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The Transport of Urate in the Small Intestine of the Rat (41471)

C. E. DUKES, D. A. STEPLOCK, A. M. KAHN, AND E. J. WEINMAN[1]

Division of Nephrology, University of Texas Medical School, P.O. Box 20708, Houston, Texas 77025

Abstract. The transport of urate in the small intestine of the rat was examined, *in vivo*, to determine if specific (mediated) transport systems are present. The secretory flux of urate was determined following the infusion of urate systemically, with or without oxanate, while the small intestine was perfused with an initially urate-free solution. The absorptive flux of urate was determined by perfusing the gut with solutions of known concentrations of urate. In some studies probenecid was infused systemically or added to the luminal perfusion solution. Over a wide range of concentrations of urate in the plasma or in the lumen, there was no evidence for saturation of either the secretory or absorptive fluxes of urate. Probenecid had no effect on either of the flux rates. These findings suggest that in the small intestine of the rat, the movement of urate out of or into the lumen occurs by passive diffusion and that, under the conditions of study, no evidence for facilitated transport can be demonstrated.

In previous studies from this laboratory, we have examined, in detail, the mechanisms subserving the excretion of urate by the kidney (1, 2). In the rat kidney, the transport of urate in both the reabsorptive and secretory directions are mediated transport processes (1, 2). Despite general agreement that the kidney is the major route of excretion of urate, there is ample data to indicate that the gastrointestinal tract also plays a role in the overall metabolism of urate (3). The nature of the transport of urate in the gastrointestinal tract, however, has not been clearly defined. In the present studies we have examined the rates of transfer of urate out of and into the small intestine of the rat in order to determine if specific transport systems for this organic anion are present.

Methods. Male Sprague–Dawley rats with free access to food and water prior to study were anesthetized with pentabarbital (50 mg/kg body wt injected intraperitoneally). A tracheotomy was performed, the femoral artery and vein were cannulated and the urinary bladder was catheterized. The abdominal cavity was opened via a midline incision and segments of the small intestine were cannulated with polyethylene catheters. Proximal segments of the small intestine were considered to be from the ligament of Trietz and extending distally 9 to 15 cm. Distal segments of the small intestine were considered to be 5 cm from the ileocecal valve and extending proximally 9 to 15 cm. Animals were placed on a heated board and body temperature was maintained at 37°. During the course of study each animal received an intravenous infusion of isotonic saline at a rate of 6 ml/hr. The small intestines were perfused at a rate of 12 ml/hr with a solution containing sodium 130 meq/liter, bicarbonate 30 meq/liter, potassium 5 meq/liter, and chloride 105 meq/liter. [^{3}H]Polyethylene glycol (PEG) (New England Nuclear Corp., Boston, Mass.) was added as a volume marker. The collection periods were 10 to 20 min in duration.

In 30 animals the secretory flux of urate was determined by the systemic infusion of urate with or without oxanate, an inhibitor of uricase, while perfusing the intestine with an initially urate free solution. In order to obtain a range of plasma concentrations of urate, sodium urate in concentrations of 10, 25, or 50 mg% and oxanate in concentrations of 0.5 or 1.0 g% were added to the

[1] To whom correspondence should be addressed.

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intravenous infusion solution. Where examined, probenecid was also added to intravenous infusion solution in concentrations calculated to deliver a dose equal to 100 mg/kg body wt/hr. In other studies, probenecid (28.5 mg%) was added to the intestinal perfusion solution.

In the studies examining the absorptive flux of urate, no urate or oxanate was infused systemically (20 animals). Sodium urate was added to the intestinal perfusion solution in concentrations of 5 to 20 mg%. Where examined, probenecid was infused systemically or added to the luminal perfusion solution in the amounts previously indicated.

