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INTESTINAL ABSORPTION AND METABOLISM OF 6-MERCAPTOPURINE IN THE RAT SMALL INTESTINE

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Abstract—The intestinal absorption of 6-mercaptopurine was examined in the rat by *in vitro* and *in situ* techniques for the purpose of establishing absorption characteristics which might explain the poor systemic oral availability of this drug. Experiments were designed to evaluate the significance of intestinal metabolism and active secretion processes. Since mucosal/serosal drug concentration gradients across *in vitro* segments were not significantly different ($P > 0.05$) for everted and noneverted preparations, with values of 1.21 ± 0.27 and 0.91 ± 0.12, respectively, it was concluded that active secretion or absorption mechanisms were absent. Varying the concentration of 6-mercaptopurine from 0.24 to 5.88 mM demonstrated saturability of the biotransformation of 6-mercaptopurine to 6-thiouric acid with a maximum rate of 1.6×10^{-5} mmoles per min per g for jejunal portions. Distal segments displayed 85% higher rates of biotransformation at concentrations of 1.47 mM. Inclusion of allpurinol (2 mM) completely inhibited biotransformation. With *in situ* loops perfused with 1.47 mM drug, collection of mesentery blood showed that absorption rates of 6-thiouric acid were 0.67 that of the parent drug.

6-Mercaptopurine (6-MP) is an analogue of hypoxanthine and has been shown to produce remissions of various types of leukemias. The drug is almost exclusively given by the oral route in the form of 50 mg tablets. Clinical studies in man by Loo *et al.* [1] have shown the drug to exhibit a low extent of absorption following oral administration of tablets. These investigators reported that total recovery of parent drug and its metabolites in the urine was approximately 50% of that by the intravenous route. They postulated that 6-mercaptopurine experiences complications in its gastrointestinal absorption process. Ding and Benet [2] reported systemic availability of 6-mercaptopurine to be 12% following oral administration to rhesus monkeys.

In vitro techniques [3] have suggested that the flavin enzyme xanthine oxidase is present in such tissues as the liver, intestine, spleen, bone marrow, and serum. This enzyme has low specificity since it interacts with numerous substrates including purines, pterins, aldehydes, and pyridine nucleotides. Xanthine oxidase activity in the hamster intestine has been reported to promote the oxidation of hypoxanthine and xanthine to uric acid [4, 5]. After intravenous injection of 6-mercaptopurine to man and rodents, the drug is rapidly metabolized by xanthine oxidase [6, 7] to 6-thiouric acid (6-TUA), an inactive product. When 6-mercaptopurine is coadministered with allopurinol, a xanthine oxidase inhibitor, the dose of mercaptopurine has been recommended to be reduced by 33–50% [8].

Studies in the rat by Sackler [3] demonstrate that two different electrophoretic forms of xanthine oxidase exist, with one form in hepatic tissue and the second in small intestinal tissue. With hypoxanthine as a substrate in the study, the oxidative activity per gram wet weight tissue was 100-fold greater for the intestine. If a similar situation is present in man, it would appear possible that the major site of 6-mercaptopurine biotransformation after oral ingestion may be at the absorption site in the intestinal mucosa.

Xanthine oxidase activity in the rat and hamster small intestine is almost exclusively confined to the columnar epithelial cells [3–5]. Staining techniques regionalize the enzyme to the brush border area of these cells [3]. Thereby, the activity of the enzyme may directly alter the amount of intact drug in the luminal contents.

Studies of hypoxanthine and xanthine absorption across rodent jejunal sacs by Berlin and Hawkins [4, 5] demonstrated 5-fold greater serosal to mucosal flux of purine compared to that in the opposite direction. The postulated purine secretion process was greatly reduced by dinitrophenol, anaerobic conditions, and chilling of buffer solutions. Inclusion of allopurinol in intestinal buffers inhibited the conversion of the xanthines to the uric acid end product but was not found to alter the secretory process or generated mucosal–serosal purine gradients. However, other investigators [9] have been unable to show concentration gradients for purines across rodent intestinal tissue. It was, therefore, of interest to also determine whether or not 6-mercaptopurine is a substrate for an intestinal secretion mechanism since such a mechanism would support observations of low oral availability and poor urinary recovery.

