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The Transport of Urate in Frog Gastric Mucosa (41443)

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Abstract. The mechanism for the transport of urate was examined using the gastric mucosa of the frog. After short circuiting the tissue to zero potential, both the unidirectional serosal to mucosal and the mucosal to serosal flux of urate were found to be a direct function of the initial concentration of urate. The serosal to mucosal permeability coefficient averaged $6.5 \pm 0.8 \times 10^{-10}$ cm/sec; a value not different from the mucosal to serosal permeability coefficient of $7.7 \pm 0.8 \times 10^{-10}$ cm/sec. These results suggest that urate passes through the gastric mucosa by passive diffusion only. When compared to the small intestine and proximal tubule of the rat the permeability coefficient for urate is significantly lower. Though the gastrointestinal tract is a potential route for the elimination of uric acid, the stomach does not appear to play a major role.

A saturable carrier transport system has been reported to mediate the transport of urate in the proximal convoluted tubule of the kidney in several species (1–3). Since the gastrointestinal tract is another route for the elimination of uric acid, the possibility exists that urate transport in this organ is also carrier mediated (4). To date, a saturable system for urate has not been unequivocally demonstrated in the gastrointestinal tract. Indirect evidence would suggest that in some, but not all species, the transport of urate in the small intestine is a facilitated transport process (5, 6). We are unaware, however, of any data which examine the transport of urate through the mucosa of stomach. The present *in vitro* experiments, therefore, were designed to determine the bidirectional fluxes of urate across the stomach of the frog.

Methods. Gastric mucosa from frogs (rana pipiens) were prepared for study in the following manner. After decapitation, a portion of the fundic region was excised and placed in a saline solution at room temperature. The mucosal layer was dissected from the serosal muscle coat with scissors and forceps. The mucosa was then mounted in two 10-ml half-chambers with 1 cm^2 of exposed tissue in each half chamber. Bathing solutions at room temperature were immediately introduced into the chambers and gassed with 95% O_2–5% CO_2.

The bathing solutions consisted of 102 m*M* sodium, 4 m*M* potassium, 1 m*M* calcium, 0.8 m*M* magnesium, 81 m*M* chloride, 25 m*M* bicarbonate, 1 m*M* phosphate, 0.8 m*M* sulfate with a final osmolality of 215 mosm/kg H_2O. (The pH of the solutions was 7.2 when gassed with 95% O_2 and 5% CO_2.) Since a buffered solution was used on the mucosal side, spontaneous hydrogen ion secretion would not change the pH of this solution throughout the course of the experiment.

The potential difference across the mucosa was measured with an automatic voltage clamp apparatus having a 100-mV sensitivity full scale and less than 0.5 mV error (7). External current was delivered through Ag/AgCl electrodes in 3% agar saline separated from the bathing solutions by the gastric mucosa diffusion barrier. When the potential difference stabilized (approximately 30 min after mounting), the mucosa was short circuited to zero potential by the automatic voltage clamp apparatus. The current was interrupted periodically to determine the spontaneous transmucosal potential difference. The electrical tissue resistance was calculated as the ratio of open circuit potential difference to short circuit current and was corrected for a solution resistance of 80 ohm·cm^2 (8).

After a 30-min equilibration period in so-

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lutions not containing urate, the solution on one side was replaced with a solution containing [^{14}C]urate. At 30-min intervals, 50-μl samples were obtained from the chamber to which the urate was added and 1-ml samples were obtained from the other chamber. The volumes of fluid removed were replaced quantitatively. The samples were transferred to scintillation fluid (Aquasol, New England Nuclear) and counted in a liquid scintillation spectrometer (Beckman 25-233). The unidirectional fluxes of urate from the chamber in which radioactive urate was originally placed to the opposite chamber were calculated by

$$J_{urate} = \text{cpm collected} \times \text{S.A.}^{-1} \times 1\ \text{cm}^{-2} \times \text{hr}^{-1},$$

where S.A. is the specific activity of urate in the chamber initially containing radioactive urate. The permeability coefficient was calculated by dividing the measured flux of urate by the concentration of urate. The concentration of urate in the chamber initially containing urate did not change significantly throughout the 30-min period.

In order to insure that the radioactive urate was not metabolized to another radioactive product, the chemical concentration of urate in the chamber toward which transport was occurring was determined in some samples by high-performance liquid chromatography with electrochemical detection as previously described (9). The mobile phase buffer was ammonia phosphate (0.05 *M*, pH 4.5). The stationary phase was a reverse phase column (Customsphere 5u OD 5, Custom LC, Inc., Houston, Tex.). Statistical significance was determined by the *t* test for paired data. Results are expressed as mean ± SEM.

Results. To establish that the uric acid remained metabolically unaltered in passage through the gastric mucosa, the chemical concentration was determined directly by high-performance liquid chromatography and this determination was compared to the concentration calculated from the isotope counts. In 15 samples, the calculated to measured concentration of urate was 1.01 ± 0.06. These results suggest that urate remains unchanged in passing from one side of the gastric mucosa to the other.

