

[Chem. Pharm. Bull.]
34(10)4265—4272(1986)

Intestinal Absorption Characteristics of 5-Fluorouracil, Ftorafur and 6-Mercaptopurine in Rats

HITOSHI SASAKI, JUNZO NAKAMURA, RYOJI KONISHI
and JUICHIRO SHIBASAKI*

Faculty of Pharmaceutical Sciences, Nagasaki University,
Bunkyo-machi 1-14, Nagasaki 852, Japan

(Received March 8, 1986)

The absorption characteristics of three anticancer antimetabolites, 5-fluorouracil (5-FU), ftorafur and 6-mercaptopurine (6-MP), were investigated by *in vitro* and *in situ* absorption techniques in rats. Absorption of a nucleic acid, uracil, was also investigated. In the *in situ* experiment, the disappearance ratios of uracil, 5-FU and 6-MP from the lumen showed a dependency on initial drug concentration, although ftorafur was absorbed at a constant rate regardless of the initial concentration. At low concentrations, Michaelis-Menten type processes were involved in the disappearance of uracil, 5-FU and 6-MP as judged from reciprocal plots. No metabolite of uracil, 5-FU or ftorafur was detected in the experiments. Most of the 6-MP that was lost from the lumen appeared as its metabolite, 6-thiouric acid, in the lumen. The *in vitro* data supported the results of the *in situ* experiment. In particular, uracil and 5-FU showed the ability to permeate at low concentrations against a concentration gradient. A small serosal transfer of 6-MP and the selective appearance of 6-thiouric acid on the mucosal side were confirmed. Metabolic inhibitors, 2,4-dinitrophenol and ouabain, strongly depressed the disappearance and transfer of uracil and 5-FU, but not ftorafur. The absorption and metabolism of 6-MP were affected by 2,4-dinitrophenol, but not ouabain.

Keywords—antimetabolite; 5-fluorouracil; ftorafur; 6-mercaptopurine; intestinal absorption; *in vitro* technique; *in situ* technique; intestinal metabolism; 2,4-dinitrophenol; ouabain

Antimetabolites have been widely used in cancer chemotherapy because these drugs inhibit the growth of cancer cells selectively; analogs of nucleic acids are examples.¹⁾ The similarity of these compounds to biological components often results in complex behavior as regards absorption, metabolism and uptake by cells.²⁾ 5-Fluorouracil (5-FU) and 6-mercaptopurine (6-MP) are typical pyrimidine and purine analogs, respectively, and are mainly used as oral dosage forms.³⁾ Ftorafur was developed as a lipophilic masked derivative of 5-FU to improve the absorbability and retention of effect after oral administration.⁴⁾ Assessment of the absorption characteristics is important for the design of an effective administration regimen and the development of suitable dosage forms because absorption is the first step in the drug action.

Therefore, in the present study, the absorption characteristics of uracil, 5-FU, ftorafur and 6-MP were investigated by means of *in vitro* and *in situ* absorption techniques in rats.

Experimental

Materials—Uracil and 5-FU were supplied by Kyowa Hakko Kogyo Co. Ftorafur and 6-MP were supplied by Taiho Yakuin Co. and Sankyo Company, Ltd., respectively. Authentic 6-thiouric acid, which is a metabolite of 6-MP, was prepared and purified as described by Elion *et al.*⁵⁾ All other chemicals were of reagent grade.

Detection of Metabolite by Thin Layer Chromatography (TLC)—*In situ* and *in vitro* perfusate at the end of an experiment was lyophilized and the residue was dissolved in a little acetone. An aliquot of the acetone solution was applied to a TLC plate (Kieselgel 60 HF₂₅₄, Merck) and developed with the following developers: for pyrimidines, (1)

ethyl acetate : acetone : water = 30 : 25 : 5, (2) ethyl acetate : formic acid : water = 65 : 5 : 5, (3) *n*-butanol : acetic acid : water = 50 : 5 : 15; for purine, (4) acetonitrile : dil. NH_4OH = 7 : 3, (5) *n*-butanol : benzene : water = 2 : 1 : 1.