MATERIALS AND METHODS

Preparation of in vitro *intestinal sacs.* In all studies, the male Sprague–Dawley rats weighing 250 g were

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fasted for 24 hr prior to the day of the experiment with water allowed *ad lib*. Under ether anesthesia, the intestine was removed and the animal killed. The whole intestine was thoroughly rinsed and then everted over a glass rod.

Ten-centimeter sacs were prepared in accordance with the method of Wilson and Wiseman [10] or the method of Crane and Wilson [11] as modified by Ravis and Feldman [12]. Segments were assigned numbers and to regional areas. The first 20 cm below the pylorus was termed proximal while the next two 40-cm segments were referred to as midsectional (jejunal) and distal respectively. In studies examining differences between regional segments, care was taken to alter the order of sac preparation. For the preparation of closed sacs, everted or noneverted 10-cm segments were filled with 1 ml of a drug-free buffer or a buffer containing 6-mercaptopurine with the aid of a syringe and incubated in 1 or 4 ml of the same buffer containing 6-mercaptopurine or 6-mercaptopurine with 2 mM allopurinol. Three initial concentrations of 6-mercaptopurine were chosen for the studies. The middle concentration of 1.47 mM corresponds to the expected luminal concentration of 2.5 mg/kg dissolved in 2.5 ml of mucosal buffer. For studies utilizing cannulated sacs, everted segments were hung from glass cannulae and filled with 2 ml of drug-free buffer. Sampling times were chosen to maintain serosal drug concentration less than 10% of mucosal concentration. After a 30-min (closed segments) or a 60-min (cannulated segments) incubation period, samples of the serosal buffer, mucosal buffer, and intestinal tissue were saved.

[^{14}C]- or [^{35}S]-6-Mercaptopurine (Amersham/Searle Corp., Arlington Heights, IL), unlabeled 6-mercaptopurine (Sigma Chemical Co., St. Louis, MO) and allopurinol (Sigma Chemical Co., St. Louis, MO) were dissolved in a Krebs–bicarbonate buffer, pH 7.4, containing 0.2% *d*-glucose. Freshly prepared buffers were bubbled with 95% oxygen–5% carbon dioxide, and the solutions were continuously bubbled with 95% oxygen–5% carbon dioxide during the experiments.

In situ *absorption studies*. *In situ* rat intestinal loops were prepared as described by Kiyasu *et al.* [13] with modification reported by Doluisio *et al.* [14] and Barr and Riegelman [15] for rabbits. Rats weighing 325–375 g were administered pentobarbital by intraperitoneal injection. After complete anesthesia, a midline incision was made, and a midsection of jejunum with a single mesentery vein was isolated by two ligatures. Into the draining mesentery vein a small gauge heparin-treated polyethylene tube was placed with the aid of a syringe needle. Two 10-ml glass syringes were attached by ligated cannulae to either end of an approximate 10-cm intestinal section. A 5-ml buffer containing a 1.47 mM concentration of labeled and unlabeled 6-mercaptopurine was placed into one of the syringes. With gentle pressure the isolated intestinal segment was filled. To assure mixing, the buffer was exchanged between the two syringes every 7.5 min. Care was taken to only slightly distend the intestinal segment during each exchange.

Mesentery blood was collected at 15-min intervals, providing collection of approximately 2 ml of blood. At the time of each mesentery collection, a sample of the mucosal buffer contained in the syringe was obtained. Upon termination of the study, a cardiac blood sample was obtained by puncture to confirm isolation of the *in situ* drug. To assure the integrity of the rat during the procedure, experiments were terminated after 30 min.

