Br. J. Pharmac. (1982), **77**, 413–417

THE KINETICS OF UPTAKE OF 5-FLUOROURACIL BY RAT LIVER

LORNA S.F. HSU

Toxicology Laboratory, St Stephen's Hospital, Fulham Road, London SW10 9TH

1 Slices of rat liver weighing 15–30 mg were incubated at 37°C in 5-fluorouracil (5-FU) solution.

2 The 5-FU taken up was measured by high pressure liquid chromatography.

3 Uptake of 5-FU was found to approximate to Michaelis-Menton kinetics with the values of K_t and V_{max} equal to 15.39 mmol l^{-1} and 1.96 μmol g^{-1} min^{-1} respectively.

4 Transport of 5-FU was saturable with the uptake being much greater in the non-fasting rats.

5 Intracellular concentration of 5-FU after 24 min incubation approached but did not significantly exceed that in the extracellular fluid.

6 Enzymatic destruction of 5-FU at 2 min was negligible but substantial after a long incubation period.

7 2,4-Dinitrophenol was found to inhibit transport of 5-FU significantly.

8 Results suggested that the mechanism of uptake of 5-FU by rat liver is an energy requiring process.

Introduction

Schanker & Tocco (1959) showed that pyrimidines were absorbed more rapidly by the rat intestine than would be predicted by their lipid solubility. They therefore suggested that uptake was by a combination of active transport and passive diffusion. Later it was shown that uptake of uracil was an active process in the intestines of several other species, requiring oxygen and being inhibited by other pyrimidines as well as by a variety of metabolic inhibitors including 2,4-dinitrophenol (Schanker & Tocco, 1962). Schanker & Jeffrey showed that foreign pyrimidine derivatives such as 5-fluorouracil were absorbed by an active process in the rat (Schanker & Jeffrey, 1961) and also that uracil and several 5-substituted derivatives, including 5-fluorouracil (5-FU), were transported at similar rates and competitively inhibited the transport of each other (Schanker & Jeffrey, 1962).

5-FU can be absorbed to a substantial extent in the stomach but the kinetics of the process are not known (Inoue, Kondo, Isoya & Tanaka, 1978). It has been reported that the renal clearance of 5-FU in man is greater than the glomerular filtration rate suggesting that renal tubular secretion of the drug occurs (Clarkson, O'Connor, Winston & Hutchinson, 1964) but entry into human leukocytes (Kessel & Hall, 1967) and Ehrlich ascites carcinoma cells is by passive diffusion (Jacquez, 1962).

We have applied a high pressure ion-exchange liquid chromatographic method (Marrs & Hsu, 1980; Hsu & Marrs, 1980) to the measurement of 5-FU in rat liver slices in order to investigate the kinetics of uptake. Since a chromatographic method of estimation of 5-FU was used it was possible to measure distribution of the intact pyrimidine.

Methods

Uptake experiments

Male Wistar rats (Tuck & Sons, Battle Bridge, Essex) non-fasting except in one part of the experiment and weighing 150–200 g were killed with ether. The liver was removed through an abdominal incision and bisected transversely. Liver slices, about 0.4 mm thick and weighing 15–30 mg, were cut (Marrs 1977) and washed free of blood with oxygenated Tris buffer, pH 7.4. They were incubated at 37°C in 1.0 ml of the same buffer containing 5-FU. After the incubation period the slices were washed with buffer, dried with tissue paper, weighed and transferred to Pyrex conical centrifuge tubes containing 1.0 ml of 0.014 mol l^{-1} ammonium dihydrogen phosphate buffer, pH 3.5. They were then boiled for 5 min and after cooling the tubes were placed on a vortex mixer for 20 s. After centrifugation at 3000 rev/min for 5 min the 5-FU was estimated by high pressure ion-exchange chromatography. This was carried out by a previously described method (Marrs & Hsu, 1980; Hsu & Marrs, 1980) but because it was found unnecessary to perform the full extraction procedure,