***In Situ* Absorption Experiment**—*In situ* absorption was carried out according to Kakemi *et al.*⁶⁾ Male Wistar albino rats (150–200 g) were fasted overnight and allowed free access to water. Animals were anesthetized with pentobarbital, given intraperitoneally. A midline incision was made, then the intestine was cannulated at the duodenal and ileal ends, and the bile duct was ligated. The intestine was replaced in the abdomen and the incision was closed. The luminal side of the intestine was washed with warm saline and drug solution prior to recirculation. Drug solution (50 ml), maintained at 37 °C, was passed through the intestine, from the pylorus to the ileocecal junction, by using an infusion pump at a rate of 10 ml/min for 90 min. The perfusate was an isotonic pH 7.2 buffer (NaCl 145 mM, KCl 4.56 mM, CaCl_2 1.25 mM, Na_2HPO_4 1.33 mM, NaH_2PO_4 0.33 mM) containing various amounts of drug and phenol red as an indicator of water movement, as reported by Schanker and Tocco.⁷⁾ Rectal temperature was maintained at 37 ± 1 °C. After recirculation for 90 min, the sample solution was withdrawn. The concentrations of the drug and phenol red in samples were determined spectrophotometrically. The disappearance of the drug was corrected based on the change of phenol red concentration.

***In Vitro* Absorption Experiment**—*In vitro* intestinal everted sacs were prepared as reported by Wilson and Wiseman.⁸⁾ Rats (160–230 g) were treated as in the *in situ* absorption experiments. Krebs–Ringer bicarbonate solution (pH 7.4) containing 0.1% glucose was used as the incubation medium. The small intestine was washed with buffer solution, maintained at 37 °C, and isolated. An intestinal segment (jejunum, 25 cm) was everted with a wire so that the mucosal surface was on the outside, ligated at both ends, and filled with 3 ml of pH 7.4 buffer solution in the serosal lumen. Immediately after ligation, each everted sac was placed in a centrifuge tube containing 10 ml of drug solution at various concentrations and incubated for 60 min at 37 °C. The drug solution was saturated with 95% O_2 and 5% CO_2 . The amount of drug that disappeared from the incubation medium was calculated as the difference in concentration between the initial solution and final solution. At the end of the incubation period, the buffer volumes on the serosal and mucosal sides were determined.

In the experiment on inhibitor effect, 2,4-dinitrophenol (DNP) or ouabain was contained in both the mucosal and serosal solutions at a concentration of 0.2 or 2.0 mM, respectively.

Analytical Methods—Uracil and 5-FU were assayed by the spectrophotometric method reported by Schanker and Tocco.⁷⁾ Samples were diluted with 0.1 M HCl and washed with ethyl acetate, then the drug concentration in the aqueous layer was determined from the absorbance difference at 260 nm and 280 nm. Ftorafur was also assayed spectrophotometrically. The sample was extracted with chloroform in pH 5.2 acetate buffer (0.1 M) and the organic layer was re-extracted with pH 11.0 carbonate buffer (0.1 M). The drug concentration in the aqueous phase was determined spectrophotometrically at 270 nm.

Separate assays of 6-MP and 6-thiouric acid were carried out by spectrophotometric determination at 327 nm and 355 nm in the aqueous phase after washing with ethyl acetate. The concentrations were calculated by means of the following two equations.

$$\text{absorbance at 327 nm} = E_1 \times C_1 + E_2 \times C_2$$

$$\text{absorbance at 355 nm} = E_3 \times C_1 + E_4 \times C_2$$

C_1 and C_2 are the concentrations of 6-MP and 6-thiouric acid, respectively, and E_1 – E_4 are molar absorption coefficients determined from calibration curves of 6-MP and 6-thiouric acid at 327 nm and 355 nm, respectively.

In the experiment on inhibitor effect, DNP was removed from samples by extraction with toluene since it interfered with the determination. Ouabain did not affect these assays.

Phenol red, used as an indicator of water movement in the *in situ* experiment, was assayed at 550 nm after sufficient dilution of the sample with 1.0 M NaOH. This dye did not interfere with the above assays.

Calculation of Apparent Kinetic Parameters—Calculation of apparent parameters *in situ* was carried out with a microcomputer by the simplex method using the nonlinear least-squares program reported by Yamaoka and Nakagawa.⁹⁾

Results

Detection of Metabolite

6-Thiouric acid was formed as a metabolite of 6-MP in the *in situ* and *in vitro* experiments, although no metabolite of uracil, 5-FU or ftorafur was detected by TLC analysis.