The fluxes of urate from the serosal to mucosal and from the mucosal to the serosal side of the tissue over a wide range of concentrations of urate are summarized in Table I. As can be seen, the unidirectional fluxes in both directions are a direct function of the initial concentration of urate and no evidence for saturation of the transport process is evident. When expressed as permeability coefficients, the calculated value is the same regardless of the initial concentration. When grouped together, the serosal to mucosal permeability coefficient was 6.5 ± 0.8 × 10^{-10} cm/sec. This value is not significantly different from the mucosal to serosal permeability coefficient of 7.7 ± 0.8 × 10^{-10} cm/sec. Except for the group at 0.18 m*M* from the mucosal to serosal direction, there were no significant differences between subgroups in the electrical measurements.

Discussion. Prior studies have indicated that the renal transport of urate occurs by both a carrier mediated process and by passive diffusion (10). In addition, there have been several prior reports examining transport of urate and other purine analogs across the small intestine of several species (11, 12). Some of these studies have suggested that urate and other purine derivatives are transported by a facilitated mechanism (5, 6). There have not, however, been any reports on the nature of uric acid transport across gastric mucosa. In the kidney, recent evidence would suggest that the transport of urate represents a process of anion exchange (13). In the stomach, the high rates of hydrogen ion secretion and the subsequent generation of hydroxyl ion gradients, make this tissue perhaps a more appropriate one for study of gastrointestinal transport of urate.

Since the possibility exists that radioactive urate could be metabolized, it was necessary to determine that the isotope fluxes truly represented the transport of urate and not a radioactive metabolic product. Over a wide range of concentrations of urate, there was a good correlation between the concentration of urate determined from

TABLE I. SEROSAL–MUCOSAL URATE FLUXES

Urate (m*M*)	*J* (pmole cm^{-2} hr^{-1})	*P* (cm sec^{-1} $\times 10^{-10}$)	P.D. (mV)	ISC (μA)	*R* (Ω cm^2)	*N*
			S → M			
0.018	41.0 ± 8.3	6.3 ± 1.3	36 ± 4	74 ± 9	407 ± 10	4
0.18	428 ± 94	6.6 ± 1.5	32 ± 3	69 ± 7	380 ± 12	5
1.8	4176 ± 1002	6.4 ± 1.5	35 ± 3	76 ± 4	373 ± 33	5
			M → S			
0.018	51.8 ± 7.2	8.0 ± 1.0	33 ± 2	79 ± 4	334 ± 25	5
0.18	535 ± 108	8.3 ± 1.7	25 ± 4	51 ± 1	408 ± 6	4
1.8	4572 ± 1165	6.9 ± 1.7	30 ± 2	70 ± 8	360 ± 24	5

Note. Values represent mean ± SEM; *J* = urate flux; *P* = permeability coefficient; P.D. = potential difference; ISC = short circuit current; *R* = resistance of tissue; *N* = number of experiments.

chemical analysis and that calculated from the isotope counts. These findings suggest that the isotope fluxes represent the transport of urate and not another isotopically labeled compound and that at least under the conditions of study, the gastric mucosa does not metabolize urate.

The p*K* of uric acid is 5.75 and at pH 7.2 is mostly in the ionic form. Because there is an average transepithelial potential difference of 30 mV, there would be a large ionic gradient for urate under nonvoltage clamping conditions. Short circuiting the current, however, permits the study of this tissue without the possible effects of potential difference. Under the conditions of study, at any given concentration of urate, there was no significant difference in the rate of transfer of urate from the serosal to the mucosal and the mucosal to the serosal side of the stomach. Moreover, there was no evidence for uphill transport of urate. Finally, over the range of concentrations used in this study there was no indication of saturation of the urate flux. Thus, it would appear that the only mode of transfer of urate in the gastric mucosa is by passive diffusion.

The diffusion coefficient for urate calculated from the present studies averaged 7.1 $\times$ 10^{-10} cm/sec when all values were pooled. The diffusion coefficient calculated from its passive flux in the proximal tubule of the rat has been estimated to be of the order of 1.5 $\times$ 10^{-7} cm/sec. The small intestine of the rat also has a permeability coefficient of around 1.5 $\times$ 10^{-7} cm/sec (Weinman, unpublished). Thus, in comparison to epithelia studied in other species, the permeability coefficient of urate in the frog gastric mucosa is small, perhaps reflecting the tight junctions of this epithelium. Given the low permeability coefficient and the failure to discern a saturable carrier system, it seems unlikely that the stomach is an important route for either the elimination or the absorption of urate in the frog. No direct data are currently available on the transport of urate across the stomach of a mammalian species. Accordingly, it is not possible to extrapolate the results of the present studies in the amphibian to other species at the present time.

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