0007-1188/82/110413-05 $01.00

the internal standard (chlorpheniramine) was added directly to aliquots of the centrifuged aqueous extract of liver, after which they were chromatographed isocratically on a Pye-Unicam (Pye Unicam Ltd, York Street, Cambridge) LC-EP h.p.l.c. system with the u.v. detector at 264 nm. The analytical column (250 mm in length, i.d. 4.6 mm) was packed with a strong cation-exchange material (Partisil-10 SCX) and was protected by a pellicular cation exchange column (Whatman Ltd, Springfield, Maidstone, Kent). The mobile phase was 0.014 mol l^{-1} aqueous ammonium dihydrogen phosphate buffer, pH 3.5, at a flow rate of 1.0 ml min^{-1}.

Observed uptake was corrected for 5-FU contained in the extracellular fluid by the following formula:

$$\text{Corrected uptake} = \text{x} - \text{blank uptake} - \frac{(\text{y} \cdot \text{inulin space } \%)}{100}\ \mu\text{mol g}^{-1}$$

where x is the observed uptake and y the concentration of 5-FU in the medium. For this purpose the uptake of [^{14}C]-carboxymethylinulin was measured under identical conditions to those given above (Figure 1). The blank was carried out by incubating the tissue slice in substrate-free Tris buffer pH 7.4 and treated exactly the same throughout the experimental procedure and analysis as for the test.

Intracellular concentration of 5-FU was calculated from the following formula using the symbols as above:

$$\text{Final concentration} = \text{x} - \text{blank uptake} - \frac{(\text{y} \cdot \text{inulin space } \%)}{100}\left(\frac{100}{100 - (\text{dry weight } \% + \text{inulin space } \%)}\right)\ \text{mmol l}^{-1}$$

Dry weight was measured by drying the tissue to constant weight.

Measurement of the rate of metabolism of 5-fluorouracil by liver

After the rats had been killed, the liver was removed and washed free of blood with Tris buffer, pH 7.4. After weighing, it was transferred into fresh buffer and homogenized with a glass tissue grinder (A. Gallenkamp & Co.) to give an homogenate of 0.5 to 1.0 g (ml of buffer)$^{-1}$. The homogenate was centrifuged for 10 min at 3000 rev/min in a GF-8 centrifuge (Fishons-MSE, Manor Royal, Crawley, Sussex) to remove debris, the supernatant being retained. 5-FU was dissolved in Tris buffer, pH 7.4, and after both substrate and supernatant had been equilibriated at 37°C and oxygenated, equal volumes of each were pipetted into a conical flask. The resultant mixture was incubated in a 37°C water bath with constant shaking (20 times min^{-1}) and 1 ml of mixture was withdrawn after the required time interval and transferred into a conical centrifuge tube. It was then boiled for 5 min, cooled and adjusted to pH 3.5 with orthophosphoric acid, placed on a Whirlimixer for 30 s and centrifuged at 3000 rev/min for 10 min. Analysis of the supernatant for 5-FU was then calculated by subtracting the concentration of 5-FU observed in each aliquot from the initial concentration in the incubation mixture.

Equal volumes of the substrate and Tris buffer were also mixed and treated in the same way as the test throughout the whole experimental procedure. These were used as controls. Analysis of these samples showed no detectable change of concentration of 5-FU, thereby confirming that under these experimental conditions 5-FU was very stable, and that any decrease in concentration of 5-FU in the supernatant was solely due to metabolism by the liver.