In Situ Absorption Experiment

The effects of initial drug concentration on the disappearance of compounds and the appearance of the metabolite in the lumen *in situ* are shown in Fig. 1 (A: uracil, 5-FU and

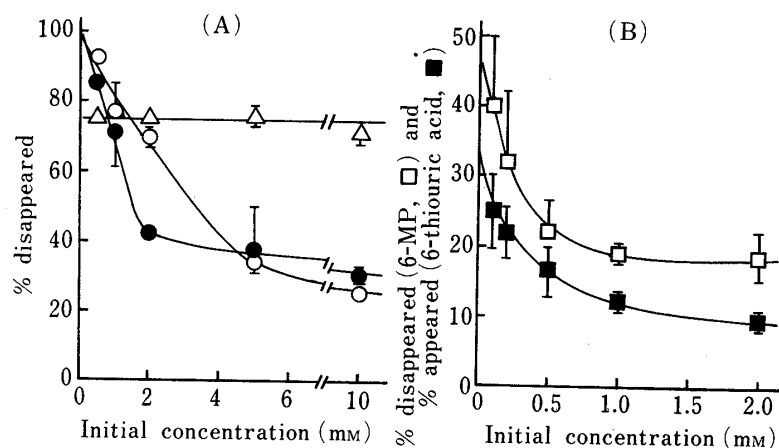


Fig. 1. Effect of Initial Concentration on the Disappearance of Uracil, 5-FU, Ftorafur and 6-MP from the Lumen, and Appearance of 6-Thiouric Acid in the Lumen *in Situ*

(A) ○, uracil; ●, 5-FU; △, ftorafur. (B) □, 6-MP disappearance; ■, 6-thiouric acid appearance. Each point represents the mean \pm S.E. of at least four experiments.

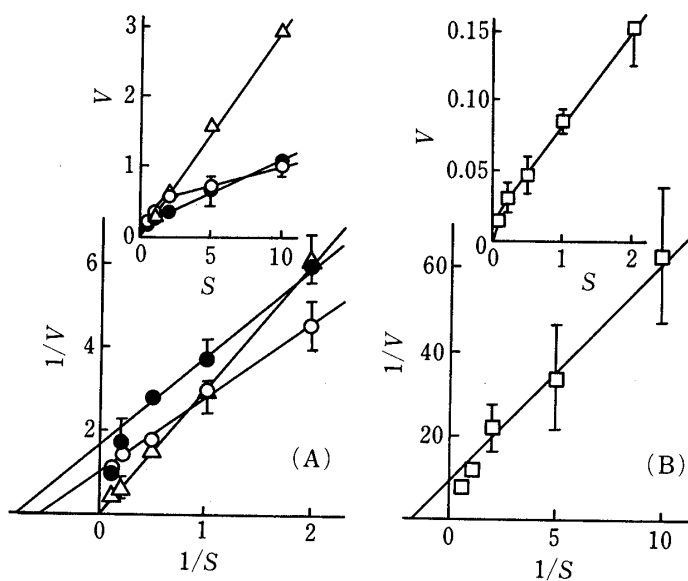


Fig. 2. Lineweaver-Burk Plot of the Disappearance Data *in Situ*

(A) ○, uracil; ●, 5-FU; △, ftorafur. (B) □, 6-MP. S : Initial concentration (mm). V : Disappearance velocity (mm/h/100 g). Each point represents the mean \pm S.E. of at least four experiments.

ftorafur. B: 6-MP and 6-thiouric acid). The absorption ratios of uracil and 5-FU were dependent on the initial drug concentration. At low concentration, uracil and 5-FU were mostly absorbed, and increase of the drug concentration decreased the disappearance. However, ftorafur was absorbed to a constant extent, regardless of concentration. The disappearance ratio of 6-MP was also dependent on initial drug concentration and most the 6-MP lost appeared in the lumen as 6-thiouric acid.

From these results, the velocities of disappearance in 1 h per 100 g body weight were calculated. The reciprocals of the velocity and initial drug concentrations in the perfusate were plotted (Lineweaver-Burk plot) as shown in Fig. 2 (A: uracil, 5-FU and ftorafur. B: 6-MP). Ftorafur gave a straight line passing through the origin, while the plots for uracil, 5-FU and 6-MP were linear with intercepts at low concentrations. The plots of the velocities *versus* initial drug concentrations are included in Fig. 2.