From a donor rat, 8 ml of blood was withdrawn by cardiac puncture into a syringe containing 0.5 ml of heparin (1:10,000). The contents of this syringe were periodically infused into the saphenous vein through a polyethylene tube. Infusions were made every 7.5 min to replace the quantity of blood loss via the mesentery vein.

Quantitation of drug and metabolite. Intestinal tissues were weighed, homogenized (Polytron model 125C, Brinkmann Instruments) with methanol and centrifuged, and the supernatant fraction was saved. Samples of mesentery and cardiac blood were collected in heparin-treated syringes or tubes. Blood was then centrifuged to obtain plasma. To 0.3 ml of plasma was added 0.05 ml of 60% trichloroacetic acid, and the samples were again centrifuged. Comparisons between the radioactivities of whole blood samples and plasma water supernatant fluids showed recovery from blood to be 92.4 ± 3.1%.

Levels of drug and metabolite in biological samples were determined by either high pressure liquid chromatography (HPLC) or thin-layer chromatography of ^{35}S or ^{14}C compounds. In experiments with ^{35}S or ^{14}C drug, samples of buffers, tissue and plasma were mixed with 7 ml of a liquid scintillation mixture (PCS, Amersham/Searle Corp.) for determination of total radioactivity. Depending on expected activity, 5-, 10- or 20-μl samples of the buffers, tissue and plasma extracts were spotted on cellulose plates (MN-300 Cellulose Plates, Analtech Labs, Newark, DE). Separation was performed with a 10% isopropyl alcohol–10% ammonium sulfate (1:1) solvent system as described by Loo *et al.* [1]. Compounds were located by ultraviolet illumination and then scraped and mixed with liquid scintillation mixture and water.

For HPLC separations, samples of buffers and tissue extracts were filtered, followed by injection of 10 μl of filtrate into a high pressure liquid chromatograph (model ALC 202, Waters Associates, Milford, MA) equipped with a C-18 column (μBondapak C_{18}, Waters Associates) and a 254 nm fixed wavelength detector (Waters, Associates). The solvent flow rate was 1.2 ml/min with a 0.05 M phosphate buffer, pH 6.6. Theophylline was employed as an internal standard, and drug concentrations were determined from standard curves. Retention times for 6-thiouric acid, allopurinol, theophylline and 6-mercaptopurine were 1.21, 1.47, 2.48 and 3.43 min respectively.

Data evaluation. Significant differences ($P < 0.05$) between levels of drug and metabolite and ratios were determined by one-way analysis of variance and the Tukey method [16].

RESULTS AND DISCUSSION

In the present study, two explanations for the poor systemic availability or orally administered 6-mer-

captopurine were investigated. While "first pass" hepatic metabolism is believed to contribute to the low extent of absorption of this drug, possible intestinal metabolism and secretion may also be involved. Intestinal 6-mercaptopurine biotransformation was first examined by observing drug metabolism by isolated intestinal segments. From *in situ* absorption studies involving sampling of mesentery blood, the significance of metabolism during the absorption process was evaluated. In addition, intestinal secretory mechanisms for purines have been reported [4, 5]. If 6-mercaptopurine is a substrate for these transport systems, the extent of absorption of the drug would be expected to be diminished and possibly dose dependent. The role of transport process in 6-mercaptopurine absorption was investigated by observing drug concentration gradients and transfer rates across *in vitro* rat intestinal segments.

The ability of xanthine oxidase in the rodent intestine to metabolize purine substrates such as hypoxanthine and xanthine has been reported [4, 5, 9]. The present study demonstrates that 6-mercaptopurine was biotransformed by the rat intestine to 6-thiouric acid, a xanthine oxidase product of hepatic metabolism [6, 7]. Thin-layer and liquid chromatographic methods showed the presence of 6-thiouric acid in buffer and tissue specimens following incubation. In everted segment studies, chromatographs of mucosal, serosal and tissue samples suggest the presence of only one intestinal biotransformation pathway with 95.3% of all chromatographed materials represented by either 6-mercaptopurine or 6-thiouric acid.