In both the secretory and absorptive flux studies, two intestinal perfusions were obtained at any given intravenous infusion rate or any given initial concentration of urate in the perfusion solution. The urate concentrations were then changed, 20 additional min allowed to elapse to allow for a new steady state and additional collections obtained. Usually three such periods at different concentrations of urate were obtained per animal. At the end of the experiments, the distance between the perfusion and collection intestinal catheters was measured. The average length of perfused segments of the small intestine averaged 11.6 ± 0.05 cm for the proximal segments and 11.4 ± 0.61 cm for the distal segments.

The radioactivity of perfusion and collection solutions was determined in Biofluor (New England Nuclear Corp.) in a liquid scintillation counter. The concentration of urate was determined by high performance liquid chromatography with electrochemical detection as previously described (4). Neither oxanate or probenecid interfered with the determination of the concentrations of urate.

The *in vivo* perfusion rate was calculated from the formula: perfusion rate (ml/hr) = collected volume (ml/hr) × CF/PF_{PEG} where CF and PF are the disintegrations per minute of PEG in the collected fluid (CF) and perfusion solution (PF), respectively. The secretory flux of urate was calculated from

$$J_s \text{ (g/min/cm)} = \text{CF urate} \times \text{collected volume} \times \text{length}^{-1},$$

where CF urate is the concentration of urate in the collected perfusion solution, and length is the distance between perfusion and collection sites in centimeters. The absorptive flux was calculated from

$$J_a \text{ (g/min/cm)} = \{(1-(\text{CF/PF urate})/(\text{CF/PF}_{PEG})\} \times \text{perfusion rate} \times \text{PF urate} \times \text{length}^{-1}$$

where PF urate is the concentration of urate in the inital perfusion solution. The results of the duplicate collections were averaged and the results presented as the mean of means ± SEM.

Results. The CF/PF_{PEG}, an index of water absorption averaged 1.04 ± 0.004 and 1.06 ± 0.006 for proximal and distal segments, respectively. The perfusion rates were also similar in both intestinal segments and averaged 12.4 ± 0.09 ml/hr for proximal segments and 13.4 ± 0.21 ml/hr for distal segments. The rates or water absorption were not effected by the presence of urate or probenecid in the lumen or by the systemic infusion of urate or probenecid.

For the sake of data presentation, the fluxes are averaged for urate concentrations over a range of 1 mg% for plasma urate concentrations from less than 1 to 5 mg% and in a 3 mg% intervals for higher plasma concentrations of urate. There were no significant differences in the rates of urate secretion in proximal and distal intestinal segments at any given plasma concentration of urate. In addition, neither the systemic infusion of probenecid nor the inclusion of probenecid in the luminal perfusion solution had any effect on the secretion of urate. The results of all secretory studies were combined and are plotted as a function of the plasma concentration of urate (Fig. 1). As can be seen, over the wide range of plasma concentrations of urate obtained, the secretion of urate appeared to be a direct function of the plasma concentration and no evidence for saturation of the secretory flux is evident.

The absorptive flux of urate was also similar in both proximal and distal segments and was not influenced by the inclusion of probenecid in the perfusion solution. Figure

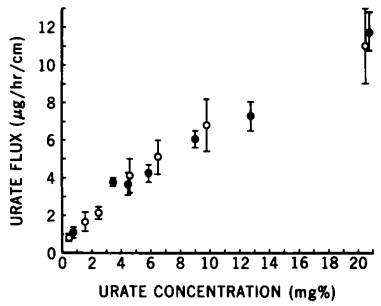

FIG. 1. The relationship between the secretory flux of urate (closed circles) or the absorptive flux of urate (open circles) and either the plasma concentration of urate in the case of the secretory studies or the arithmetic mean intraluminal concentration of urate in the absorptive studies. Values represent mean of means ± SEM. Each point is the average of 4 to 15 experimental periods.

