml	1.15±0.09	1.82±0.11	<0.005
Hematocrit, volume %	43.3±1.2	50.3±3.8	<0.005

* Values are expressed as mean±SEM. *P* indicates level of significance. Control and volume-contracted rats were receiving an infusion of isotonic saline at a rate of 1 ml/h. C_{inulin}, inulin clearance; C_{urate}, uric acid clearance; $U_{Na}V$, sodium excretion rate.

RESULTS

Effects of alterations in the ECFV on the renal handling of uric acid (*Tables I and II*). In the control rats receiving an infusion of isotonic saline at a rate of 1 ml/h the uric acid clearance averaged 122.3±8.6 μl/min/g kidney wt. The ratio of uric acid clearance to the simultaneously determined inulin clearance (C_{urate}/C_{inulin} ratio) was 0.12±0.006. Net tubular reabsorption of uric acid (hereinafter referred to as "tubular reabsorption") was 11.3±1.04.

Volume contraction resulted in a significant rise in the hematocrit from 43.3±1.2% (control) to 50.3±3.8%, a decrease in the urinary excretion of sodium from 0.24± 0.06 to 0.06±0.01 μeq/min, and an 11% loss of body wt.

As compared to controls, in volume-contracted rats receiving an infusion of isotonic saline (1 ml/h) the GFR was lower by 20% and the serum uric acid concentration was higher (1.82±0.11 vs. 1.15±0.09 mg/100 ml [$P < 0.005$]). The clearance of urate was 64.8±4.9 μl/min/g kidney wt, which was 47% lower than the control group ($P < 0.001$). The C_{urate}/C_{inulin} ratio was 0.08±0.01 and 0.12±0.006 ($P < 0.01$) in volume contracted and control rats, respectively. The tubular reabsorption of uric acid was markedly higher, being 17.9± 1.12 in volume-contracted rats compared to 11.3±1.04 μg/ml GFR in controls ($P < 0.005$).

Volume expansion with isotonic saline resulted in an increased urinary excretion of uric acid and a rise in the clearance of uric acid to 438.0±66.0 μl/min/g kidney wt from the control value of 124.8±6.8 ($P < 0.01$). The C_{urate}/C_{inulin} ratio increased to 0.47±0.08. The tubular reabsorption of urate fell 37% compared to the control period ($P < 0.05$).

The renal handling of uric acid would, therefore, appear to be affected by changes in the ECFV over the entire range of bodily hydration. During volume contraction there is an increase in the reabsorption of uric acid, the decrement in the clearance of uric acid exceeding that of inulin. By contrast, during volume expansion, there is a marked increase in the excretion of uric acid, a decrease in the rate of tubular reabsorption of uric acid, and a rise in the C_{urate}/C_{inulin} ratio.

Effect of D-*mannitol* (*Table III*). Since hypertonic mannitol is known to be uricosuric, the effect of man-

TABLE II

*Effect of Isotonic Saline Volume Expansion on Uric Acid Reabsorption**

	Control	Volume Expansion	*P*
C_{inulin}, μl/min/g kidney wt	974.0±68.0	977.3±127.0	NS
C_{urate}, μl/min/g kidney wt	124.8±6.8	438.0±66.0	<0.01
C_{urate}/C_{inulin}	0.13±0.01	0.47±0.08	<0.02
Tubular reabsorption of uric acid, μg/ml GFR	13.09±1.77	8.26±0.53	<0.05
$U_{Na}V$, μeq/min	0.21±0.11	16.6±1.17	<0.001
Serum uric acid, mg/100 ml	1.4±0.07	1.2±0.09	NS
Hematocrit, volume %	44.25±1.8	39.5±1.5	<0.005

* See Table I for legend. Four rats were studied under control conditions receiving isotonic saline infusion at a rate of 1 ml/h. Volume expansion was accomplished by the infusion of isotonic saline (10% of the body wt over 60 min).