Results

Uptake continued to increase up to 4 min after which no further uptake appeared to take place (Table 1). This was true of liver from both fasting and non-fasting rats, but there was a considerable difference in uptake in the two nutritional states, uptake being much greater in the non-fasting animals. When liver from non-fasting rats was incubated for 24 min in media containing various concentrations of 5-FU the intracellular concentration of 5-FU approached but did not significantly exceed that in the extracellular fluid (Table 2). Table 3 gives the uptake by rat liver slices of 5-FU from media containing various 5-FU concentrations. Uptake by liver slices increased with the medium concentration of 5-FU but in a non-linear fashion and when the reciprocal of the uptake was plotted against the reciprocal of the medium concentration of 5-FU the relationship suggested mediated uptake (Figure 1). The K_t for uptake was 15.39 mmol l^{-1} and the V_{max} 1.96 μmol g^{-1} min^{-1}. When 0.1 mmol l^{-1} 2,4-dinitrophenol was added to the medium and the uptake compared with uptake from media free of 2,4-dinitrophenol, inhibition was found to have occurred at each concentration of 5-FU (Figure 2), the mean percentage inhibition by 2,4-dinitrophenol being 29.1%. When the significance of the difference between uptake with and without 2,4-dinitrophenol was tested by multiple linear regression analysis, the difference was highly significant ($P < 0.01$) (Snedecor & Cochran, 1967).

Metabolism of 5-FU by liver slices was very little at 2 min, but increased steadily to the end of the experiment at 1 h (Table 4). When expressed as a percentage of the original concentration in the homogenate

Table 1 5-Fluorouracil uptake by slices of liver from fasting and non-fasting rats.

Time of incubation (min)	*Liver* *Non-fasting uptake* μmol (g final wet weight)$^{-1}$	*Fasting uptake* μmol (g final wet weight)$^{-1}$
2	0.525 ± 0.075	0.169 ± 0.036
4	0.634 ± 0.191	0.280 ± 0.060
8	0.628 ± 0.196	0.213 ± 0.008
16	0.624 ± 0.161	0.234 ± 0.040

The medium contained 5-fluorouracil, 1 mmol l^{-1}. Results are in μmol (g final wet weight)$^{-1}$ over the stated times and are means ± s.d. ($n = 6$).

Table 2 Intracellular concentration of 5-fluorouracil attained after incubation of liver slices from non-fasting rats for 24 min in media containing 5-fluorouracil at concentrations between 0.5 and 10.0 mmol l^{-1}

5-Fluorouracil concentration in the medium (mmol l^{-1})	*Intracellular concentrations* (mmol l^{-1})
0.5	0.714 ± 0.050
1.0	0.741 ± 0.044
2.5	2.483 ± 0.034
5.0	4.766 ± 0.810
10.0	8.981 ± 1.535

Results are means ± s.d. ($n = 6$).

Table 3 Uptake of 5-fluorouracil by liver slices from non-fasting rats during a 2 min incubation period

Concentration of 5-fluorouracil in medium	*Uptake* (μmol g^{-1} min^{-1}) Mean	s.d.
0.5	0.065	0.010
1	0.131	0.067
2.5	0.331	0.201
5.0	0.523	0.375
10.0	1.025	0.230
20.0	1.181	0.008

($n = 12$).

it was highest at the lowest substrate concentration. However, it seems unlikely to have affected significantly the 10 min uptake results shown in Figure 2 at any of the concentrations used.

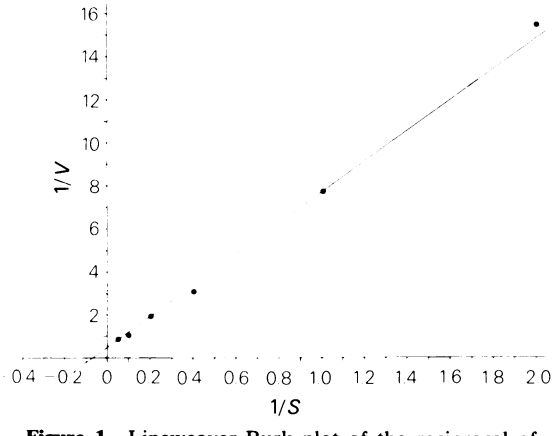

Figure 1 Lineweaver-Burk plot of the reciprocal of rate of transport ($1/V$) in 1/μmol g^{-1} final wet weight against the reciprocal of substrate concentration ($1/S$) in 1/mmol l^{-1}.