Kinetic Analysis

The *in situ* absorption data were analyzed kinetically. The apparent parameters of disappearance of each compound from the lumen were calculated by using the following equations:

$$V = K \times S \quad (1)$$

$$V = K \times S + (V_{\max} \times S)/(K_m + S) \quad (2)$$

Equation 1 was used for ftorafur and Eq. 2 was used for uracil, 5-FU and 6-MP; V , S and K are the disappearance velocity in 1 h per 100 g body weight, the initial drug amount in the perfusate, and the passive diffusion constant, respectively. K_m and V_{\max} are the Michaelis constant and maximum disappearance velocity in the particular absorption process. The computer-assessed kinetic parameters of uracil, 5-FU, ftorafur and 6-MP are summarized in Table I. The values of K_m and V_{\max} of 5-FU were lower than those of uracil. 6-MP had remarkably small values of K_m and V_{\max} . The lipophilic masked compound, ftorafur, showed a higher passive absorption constant than the parent drug, 5-FU.

In Vitro Absorption Experiment

The *in vitro* results are expressed as drug disappearance from the mucosal side and the ratio of drug concentration on the serosal side against that on the mucosal side (S/M ratio). During the *in vitro* absorption experiment, no marked change of the serosal and mucosal volumes was observed.

The effects of mucosal initial concentration on the disappearance and S/M ratio of uracil

TABLE I. Computer-Assessed Kinetic Parameters of the Absorption Processes of Antimetabolites

Substance	K_m (mM)	V_{\max} (mm/h/100 g)	K (1/h/100 g)
Uracil	1.06	0.688	0.038
5-FU	0.26	0.204	0.093
Ftorafur	—	—	0.305
6-MP	0.047	0.015	0.069

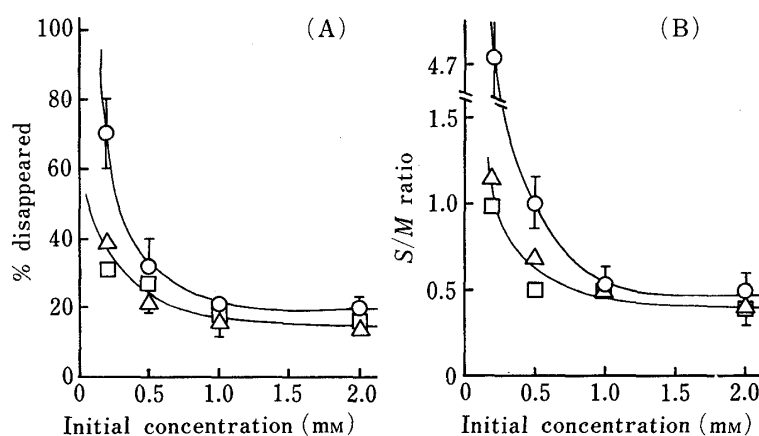


Fig. 3. Effect of Initial Concentration on the Disappearance from the Mucosal Side and the S/M Ratio of Uracil with or without Inhibitor *in Vitro*

(A) Disappearance ○, uracil alone; △, with DNP; □, with ouabain. (B) S/M Ratio ○, uracil alone; △, with DNP; □, with ouabain. Each point represents the mean \pm S.E. of at least three experiments except in some cases (two experiments; 0.2 and 0.5 mM with DNP and with ouabain).

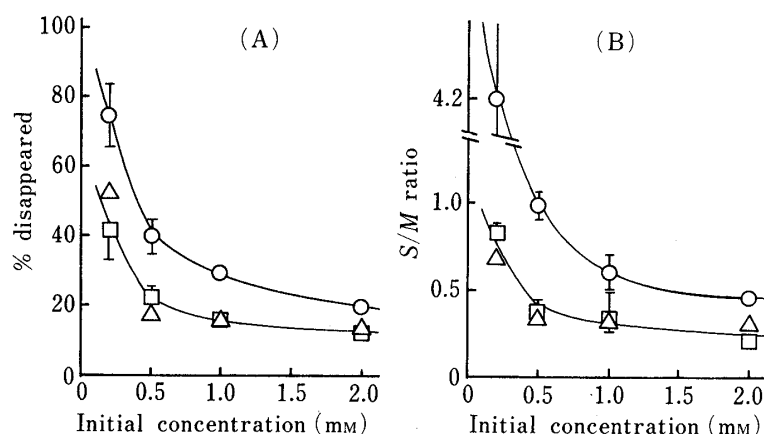


Fig. 4. Effect of Initial Concentration on the Disappearance from the Mucosal Side and the S/M Ratio of 5-FU with or without Inhibitor *in Vitro*