TABLE III

*Effect of 5% D-Mannitol in Isotonic Saline on Uric Acid Reabsorption in Normal and Volume-Contracted Rats**

	Control (5)		Volume contracted (4)	
	Saline	Mannitol	Saline	Mannitol
C_{inulin}, μl/min/g kidney wt	1,079.0±88.2	1,176.0±132.4	860.0±45.8	1,083.0±97.4‡
C_{urate}, μl/min/g kidney wt	121.0±4.8	213.0±27.3‡	65.7±6.2	112.5±22.1‡
C_{urate}/C_{inulin}	0.11±0.008	0.19±0.02‡	0.07±0.01	0.10±0.01‡
Tubular reabsorption of uric acid, μg/ml GFR	10.5±1.4	9.92±1.9	17.8±1.4	16.9±1.9
$U_{Na}V$, μeq/min	0.20±0.07	0.33±0.08‡	0.06±0.015	0.05±0.003
Serum uric acid, mg/100 ml	1.15±0.11	1.09±0.11‡	1.82±0.13	1.74±0.19
Hematocrit, volume %	44.4±1.8	43.9±1.5	50.9±1.2	49.5±1.3

* See Table I for legend. Numbers in parentheses indicate number of animals studied. All animals were infused at a rate of 1 ml/h.

‡ Significant differences at $P < 0.05$ as determined by paired t-test.

nitol was first evaluated by clearance techniques (10). In normal rats, the infusion of 5% D-mannitol at a rate of 1 ml/h resulted in a modest rise in sodium excretion from 0.20±0.07 to 0.33±0.08 μeq/min ($P < 0.005$) and an increase in urate clearance from 121.0±4.8 to 213.0± 27.3 μl/min/g kidney wt ($P < 0.02$). The C_{urate}/C_{inulin} ratio also increased but there was no significant change in the tubular reabsorption of uric acid.

In the volume-contracted rats, mannitol infusion caused an increase in the GFR from 860±45.8 to 1,083± 97.4 μl/min/g kidney wt ($P < 0.05$). Urate clearance was also increased, as was the C_{urate}/C_{inulin} ratio. There was a decrease in the tubular reabsorption of uric acid from 17.8±1.4 to 16.9±1.9 μg/ml GFR after mannitol infusion, but this change was not significant. In response to mannitol infusion, there was a rise in the clearance of uric acid in both the control and volume-depleted animals. This rise is most likely a result of the increase in the rate of filtration, especially in the volume-depleted animals. It is also possible that there was some decrease in net reabsorption which could have contributed to the increased clearance, although the differences in tubular reabsorption failed to reach statistical significance.

Comparing mannitol infusion in control and in volume-contracted rats demonstrates the same pattern of change as in the same groups of animals receiving isotonic saline infusions. In the volume-contracted rats the uric acid clearance was lower than in controls (112.5±22.1 vs. 213.0±27.3 μl/min/g kidney wt [$P < 0.05$]). The C_{urate}/C_{inulin} ratio was also lower (0.10±0.01 vs. 0.19± 0.02 [$P < 0.05$]), and the tubular reabsorption of uric acid higher (16.9±1.9 vs. 9.9±2.3 μg/ml GFR [$P <$ 0.05]). Interestingly, the urinary excretion of sodium in volume-contracted rats did not increase in response to mannitol, probably due to increased distal reabsorption of sodium delivered out of the proximal tubule.

Microinjection studies. To examine the effect of alterations in the state of hydration on the proximal and distal tubular reabsorption of uric acid, microinjection studies were performed in twelve control, nine volume-depleted, and seven volume-expanded rats. The time-course for the excretion of [2-^{14}C]urate paralleled that of [*methoxy*-^{3}H]inulin in all groups of animals studied. Delayed excretion ranged from 3±2% to 9±3%, without a significant difference in delayed excretion between the groups of rats or between the microinjection sites. Since no significant differences in delayed excretion could be demonstrated, the results in the experimental groups of rats are expressed as total recovery of [2-^{14}C]urate only and are summarized in Fig. 2.

In the control animals, the total recovery of [2-^{14}C]-urate was 66±2% after microinjections into the early proximal tubular sites and 75±3% after microinjections into late proximal tubular sites. Recoveries

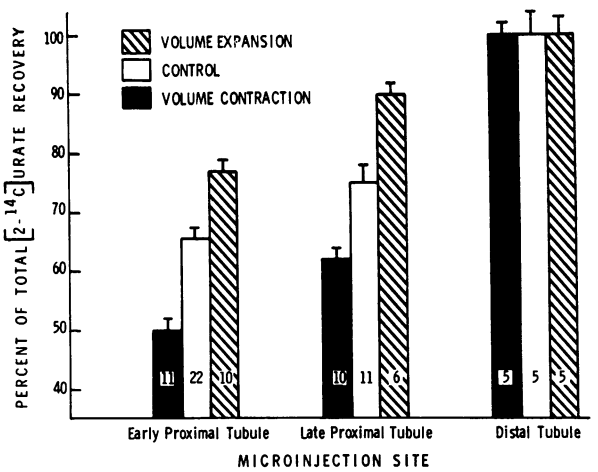

FIGURE 2 Total recoveries of [2-^{14}C]urate in control, volume-contracted, and volume-expanded animals after intratubular microinjections into the early or late portion of the proximal tubule or into the distal tubule.