Three initial incubation concentrations (serosal and mucosal) of 6-mercaptopurine varying over a 25-fold range, were chosen for the purposes of investigating metabolic saturability and mucosal to serosal concentration gradients for parent drug and metabolite. Final percentages of initial radioactivity present as 6-thiouric acid and ratios of metabolite/parent drug of jejunum after 60 min of incubation appear in Table 1. Inspection of Table 1 reveals that, as the drug concentration increased, the final ratio of 6-TUA/6-MP decreased in both the buffers and tissue. Intestinal biotransformation appeared saturable with final percentages of initial radioactivity present as 6-TUA of 39, 11 and 5% for concentrations of 0.24, 1.47 and 5.88 mM respectively. While 6-TUA/6-MP tissue ratios were variable and not significantly different from corresponding ratios for either the serosal or mucosal buffers at two of the three concentrations, significantly larger ratios ($P \leq 0.05$) were noted for the mucosal buffers compared to serosal buffers at all concentrations. This observation would be consistent with the studies of Sackler [3] which localized xanthine oxidase at the brush border area of the mucosal epithelial cells. In contrast, glucuronide conjugation of salicylamide has been reported to occur below the mucosal layer [15].

Small mucosal volumes were initially chosen to enhance the ability to detect metabolite and examine tissue uptake. Since at an initial concentrations of 0.24 mM approximately 63% of the final mucosal radioactivity was metabolite, the volume of the mucosal buffer was increased to 4 ml to prevent substrate limitation of observed metabolic rates in

Table 1. Distribution of 6-mercaptopurine (6-MP) and 6-thiouric acid (6-TUA) across the *in vitro* everted rat intestine* as a function of initial concentration

Initial 6-MP concn† (mM)	Ratio 6-TUA/6-MP			Ratio mucosal/serosal		% Radioactivity	
	Mucosal	Serosal	Tissue	6-MP	6-TUA	6-TUA	6-TUA + 6-MP
0.24	1.714 ± 0.223‡	0.243 ± 0.097	1.400 ± 0.608	0.55 ± 0.13	4.02 ± 0.59	39.1 ± 3.1	95.3 ± 5.5
1.47	0.239 ± 0.022	0.040 ± 0.011	0.269 ± 0.114	0.91 ± 0.07	5.65 ± 2.27	11.2 ± 2.4	97.5 ± 3.4
5.88	0.066 ± 0.005	0.021 ± 0.010	0.135 ± 0.105	1.06 ± 0.05	3.64 ± 1.23	5.3 ± 1.1	99.1 ± 6.1

* Ten-centimeter jejunum segments, 30-min incubation.
† Initial 6-MP concentration of serosal and mucosal solutions (1 ml).
‡ Mean ± S.D.

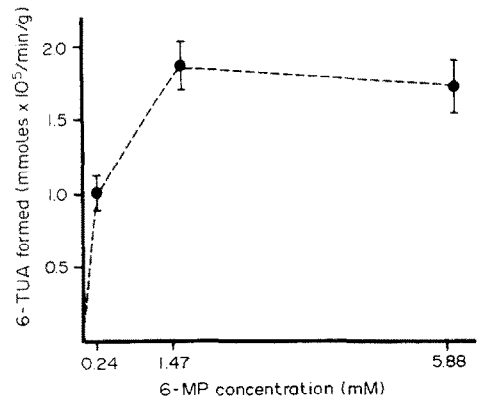

Fig. 1. Formation rate of 6-thiouric acid (6-TUA) by jejunal segments as a function of initial 6-mercaptopurine concentration. Each point represents the mean rate ± S.D. for eight segments.

studies of regional differences in xanthine oxidase activity. A plot of 6-thiouric acid formation rates versus initial 6-mercaptopurine concentration appears in Fig. 1 for jejunal sacs. As illustrated, saturation seems to occur between drug levels of 0.24 and 1.47 mM. The rates of metabolite formation per 10-cm segment as a function of section and concentration appear in Fig. 2. While at the lowest concentration (0.24 mM) no significant differences between segments were observed, at initial levels of 1.47 mM, the distal portion displayed rates 85 and 58% greater than proximal and jejunal sections respectively. Allopurinol was extremely effective in inhibiting 6-mercaptopurine oxidation by xanthine oxidase. With allopurinol (2 mM) included in both mucosal and serosal buffers, levels of 6-thiouric acid were not significantly different from zero as noted by both thin-layer and liquid chromatographic methods.