(A) Disappearance \circ , 5-FU alone; \triangle , with DNP; \square , with ouabain. (B) S/M Ratio \circ , 5-FU alone; \triangle , with DNP; \square , with ouabain. Each point represents the mean \pm S.E. of at least three experiments except in some cases (two experiments; 0.2, 0.5 and 1.0 mM with DNP and with ouabain).

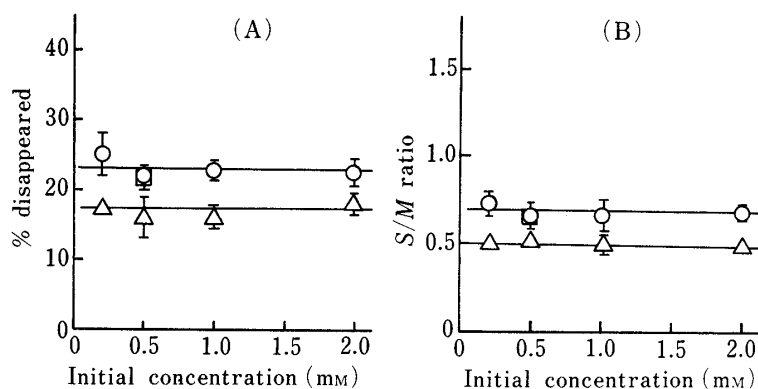


Fig. 5. Effect of Initial Concentration on the Disappearance from the Mucosal Side and the S/M Ratio of Ftorafur with or without Inhibitor *in Vitro*

(A) Disappearance \circ , ftorafur alone; \triangle , with DNP; \square , with ouabain. (B) S/M Ratio \circ , ftorafur alone; \triangle , with DNP; \square , with ouabain. Each point represents the mean \pm S.E. of at least three experiments except in some cases (two experiments; 0.2 and 0.5 mM with DNP and with ouabain).

with or without inhibitors are shown in Fig. 3 (A, B). Disappearance of uracil from the mucosal side was dependent on its initial concentration, and its S/M ratio was over 1 at 0.2 mM. Coadministration of DNP or ouabain with uracil depressed the disappearance and the ratio.

Figure 4 (A, B) shows the *in vitro* absorption behavior of 5-FU. 5-FU also showed dependence of the disappearance on mucosal drug concentration and gave a high S/M ratio at lower concentrations. The metabolic inhibitors depressed the permeation of the drug.

In contrast, ftorafur disappeared at a constant rate regardless of the initial concentration. The S/M ratio was constant below 1 as shown in Fig. 5 (A, B). Ouabain did not inhibit the absorption of 0.5 mM ftorafur, although DNP showed a slight but constant inhibition regardless of the initial concentration.

The disappearance and S/M ratio of a purine compound, 6-MP, are presented in Fig. 6 (A, B). 6-MP disappearance depended on the initial concentration, and the S/M ratio was small. Ouabain affected the behavior of 6-MP slightly. DNP increased the S/M ratio, although it decreased the disappearance from the mucosal side.

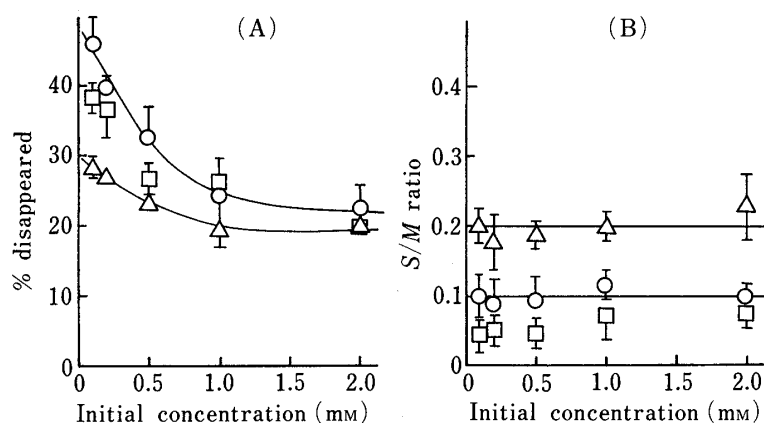


Fig. 6. Effect of Initial Concentration on the Disappearance from the Mucosal Side and the S/M Ratio of 6-MP with or without Inhibitor *in Vitro*