TABLE IV

*Recovery of [2-^{14}C] Urate after Intratubular Microinjection in Nondiuretic Control Rats**

Recoveries	Early proximal (n = 22)	Late proximal (n = 11)	Distal (n = 5)
Total	66±2 ($P < 0.05$)	75±3 ($P < 0.005$)	100±4
Direct	60±2 ($P < 0.01$)	70±2 ($P < 0.005$)	91±3
Delayed	6±1 (P = NS)	6±2 (P = NS)	9±3

* Values indicate mean ± SEM. Numbers in parentheses indicate number of observations. P values indicate statistical differences between corresponding values from early or late proximal tubule microinjections or distal tubule microinjections.

after distal tubular injections were 100±4% (Table IV). These results indicate that uric acid is reabsorbed primarily in the proximal convoluted tubule and also at a site distal to the accessible portion of the proximal tubule. No evidence for distal tubular reabsorption was demonstrated.

Volume depletion was evidenced by a rise in hematocrit from 46.8±2.1% in control rats to 56.1±0.5% ($P < 0.01$) in volume-contracted rats. The urine volume per kidney was 2.95±0.26 μl/min in volume-contracted rats vs. 4.5±0.5 in controls ($P < 0.01$). The transit time of lissamine green dye was prolonged from 9.6±0.4 (controls) to 19.3±3.4 s ($P < 0.005$).

In the volume-contracted rats, there were significant decreases in the total recovery of uric acid after microinjections into early or late portions of the proximal tubule (Fig. 2). Recoveries averaged 50±2% after early and 62±2% after late proximal tubular injections. The recovery of urate was 100±2% after distal tubular microinjections.

Volume expansion with isotonic saline resulted in a fall in the hematocrit to 39.3±1.2%, a shortening of the lissamine green dye transit time to 7.67±0.3 s, and an increase in the rate of urine flow per kidney to 19.4±3.35 μl/min. All of these values are significantly different from the corresponding control values. Total recoveries of uric acid in the urine were significantly increased after microinjections into the proximal tubule (Fig. 2). 77±2% of the injected urate was recovered after early proximal injections. After late proximal tubular microinjections, 90±2% of the injected urate was recovered. There was no evidence of distal tubular reabsorption, and recoveries averaged 100±3% after distal tubular microinjections.

It is apparent, therefore, that in volume expansion, as in volume contraction, the proximal tubule is the major site of the adjustments of uric acid reabsorption in the rat. Previous studies, employing an identical model of volume contraction in the rat, have demonstrated impressive increases in the reabsorption of salt and water in the proximal tubule (6). Similarly, volume expansion with isotonic saline has repeatedly been demonstrated to result in a decreased fractional reabsorption of sodium in the proximal tubule (11, 12). Over a large range of states of hydration, therefore, urate reabsorption parallels that of sodium and this is attributable to alterations in the rates of reabsorption of both uric acid and sodium in the proximal convoluted portion of the nephron.

DISCUSSION

The results of the present study clearly indicate that the state of hydration of the extracellular fluid compartment strongly influences the renal handling of uric acid. Since we were unable to detect any evidence of urate reabsorption in the distal tubule, it would appear that the proximal portion of the nephron is the major nephronal site for the adjustments in urate excretion in response to alterations in the ECFV. In the microinjection studies, there was a significant decrease in proximal urate reabsorption when the ECFV was expanded. Conversely, the rate of urate reabsorption at the same site was increased after contraction of the ECFV. These findings, therefore, would expand the accumulating experimental evidence that there is a close parallelism between the reabsorption of sodium and the reabsorption of a number of constituents of the glomerular filtrate when the ECFV is altered (1–3).