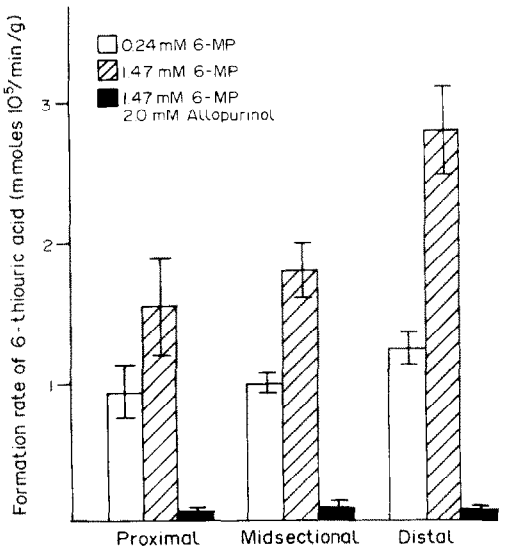

Fig. 2. Rates of 6-thiouric acid formation by the rat small intestine as a function of intestinal region and 6-mercaptopurine concentration. Each bar represents the mean ± S.D. for eight segments.

While observations in differences in xanthine oxidase occurring among sections might suggest differences in the nature and quantity of the enzyme, whole tissue metabolic rates may be influenced and complicated by drug and metabolite tissue uptake and binding. No further evaluation of the kinetics of 6-mercaptopurine intestinal metabolism by whole tissue was attempted since permeability and tissue binding might alter the access of the drug to the metabolic site. Furthermore, interpretation of rate data as a function of substrate concentration is complicated by reports [17] that xanthine oxidase shows substrate inhibition.

Saturable or nonlinear absorption characteristics are expected for agents which transverse the gastrointestinal membrane by carrier mediated absorption mechanisms. Utilizing cannulated intestinal segments from various regions, mucosal to serosal clearance rates were evaluated over a mucosal concentration range of 50-fold (0.03 to 1.5 mM) in the presence of allopurinol. With xanthine oxidase inhibition, loss of drug as a result of metabolism could be prevented, allowing specific examination of mucosal transfer of 6-mercaptopurine. Berlin and Hawkins [4, 5] reported that allopurinol prevented the biotransformation of hypoxanthine and xanthine, but it did not alter purine secretion processes or gradient generating capabilities of the hamster intestine. Cumulative amounts of drug transferred and clearance rates calculated over 60 min are shown in Fig. 3. Clearance values calculated for each 30-min period as well as for the 120-min experiment were not significantly different among the three concentrations chosen. Therefore, no evidence of saturable mucosal to serosal transfer can be reported. Gibaldi and Grundhofer [18, 19] reported *in vitro* intestinal clearance rates of less than 100×10^{-4} ml/min for drugs such as riboflavin and methylene blue which display low mucosal to serosal permeability and are termed as membrane-limited.

Observed average 2-hr clearance values of 54.59×10^{-4} ml/min for 6-mercaptopurine would suggest that this drug also could be classified as displaying low mucosal to serosal transfer. The increases in transfer rates with incubation time may result from the progressive loss of integrity of the mucosal brush border with incubation [18]. Such increases with incubation time would be expected for drugs which are membrane-limited in their gastrointestinal absorption [18].