(A) Disappearance \circ , 6-MP alone; \triangle , with DNP; \square , with ouabain. (B) S/M Ratio \circ , 6-MP alone; \triangle , with DNP; \square , with ouabain. Each point represents the mean \pm S.E. of at least three experiments.

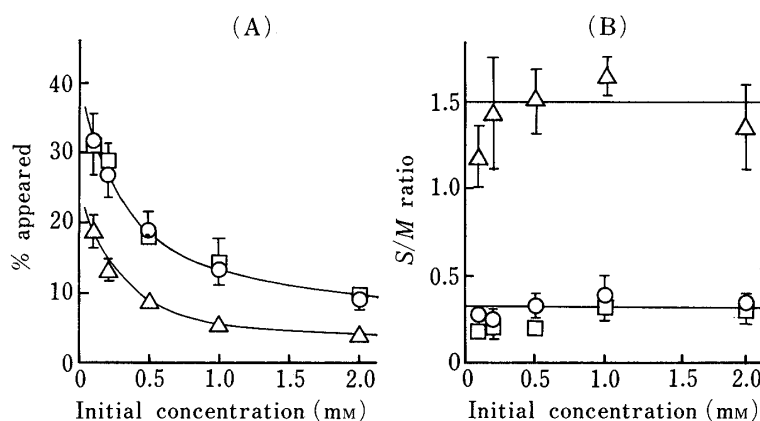


Fig. 7. Effect of Initial Concentration of 6-MP on Appearance on the Mucosal Side and the S/M Ratio of 6-Thiouric Acid with or without Inhibitor *in Vitro*

(A) Appearance \circ , 6-MP alone; \triangle , with DNP; \square , with ouabain. (B) S/M Ratio \circ , 6-MP alone; \triangle , with DNP; \square , with ouabain. Each point represents the mean \pm S.E. of at least three experiments.

The behavior of 6-thiouric acid in the same experiment is shown in Fig. 7 (A, B). 6-Thiouric acid appearance on the mucosal side was also dependent on the initial concentration of 6-MP. Most of the lost 6-MP appeared on the mucosal side as its metabolite, 6-thiouric acid, especially at low initial concentrations. The S/M ratio was small. Ouabain had no influence on the behavior of 6-thiouric acid. DNP suppressed the production of 6-thiouric acid.

Discussion

Generally, the absorption behavior of most drugs is consistent with the pH-partition theory. Furthermore it is well known that some substances are absorbed by specific mechanisms. The existence of active transport was reported for purine, pyrimidine and their analogs.¹⁰⁾ However, few comparative studies on the absorption characteristics using both *in vitro* and *in situ* techniques have been done. In this work, we investigated the absorption processes of uracil, 5-FU, ftorafur and 6-MP.

The synthetic pyrimidine analog, 5-FU, has been used clinically for the treatment of

carcinoma of the breast and gastrointestinal tract.¹¹⁾ Schanker *et al.*¹⁰⁾ and Muranishi *et al.*¹²⁾ demonstrated that 5-FU crossed the small-intestinal epithelium by active transport as well as passive diffusion. In the present study, the absorptions of uracil and 5-FU showed a dependency on drug concentration (Fig. 1A). The reciprocal plot was linear at lower concentrations with a positive intercept, suggesting that the absorption occurs by a Michaelis–Menten type process (Fig. 2A). The values of K_m and V_{max} of 5-FU absorption were slightly smaller than those of uracil absorption (Table I).