Alterations of the ECFV have previously been demonstrated to lead to changes in the net tubular reabsorption of sodium. To a large extent, these effects are mediated by alteration in the rates of reabsorption by the proximal convoluted portion of the nephron (11, 12). Since the major alterations in urate reabsorption occur at the same nephronal sites, it would seem that sodium is somehow pivotal in the reabsorption of uric acid. Alternatively, the physical factors which govern sodium reabsorption, such as the peritubular oncotic pressure and peritubular hydrostatic pressure, may also affect the reabsorption of uric acid. This may be due to alterations in the rate of capillary uptake from the interstitium and/or in the rate of backflux into the lumen (7, 13, 14).

The model of contraction of the ECFV employed in the present studies has previously been demonstrated to be a stable model in which to evaluate the influence of volume depletion on sodium reabsorption. The changes in GFR, hematocrit, and in the urinary excretion of sodium are similar to those reported (6, 7). Since the reabsorption of sodium in the proximal tubule is increased in these animals (6), it was of interest to use this model for studying changes in urate reabsorption in response to contraction of the ECFV. After contraction, there was a fall in the clearance of uric acid. The C_{urate}/C_{inulin} ratio also fell, indicating that the decrement in the urate clearance exceeded that of the

GFR. This is reflected in the significant rise in the tubular reabsorption of uric acid.

In the volume-contracted animals, the serum concentration of uric acid was also elevated. While the increase in the serum uric acid level may be due, in part, to the decrease in the GFR, the infusion of 5% mannitol in volume-contracted rats restores the filtration rate to near normal. Despite the correction of the filtration rate, the serum uric acid remained elevated and the tubular reabsorption of urate, expressed in micrograms per milliliter of GFR, was still significantly increased. This would suggest that both the fractional and absolute rates of uric acid reabsorption are increased in response to contraction of the ECFV.

To localize the site of the increased tubular reabsorption of uric acid, the microinjection technique of Gottschalk et al. was employed (8). The total recovery of [2-^{14}C]urate after microinjections into the early proximal tubule was significantly decreased in the volume-contracted rats. Recoveries were also decreased, although to a lesser degree, after microinjections into the late portions of the proximal tubule. Thus, in volume-contracted rats the nephronal site of accelerated reabsorption of urate is the proximal convoluted tubule. A contribution to this increased reabsorption may also have been made at a site distal to the accessible portion of the proximal tubule, presumably in the pars recta or the loop of Henle.

The renal response to expansion of the ECFV with isotonic saline was an increase in the clearance of uric acid without a significant change in the GFR. The tubular reabsorption of uric acid was decreased by 37% and the C_{urate}/C_{inulin} ratio was increased from 0.13 in the nondiuretic state to 0.47 after volume expansion. In the microinjection studies, the urinary recoveries of uric acid from proximal tubular sites were increased in the volume-expanded rats. The decrease in the tubular reabsorption in response to volume expansion would therefore appear to be due to a decrease in the reabsorption of urate by the proximal convoluted tubule.

In none of the experimental conditions examined was evidence for distal reabsorption found, and recoveries of [2-^{14}C]urate after microinjections into the distal tubule averaged 100%. When viewed together, the results of the clearance and micropuncture experiments support the conclusion that the alterations in the rates of uric acid reabsorption in response to alterations in the ECFV are mediated in the proximal portion of the nephron.

A comparison of the fractional rates of urate reabsorption by clearance measurements and by microinjection methods reveals a marked disparity. Clearly, more urate is being reabsorbed by the kidney, both in control and experimental rats, than is evident from the microinjection studies. This may be due to several factors. First, the initial part of the proximal tubule, that portion which is below the surface and unavailable to micropuncture, may make a disproportionate contribution to urate reabsorption. Evidence favoring this interpretation has been advanced and similar findings for sodium and phosphate reabsorption by the proximal tubule have also been reported (15–17). Another possibility would be that the redistribution of blood flow and glomerular filtration which occurs in response to depletion of the ECFV results in a greater reabsorption of uric acid by the deeper nephrons of the kidney (18). A final consideration is that [2-^{14}C]urate, when injected into the tubule, is not handled in an entirely analogous manner as is the endogenously produced uric acid filtered at the glomerulus. The injectate and techniques employed, however, were comparable in control and experimental rats and the differences in the rates of urate reabsorption must have reflected alterations in the rates of reabsorption due to either one of the first two possibilities.