1 summarizes the results of these studies and illustrates the absorptive flux as a function of the arithmetic mean intraluminal concentration of urate. In these studies, the plasma concentration of urate was less than 1 mg%. The values illustrated were grouped in a manner identical to that of the secretory studies. As was the case in the secretory studies, the absorptive flux is a direct function of the mean intraluminal concentration of urate and no evidence for saturation of the absorptive flux is evident. At any given concentration of urate, the absorptive flux approximates the secretory flux.

Discussion. It is known that the ingestion of a high purine diet increases the urinary excretion of urate and, in some individuals, exacerbates hyperurecemia. This response presumably reflects absorption of urate precursors which are metabolized to urate in the liver. It has also been demonstrated that urate itself can be reabsorbed from the gastrointestinal tract (3). The question of gut absorption of urate might be rendered moot when it is appreciated that diet of neither man nor rat contains any preformed urate. On the other hand, the gut is thought to dispose of 25 to 33% of the daily production of urate (3). Reabsorption of this component of urate in the lumen could be of potential importance. The mechanism whereby urate enters the intestine has not been clarified. Conceivably, urate could enter as components of gastrointestinal secretions, passively diffuse from the blood into the gastrointestinal tract, or be secreted by specific mediated transport systems analogous to those present in the kidney (1, 2).

Kolassa *et al.* have recently presented evidence that hypoxanthine and xanthine are actively secreted by the jejunum of the guinea pig (5). The transport of urate itself, however, was not investigated in these studies. Berlin and Hawkins have also reported active secretion of hypoxanthine and xanthine in the small intestine of the golden hamster (16). Although less definitive than for hypoxanthine and xanthine, these studies also suggested that the carrier had specificity for urate as well. Scharrer *et al.* have demonstrated an active absorptive flux for hypoxanthine in the jejunum of the lamb (7). The transport of urate was not specifically examined in these studies but urate had no effect on the transport of hypoxanthine. Active absorption of some purines and pyrimidines has also been demonstrated in the chiton but no evidence for the active absorption of urate was found in this species (8).

Shanker *et al.* demonstrated that the rat intestine was capable of active absorption of uracil and that certain purine and purine-like compounds could inhibit its transport (9). Although it was not possible to demonstrate unequivocally that urate itself was transported, urate did inhibit, albeit weakly, the transport of uracil suggesting a common transport mechanism. Wilson and Wilson, Kahn *et al.*, and Oh *et al.*, however, have all presented evidence that urate transport across the small intestine of the rat was by passive diffusion only and no active or mediated transport systems could be discerned (10–12). The validity of the conclusions of these studies has been questioned in some of these experiments since the studies were performed *in vitro* and no data were presented to indicate that the tissues examined were capable of active transport of other substrates (5). Moreover, these studies employed [2-^{14}C]urate which can conceivably undergo

metabolic transformation to radioactive allantoin.

Since some uncertainty as to whether or not the intestine of the rat is capable of transport of urate, and since the intestine may be important in the overall disposition of urate, we elected to reinvestigate this question using the techniques of *in vivo* intestinal perfusion. In the rat, urate is converted to allantoin and, as a consequence of this conversion, the infusion of urate systemically does not lead to predictable elevations in the serum concentration of urate. Moreover, renal function was intact in our animals and no doubt the urinary excretion of urate was increased. However, by infusing varying amounts of urate with or without oxanate, a hepatic inhibitor of uricase, we were able to study the recovery of urate in perfused segments of the gut in response to a wide range of plasma concentrations. The secretory flux of urate into the small intestine, at any given plasma concentration of urate, was similar in proximal and distal segments of the small intestine. As summarized in Fig. 1, no clear evidence of saturation of the secretory process was evident although some flattening of the relationship is noted at the highest plasma concentrations of urate obtained. It might be argued that a low capacity transport system does exist and was nearly saturated even at the lowest concentrations examined. It is also possible that a carrier system is present, one which has a K_m above that of the plasma concentrations obtained in the present study. Against these suggestions is the fact that probenecid in concentrations or amounts known to inhibit the renal transport of urate, had no influence on transport in the small intestine. To the degree that probenecid is a reasonably specific inhibitor of organic anion transport in a variety of tissues and based upon the relationship between the secretory flux and the plasma concentration, it seems reasonable to conclude that urate enters the lumen of the intestine by a passive mechanism and that a specific mediated transport system cannot unequivocally be demonstrated. We have also investigated the absorptive flux of urate over a range of concentrations of urate in the lumen. The imposition of a lumen to blood gradient for urate results in absorption of luminal urate. There was a direct relationship between the average luminal concentration and the absorptive flux over a wide range of urate concentrations. There was no clear evidence however for saturation of the absorptive process. Moreover, as in the secretory studies, the absorptive flux of urate was not influenced by the infusion of probenecid systemically or the inclusion of probenecid in the luminal solution. Using analogous considerations to those already discussed, it seems likely that the absorption of urate is also occurring by passive permeation only. The conclusions about both the nature of the secretory and the absorptive flux are entirely consistent with some of the evidence already cited from studies performed in the rat (10–12).