The dependency of the absorption ratio of uracil or 5-FU on initial drug concentration was supported by the *in vitro* experiment (Fig. 3A and Fig. 4A). Especially at low concentrations, uracil and 5-FU were transferred from the mucosa to the serosa against a concentration gradient, suggesting that active transport occurs (Fig. 3B and Fig. 4B). Active transport is usually affected by various chemical substances.¹³⁾ Therefore, the effects of DNP and ouabain were investigated (Fig. 3A, B and Fig. 4A, B). DNP is an uncoupler of the respiratory chain and oxidative phosphorylation, and ouabain is an inhibitor of ATPase. Both inhibitors reduced the disappearance from the mucosal side as well as the S/M ratio for uracil and 5-FU, especially at low initial concentrations. The S/M ratio at 0.2 mm was not higher than about 1. Therefore, active transport is the predominant mode of absorption at low concentrations of these compounds, whereas passive diffusion predominates at high concentrations, at which the active process is saturated. These *in vitro* and *in situ* results are consistent with the previous results.^{10,12)}

Ftorafur was developed as a lipophilic masked derivative of 5-FU for oral use, and shows significant activity against several adenocarcinomas with lower myelotoxicity than 5-FU.¹⁴⁾ Few data are available on the absorption of ftorafur, although the mechanism was considered to be passive diffusion because of the good drug absorbability *in vivo*.¹⁵⁾ In this absorption study, ftorafur was absorbed at a constant rate irrespective of the initial concentration, *in situ* and *in vitro* (Fig. 1A and Fig. 5A). The *in situ* reciprocal plot was linear, passing through the origin (Fig. 2A). The *in vitro* S/M ratio was also constant, below 1 (Fig. 5B). Ouabain did not inhibit the absorption of 0.2 mm ftorafur. DNP showed slight inhibition, but with no concentration dependency. These *in vitro* and *in situ* results support the view that ftorafur was absorbed by passive diffusion. The passive diffusion constant, determined from the *in situ* results, was higher than that of 5-FU (Table I), suggesting that the lipophilic prodrug approach is useful for improving drug absorbability. We have also reported several examples of a lipophilic prodrug approach.¹⁶⁾

Using the same techniques, 6-MP, a purine-type antimetabolite was evaluated. 6-MP is used clinically against various leukemias and is administered orally, though little work has been done on its absorption.¹⁷⁾ In the present study, the disappearance of 6-MP was not large, and was dependent on the initial concentration (Fig. 1B). The reciprocal plot indicated that the disappearance at the lower concentrations followed Michaelis–Menten type kinetics (Fig. 2B). However, the absorption constants of 6-MP, K_m , V_{max} and K , were small in comparison with those of pyrimidines (Table I). The dependence of 6-MP disappearance on initial concentration and the small S/M ratio were supported by the *in vitro* results.

6-MP is normally metabolized to thiouric acid by xanthine oxidase *in vivo*.¹⁸⁾ In fact, a large part of the lost 6-MP appeared in the lumen as 6-thiouric acid both *in vitro* and *in situ* (Fig. 1A and Fig. 7). These results indicate that the intestinal first-pass metabolism of 6-MP will be large after oral administration. Sackler¹⁹⁾ demonstrated that the oxidative activity toward hypoxanthine per gram wet weight of tissue was 100 fold greater in the intestine than in hepatic tissue. Ding and Benet²⁰⁾ reported the systemic availability of 6-MP after oral administration to be 12% in rhesus monkey.

The appearance of 6-thiouric acid on the mucosal side was greater than that on the serosal side (Fig. 7B). No conversion of 6-MP to 6-thiouric acid in the perfusate was observed,

and authentic 6-thiouric acid was hardly absorbed *in vitro* or *in situ* (data not shown). Thus, the oxidative enzyme may directly convert luminal 6-MP to 6-thiouric acid. Sackler¹⁹⁾ reported that xanthine oxidase is localized at the brush border area of the mucosal epithelial cells. Consequently, DNP could decrease both the production of 6-thiouric acid and the disappearance of 6-MP in the lumen by inhibition of the enzyme in the brush border area (Figs. 6 and 7). However, there is another possibility. Berlin and Hawkins²¹⁾ demonstrated that a purine secretion system exists in rodent intestine, and this process was greatly reduced by DNP. The metabolite of 6-MP, 6-thiouric acid, might be a substrate for the intestinal secretion mechanism. Yasuhara *et al.*²²⁾ reported that a metabolite of *p*-aminobenzoic acid in the intestinal tissue is released selectively on the luminal side. Although further work is necessary, the present results should contribute to the design of oral dosage forms of anticancer antimetabolites.