Discussion

The results suggest that an active mechanism exists for uptake of 5-FU by rat liver. It was not possible to demonstrate the formation of a concentration-gradient of the drug across the membrane of the liver cell; however, the substantial metabolism of 5-FU which occurred after long incubation periods may have masked this. Another factor preventing attainment of higher intracellular concentrations may have been outward leaking of 5-FU. 2,4-Dinitrophenol inhibited the uptake process at a concentration of 0.1 mmol l^{-1}, a concentration considerably below that found by Schanker & Tocco (1959) to inhibit transport of [^{14}C]-uracil by small intestine. This suggests that the process is an energy requiring one. The marked difference in uptake between liver slices from fasting and non-fasting rats would then be due to lack of an energy source in the former preparation. Although no studies have been reported of the effect of nutritional status on the uptake of pyrimidine by the liver or the intestine, it must be noted that administration of 5-FU to rats is followed by a severe reduction in food intake and that food restriction or

Table 4 Rate of metabolism of 5-fluorouracil by liver homogenate

Substrate concentration *Time* (min)	*% metabolized by homogenate* 1 mmol l^{-1}	0.5 mmol l^{-1}	0.25 mmol l^{-1}
2	4.4	13.4	32.0
10	10.6	19.8	34.0
20	11.9	21.4	38.0
60	17.9	21.0	44.0

Results are expressed as a percentage of the 5-fluorouracil initially present and are means of 6 observations.

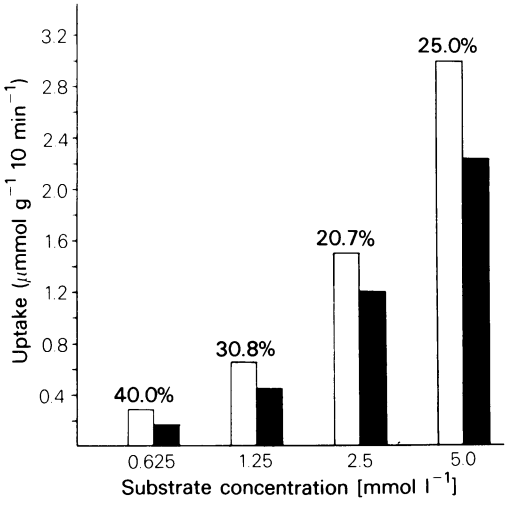

Figure 2 Inhibition of 5-fluorouracil uptake by 2,4-dinitrophenol (DNP). The solid columns represent uptake with DNP; the open columns without DNP. Each column represents the mean of 3 estimations, and while the differences were not significant when uptake was compared with and without DNP at each substrate concentration, they were when compared by multiple linear regression analysis at all concentrations.

starvation *per se* are both known to cause changes in the absorptive and enzymatic activities of the small intestine (Kershaw, Neame & Wiseman, 1960; McManus & Isselbacher, 1970; Newey, Sanford & Smyth, 1970; Lis, Matthews & Crampton, 1972; Kim, McCarthy, Lane & Fong, 1973; Debham & Levin, 1975), the intestine is reported to show decrease uptake of some nutrients (Schedl, Burston, Taylor & Matthews, 1979).

I would like to thank Dr T.C. Marrs for his help in tabulation of the results, and his advice and review of the manuscript; Mr S. Brown for the preparation of the figures and Miss K. Iddon for the typing. I am grateful to Professor J.R. Hobbs for the support in purchasing the experimental animals. Finally I would like to thank Dr D. Burston for his technical assistance.

References

CLARKSON, B., O'CONNOR, A., WINSTON, L.R. & HUTCHINSON, D. (1964). The physiologic description of 5-fluorouracil and 5-fluoro-2-dioxyunidine in man. *Clin. Pharmac. Ther.*, **5,** 581–610.