Intestinal active absorption and secretion mechanisms can be examined by observing the ability of isolated intestinal segments to generate drug mucosal/serosal gradients. Prior incubation of intestinal tissue in buffers of 6-mercaptopurine and allopurinol, followed by preparation of everted and noneverted sacs had the advantage of minimizing effects of tissue equilibrium and thereby allowing better evaluation of drug intestinal concentration gradients and drug uptake. As illustrated in Fig. 4, final mucosal/serosal gradients were 1.21 ± 0.27 and 0.91 ± 0.12 for everted and noneverted segments. No differences were noted between regional areas of the small intestine. For drug gradients across both noneverted and everted mucosal/serosal gradients were not significantly different from one another.

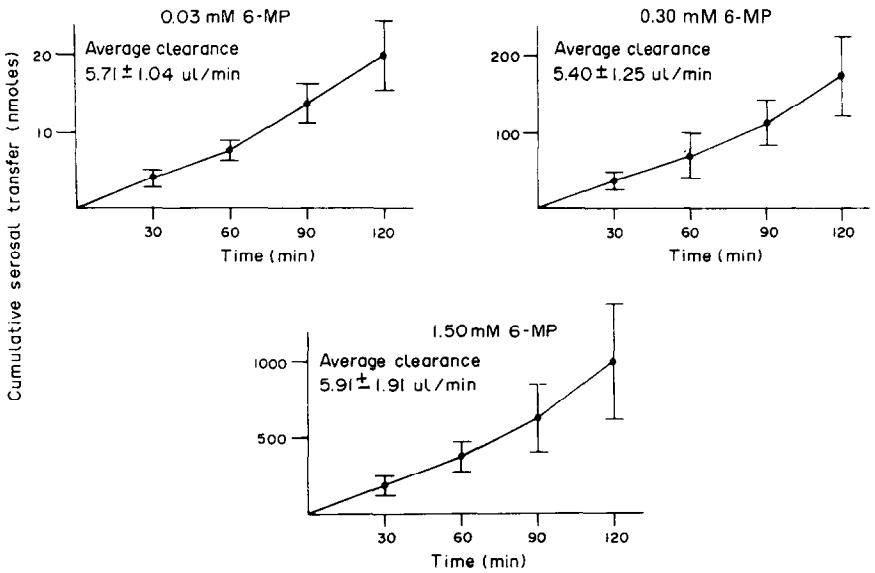

Fig. 3. Cumulative amounts of 6-mercaptopurine transferred across 10-cm cannulated intestinal segments in the presence of 2 mM allopurinol. Each point represents the mean amount ± S.D. for four segments.

indicating that a consistent drug gradient could not be demonstrated. The present results would, in part, support the observations of Khan *et al.* [9] that the rat intestine lacks active secretion or absorption processes for purines.

The utilization of *in vitro* intestinal preparations allows examination of drug metabolism and absorption under controlled conditions. However, extrapolation of results to *in vivo* absorption is not always possible [20, 21]. To better characterize the significance of 6-mercaptopurine intestinal metabolism, an *in situ* method permitting sampling of mesentery blood and luminal buffer was employed. Concentrations of drug and metabolite in the luminal buffer and amounts of each in the mesentery blood drained from *in situ* loops are illustrated as a function of time in Fig. 5. While 60-min experiments were first attempted, despite blood infusion, the condition of the animal was questionable during the last 15 min. For this reason, experiments were terminated after 30 min. Assay of radioactivity of systemic blood obtained by cardiac puncture at the conclusion of the studies demonstrated the isolation of radioactive species from the general circulation. Approximately 40% of all the radioactive species determined in the mesentery blood was the metabolite, 6-thiouric acid. Luminal disappearance rates of parent drug were calculated from the amounts of drug initially contained in syringes minus the final amount. Rates of