Although not specifically examined in the present study, alterations in the rate of uric acid secretion would not seem to explain our results. Unequivocal evidence for urate secretion in the rat is difficult to demonstrate. Tissue slice studies on the rat kidney have failed to demonstrate uric acid uptake, although uptake could be demonstrated in animals such as the chicken, the rabbit, and the guinea pig, which show unequivocal evidence of net secretion *in vivo* (19). Precession studies in the rat utilizing [2-^{14}C]urate have also failed to demonstrate evidence for a urate secretory mechanism in this species (9). By contrast, evidence for urate secretion in the rat has been advanced by Greger, Lang, and Deetjen, who were able to demonstrate that the proximal tubular fluid-to-plasma urate ratio divided by the tubular fluid-to-plasma inulin ratio exceeded unity. Since these animals also demonstrated net urate reabsorption, it was proposed that uric acid reabsorption occurs at a site distal to the proximal tubule (20). The analytical problems of determining uric acid in microsamples of tubular fluid are considerable and the above studies await confirmation. The bulk of evidence would favor the view that uric acid secretion in rats is limited if, in fact, it exists at all. The absence of significant differences in delayed excretion in the current study is consistent with this conclusion.

The rat has an active hepatic uricase enzyme system and can rapidly convert uric acid into allantoin. The validity of the microinjection technique for evaluating the reabsorption of uric acid, therefore, is dependent upon the demonstration that there is no intrarenal conversion of uric acid to allantoin. We were unable to recover any radioactive allantoin after microinjections of [2-^{14}C]urate in control and dehydrated animals during

the time-course of the collection periods. Utilizing the thin-layer chromatographic methods developed, we were able to separate urinary uric acid from allantoin, yet we were unable to detect any conversion of the radioactive [2-^{14}C]urate to allantoin. Nevertheless, it is likely that some conversion could have occurred, especially when the uric acid was recirculated. Since the amount of recirculated uric acid was small and corrections were made for the amount of ^{14}C label appearing from the nonmicropunctured kidney, the microinjection technique would seem to be a valid method for evaluating the renal handling of uric acid.

The values for total recoveries after microinjections into early and late proximal sites and into the distal tubule in nondiuretic and volume-expanded rats are in close agreement with the results of Kramp et al. (9). Although delayed recoveries in our studies varied between 3 and 9%, we were unable to confirm the significant changes in this parameter that were present in their studies. It has been pointed out, however, that compared to the total recoveries, the calculations of direct and delayed recoveries are less reliable due to their dependence upon the ratio of the isotopes in a single urine collection and this may have contributed to the disparity (9).

The renal clearance of uric acid in the rat was also determined in the studies of Kramp et al. from the clearance of [2-^{14}C]urate. These results are in marked contrast to the urate clearances of the present study. When the clearance of uric acid, as determined by a chemical method, is compared to the simultaneously obtained clearance of [2-^{14}C]urate, the latter measurement overestimates the true uric acid clearance by a factor of 3–6. This suggests that after a sustained intravenous infusion, significant amounts of the radioactive labeled uric acid are converted to radioactive allantoin. This conclusion is further supported by the findings that almost two-thirds of the radioactive counts are associated with the allantoin spot on the thin-layer chromatographic plates after a sustained intravenous infusion of [2-^{14}C]urate. Only one-third of the counts are clearly located in the uric acid spot. It would appear, then, that the clearance of [2-^{14}C]urate cannot be used as a measure of uric acid clearance. The concentration of uric acid in the serum reported in the present study averaged 1.15 mg/100 ml and is similar to that previously reported. The control values for the uric acid clearance and the tubular reabsorption of urate are also comparable to those obtained by Boudry in the nondiuretic rat (21, 22).

To date, the understanding of the renal handling of uric acid has been incomplete and somewhat dependent upon implications derived from the use of pharmacologic inhibitors. The results of the present study suggest that the intratubular microinjection technique is a valid method for assessing qualitative changes in absorption and in localizing the nephronal sites of these alterations. The results also demonstrate the significant influence of the state of hydration on uric acid reabsorption in the rat kidney. Of interest was the observed increase in net proximal tubular reabsorption of uric acid after depletion of the ECFV. Although significant differences in urate metabolism may exist between the rat and man, a similar sequence of events has also been proposed to explain the hyperuricemia of chronic diuretic administration (23).

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