DEBHAM, E.S. & LEVIN, R.J. (1975). Effects of fasting and semi-starvation on the kinetics of active and passive sugar absorption across the small intestine *in vivo*. *J. Physiol.*, **272,** 681–700.

HSU, L.S.F. & MARRS, T.C. (1980). Determination of 5-fluorouracil in human plasma by high-pressure ion exchange chromatography. *Ann. Clin. Biochem.*, **11,** 272–276.

INOUE, A., KONDO, A., ISOYA, M. & TANAKA, M. (1978). Studies on the oral administration of 5-FU (1). The distribution of 5-FU in the gastrointestinal tracts under various conditions of administration. *Chemotherapy (Japan).*, **26,** 469.

JACQUEZ, J.A. (1962). Transport and enzymic splitting of pyrimidine nucleosides in Ehrlich cells. *Biochem. Pharmac.*, **16,** 2395–2403.

KERSHAW, T.G., NEAME, K.D. & WISEMAN, G. (1960). The effects of semistarvation on absorption by the rat small intestine *in vitro* and *in vivo*. *J. Physiol., Lond.*, **152,** 182–190.

KESSEL, D. & HALL, T.C. (1967). Studies on drug transport by normal leukocytes. *Biochem. Pharmac.*, **16,** 2395–2403.

KIM, Y.S., McCARTHY, D.M., LANE, W. & FONG, W. (1973). Alteration in the levels of peptide hydrolases and other enzymes in bruxhborder and soluble fractions of rat

small intestinal mucosa during starvation and refeeding. *Biochem. biophys. Acta*, **321,** 262–273.

LIS, M.T., MATTHEWS, D.M. & CRAMPTON, R.F. (1972). Effects of dietary restriction and protein deprivation on intestinal absorption of protein digestion products in the rat. *Br. J. Nutr.*, **28,** 443–446.

MARRS, T.C. (1977). Peptide transport: its role in intestinal absorption and occurrence in different mammalian species. *MD Thesis, University of London.*

MARRS, T.C. & HSU, L.S.F. (1980). The determination by HPLC of 5-fluorouracil in plasma. *1st World Conference, Clin. Pharmac. Ther., London,* Abstract 0290.

McMANUS, J.P.A. & ISSELBACHER, K.J. (1970). Effects of fasting versus feeding on the rat intestine. *Gastroenterology*, **59,** 214–221.

NEWEY, H. SANFORD, P.A. & SMYTH, D.H. (1970). Effects of fasting on intestinal transfer of sugars and amino acids in vitro. *J. Physiol.*, **208,** 705–724.

SCHANKER, L.S. & JEFFREY, J.J. (1961). Active transport of foreign pyrimidines across the intestinal epithelium. *Nature*, **190,** 4777–4778.

SCHANKER, L.S. & JEFFREY, J.J. (1962). Structural specificity of the pyrimidines transport process of the small intestine. *Biochem. Pharmac.*, **11,** 961–966.

SCHANKER, L.S. & TOCCO, D.J. (1959). Active transport of some pyrimidines across the rat intestinal epithelium. *J. Pharmac.*, **128,** 115–121.

SCHANKER, L.S. & TOCCO, D.J. (1962). Some characteristics of the pyrimidine transport process of the small intestine. *Biochem. biophys. Acta*, **56,** 469–473.

SCHEDL, H.P., BURSTON, D., TAYLOR, ELIZABETH & MATTHEWS, D.M. (1979). Kinetics of uptake of an amino acid and a dipeptide into hamster jejunum and ileum: the effect of semistarvation and starvation. *Clinical Sci.*, **56,** 487–492.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). Statistical Methods. Iowa State University Press: Ames, Iowa, U.S.A.

(*Received March 4, 1982.*
Revised June 18, 1982.)