It is possible that species differences account for some of the disparities in the literature and it is recognized that interspecies variability in the renal handling of organic anions exists. It is likely that such may also exist in the intestinal transport of organic anions. It seems reasonable to conclude from the present studies plus the studies performed by others that in the small intestine of the rat, urate transport is most likely due to passive diffusion. The present studies, however, do not rule out the possibility of mediated transport of urate in the terminal portion of the ileum.

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1. Weinman EJ, Sansom SC, Steplock DA, Sheth AV, Knight TF, Senekjian HO. The secretion of urate in the proximal convoluted tubule of the rat. Amer J Physiol 239 (Renal Fluid Electrolyte Physiol 8):F383–F387, 1980.
2. Sansom SC, Senekjian HO, Knight TF, Babino H, Steplock DA, Weinman EJ. Determination of the apparent transport constants for urate absorption in the rat proximal tubule. Amer J Physiol 240 (Renal Fluid Electrolyte Physiol 9):F406–F410, 1981.
3. Sorensen LB. The elimination of uric acid in man. Scand J Clin Lab Invest Suppl 54:1–214, 1960.
4. Weinman EJ, Sansom SC, Steplock DA, Sheth AV, Knight TF, Senekjian HO. The use of high per-

formance liquid chromatography for determination of urate concentrations in nanoliter quantities of fluid. Kidney Int 19:81–85, 1981.
5. Kolassa N, Schutzenberger WG, Weiner H, Turnheim K. Active secretion of hypoxanthine and xanthine by guinea pig jejunum in-vitro. Amer J Physiol 238 (Gastrointest Liver Physiol 1):G141–G149, 1980.
6. Berlin RD, Hawkins RA. Secretion of purines by the small intestine: Transport mechanism. Amer J Physiol 215:942–950, 1968.
7. Scharrer E, Raab W, Tiemeyer W, Amann B. Active absorption of hypoxanthine by lamb jejunum in-vitro. Pflugers Arch 391:41–43, 1981.
8. Hanisch ME, Lawrence AL. Purine and pyrimidine absorption by the gut of the chiton, cryptochiton Stelleri. Comp Biochem Physiol 42A:601–610, 1975.
9. Shanker LS, Jeffrey JL, Tocco DJ. Interaction of purines with the pyrimidine transport process of the small intestine. Biochem Pharmacol 12:1047–1053, 1963.
10. Wilson DW, Wilson HCW. Studies in-vitro of the digestion and absorption of purine ribonucleotides by the intestine. J Biol Chem 237:1643–1647, 1962.
11. Kahn AM, Wilson S, Crawhall J. The influx of uric acid and other purines into overted jejunal sacs of the rat and hamster. Canad J Pharmacol 53:113–119, 1975.
12. Oh JH, Dossetor JB, Beck IT. Kinetics of uric acid transport and its production in rat small intestine. Canad J Physiol Pharmacol 45:121–127, 1967.

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