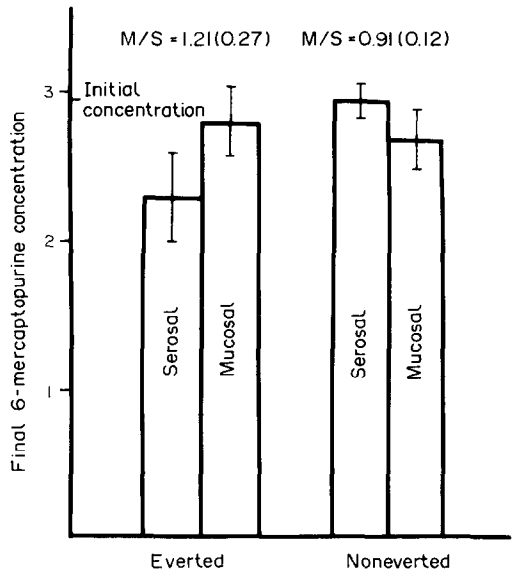

Fig. 4. Final concentrations and concentration gradients of 6-mercaptopurine across everted and noneverted sacs after preincubation in 6-mercaptopurine (2.9 mM) and allopurinol (2 mM). Each bar represents the mean ± S.D. for ten segments.

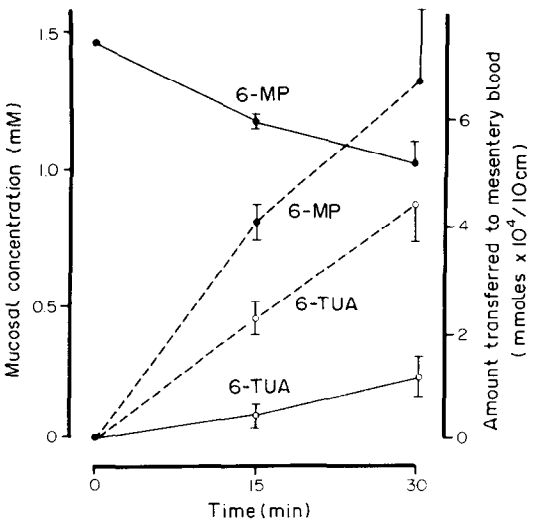

Fig. 5. Mucosal concentration (——) and amount transferred to mesentery (- - -) blood for 6-mercaptopurine (6-MP) and 6-thiouric acid (6-TUA) as a function of time. Each point represents mean ± S.D. of three subjects.

6-mercaptopurine luminal loss as calculated at 30 min were $6.98 \pm 1.23 \times 10^{-5}$ mmoles per 10 cm per min which corresponded to a 28% loss from initial amounts of 6-mercaptopurine. Approximately 37% of this loss could be accounted for by the appearance of 6-thiouric acid in the luminal buffer. Of the remaining mucosal drug loss, 48% was found in the mesentery blood as either parent drug or metabolite. Although drug levels in tissue were not determined, the $12 \pm 7\%$ difference in radioactivity between initial and final amounts is probably associated with the tissue uptake. A comparison of *in situ* and *in vitro* results was not made since *in vitro* mucosal to serosal transfer studies were performed with allopurinol present in buffers.

The *in situ* studies demonstrate that 6-mercaptopurine underwent substantial biotransformation during the absorption process. Previous studies with monkeys and humans [2, 22] have suggested that the low availability of the drug may be a consequence of presystemic hepatic and/or intestinal mucosal metabolism during the drug transfer across the intestinal wall and sequent passage through the liver from the portal circulation. The systemic availability, F, of an orally administered drug can be viewed as the product of separate extents of absorption for luminal uptake, intestinal wall transfer, and hepatic "first pass" metabolism. In this case, F equals $F_{GI} \times F_{IW} \times F_{HFP}$ where F_{GI}, F_{IW}, and F_{HFP} are the fractions of the drug which will pass from the lumen into the intestinal wall, from the intestinal wall to the mesentery veins, and from the portal system to the systemic circulation respectively. The greater the value of F_{IW} (fraction lost by intestinal metabolism), the smaller the percentage of the original oral dose which will be metabolized by hepatic "first pass". Should the human intestinal mucosa have similar features and abilities for metabolizing 6-mercaptopurine as the rat, factors such as dosage form, dosage regimen, and intestinal transit may influence the bioavailability of this drug. Considering the saturable nature and site specificity of the metabolism in the intestine, improved systemic availability would be favored by dosage forms of the drug which rapidly release in the upper gastrointestinal tract. In addition, availability may be different if allopurinol is co-administered with 6-mercaptopurine as compared with administration of each on separate occasions. Further studies are needed to evaluate the significance of intestinal metabolism of this drug in humans and its importance relative to "first pass" hepatic metabolism.