References

- 1) C. Heidelberger, *Annu. Rev. Pharmacol.*, **7**, 101 (1967); G. B. Grindey, *Cancer Treat. Rev.*, **6**, 19 (1979).
- 2) C. Benz and E. Cadman, *Cancer Res.*, **41**, 994 (1981); R. I. Walker, W. L. Horvath, W. S. Rule, J. C. Herion and J. G. Palmer, *Cancer Res.*, **33**, 755 (1973); S. Fujii, K. Ikenaka, M. Fukushima and T. Shirasaka, *Gann*, **69**, 763 (1978).
- 3) G. B. Elion, E. Burgi and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952); R. Duschinsky, E. Plevan and C. Heidelberger, *J. Am. Chem. Soc.*, **79**, 4559 (1957).
- 4) S. Hillers, R. A. Zhuk and M. Lidaks, *Dokl. Akad. Nauk SSSR*, **176**, 332 (1967).
- 5) G. B. Elion, S. Mueller and G. H. Hitchings, *J. Am. Chem. Soc.*, **81**, 3042 (1959).
- 6) T. Koizumi, T. Arita and K. Kakemi, *Chem. Pharm. Bull.*, **12**, 421 (1964).
- 7) L. S. Schanker and D. J. Tocco, *J. Pharmacol. Exp. Ther.*, **128**, 115 (1960).
- 8) T. H. Wilson and G. Wiseman, *J. Physiol. (London)*, **123**, 116 (1954).
- 9) K. Yamaoka and T. Nakagawa, *J. Pharmacobio-Dyn.*, **6**, 595 (1983).
- 10) L. S. Schanker and D. J. Tocco, *Biochim. Biophys. Acta*, **56**, 469 (1962); L. S. Schanker and J. J. Jeffrey, *Biochem. Pharmacol.*, **11**, 961 (1962); R. D. Berlin, *Science*, **168**, 1539 (1970).
- 11) C. Heidelberger and F. J. Ansfield, *Cancer Res.*, **23**, 1226 (1963).
- 12) S. Muranishi, H. Yoshikawa and H. Sezaki, *J. Pharmacobio-Dyn.*, **2**, 286 (1979).
- 13) A. Begleiter, H-Y. P. Lam, J. Grover, E. Froese and G. J. Goldenberg, *Cancer Res.*, **39**, 353 (1979); C. F. George and B. S. Gruchy, *J. Pharm. Pharmacol.*, **31**, 643 (1979).
- 14) J. A. Benvenuto, K. Lu, S. W. Hall, R. S. Benjamin and T. L. Loo, *Cancer Res.*, **38**, 3867 (1978).
- 15) S. Fujii, H. Okuda, H. Toide, N. Watanabe, K. Taira and S. Hashimoto, *Oyo Yakuri*, **8**, 589 (1974).
- 16) H. Sasaki, T. Kakutani, M. Hashida and H. Sezaki, *J. Pharm. Pharmacol.*, **37**, 461 (1985); H. Sasaki, T. Takahashi, J. Nakamura, R. Konishi and J. Shibasaki, *J. Pharm. Sci.*, **75**, 676 (1986).
- 17) W. R. Ravis, J. S. Wang and S. Feldman, *Biochem. Pharmacol.*, **33**, 443 (1984).
- 18) G. B. Elion, S. Bieber and G. H. Hitchings, *Ann. N. Y. Acad. Sci.*, **60**, 297 (1954); E. J. Sarcione and L. Stutzman, *Cancer Res.*, **20**, 387 (1960).
- 19) M. L. Sackler, *J. Histochem. Cytochem.*, **11**, 326 (1969).
- 20) T. L. Ding and L. Z. Benet, *Drug Metab. Dispos.*, **7**, 373 (1979).
- 21) R. P. Berlin and R. A. Hawkins, *Am. J. Physiol.*, **215**, 932 (1968); R. P. Berlin and R. A. Hawkins, *Am. J. Physiol.*, **215**, 942 (1968).
- 22) M. Yasuhara, H. Kobayashi, Y. Kurosaki, T. Kimura, S. Muranishi and H. Sezaki, *J. Pharmacobio-Dyn.*, **2**, 177 (1979).