In summary, investigations for a possible active secretion or absorption mechanism for 6-mercaptopurine in the rat small intestine failed to provide evidence for either process. *In vitro* and *in situ* examination of the absorption of the drug demonstrated that 6-mercaptopurine was metabolized by the rat small intestine to an extent that mesentery appearance rates of 6-thiouric acid were approximately two-thirds that of parent drug. Intestinal biotransformation to 6-thiouric acid was prevented by the inclusion of allopurinol in buffer solutions. In the rat, saturation was apparent over the 25-fold range of drug concentrations chosen. Should intestinal xanthine oxidase activity in human intestinal tissue be similar to that noted in the present studies with the rat, low extents of absorption following oral administration would be predicted as has been reported previously [1, 22].

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REFERENCES

1. T. L. Loo, J. K. Luce, M. P. Sullivan and E. Frei, *Clin. Pharmac. Ther.* **9**, 180 (1968).
2. T. L. Ding and L. Z. Benet, *Drug Metab. Dispos.* **7**, 373 (1979).
3. M. L. Sackler, *J. Histochem. Cytochem.* **11**, 326 (1969).
4. R. P. Berlin and R. A. Hawkins, *Am. J. Physiol.* **215**, 932 (1968).
5. R. P. Berlin and R. A. Hawkins, *Am. J. Physiol.* **215**, 942 (1968).
6. G. B. Elion, S. Bieker and G. H. Hitchings, *Ann. N.Y. Acad. Sci.* **60**, 297 (1954).
7. L. Hamilton and G. Elion, *Ann. N.Y. Acad. Sci.* **60**, 304 (1954).
8. *A.M.A. Drug Evaluations*, p. 1175. Publishing Science Group, Littleton, MA (1980).
9. A. H. Khan, S. Wilson and J. D. Crawhell, *Can. J. Physiol. Pharmac.* **53**, 113 (1975).
10. T. H. Wilson and G. Wiseman, *J. Physiol., Lond.* **123**, 116 (1954).
11. R. K. Crane and T. H. Wilson, *J. appl. Physiol.* **42**, 145 (1958).
12. W. R. Ravis and S. Feldman, *J. pharm. Sci.* **68**, 945 (1979).
13. J. T. Kiyasu, J. Katz and I. L. Chaikoff, *J. biol. Chem.* **224**, 935 (1957).
14. J. T. Doluisio, N. F. Billups, L. W. Dittert, E. J. Sugita and J. V. Swintosky, *J. pharm. Sci.* **58**, 1196 (1969).
15. W. H. Barr and S. Riegelman, *J. pharm. Sci.* **59**, 154 (1970).
16. B. J. Winer, *Statistical Principles in Experimental Design*, p. 198. McGraw-Hill, New York (1966).
17. B. J. Hofstee, *J. biol. Chem.* **216**, 235 (1955).
18. M. Gibaldi and B. Grundhofer, *Proc. Soc. exp. Biol. Med.* **141**, 546 (1972).
19. M. Gibaldi and B. Grundhofer, *J. pharm. Sci.* **61**, 116 (1972).
20. R. R. Levine, W. F. McWary, P. J. Korngrith and R. LeBlanc, *Eur. J. Pharmac.* **9**, 211 (1978).
21. D. L. Wolfe, S. C. Forland and L. Z. Benet, *J. pharm. Sci.* **62**, 200 (1973).
22. S. Zimm, J. M. Collins, R. Riccardi, D. O'Neill, P. K. Narang, B. Chabner and D. G. Poplack, *New Engl. J. Med.* **308**, 1005 (1983).