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THE TRANSPORT OF PYRIMIDINES INTO TISSUE RINGS CUT FROM RAT SMALL INTESTINE

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SUMMARY

1. At low concentrations (0·1 mM) the transport of uracil, 5-fluorouracil and thymine into jejunal tissue rings is an active process.

2. The transport of 5-fluorouracil into tissue rings cut from the duodenum and jejunum was greater than the transport into rings cut from the ileum. This difference was abolished by starving the rats for 48 h before the experiment.

3. The active transport can be abolished by replacing the Na^+ in the incubation medium with either K^+ or mannitol, or by increasing the concentration of the pyrimidine to 1·0 mM.

4. The accumulation of uracil or 5-fluorouracil into the jejunal rings was identical when determined by radioactive tracer or by high-performance liquid chromatography.

5. The apparent Michaelis constant (K_m) for 5-fluorouracil transport into jejunal rings was 0·074 mM in the standard Na^+ bicarbonate Krebs–Ringer solution and 0·394 mM in the K^+-substituted bicarbonate Krebs–Ringer solution.

6. Both thymine and uracil inhibited the transport of 5-fluorouracil into jejunal tissue rings; however, cytosine and orotic acid did not.

INTRODUCTION

There are two natural sources for the nucleic acids and their components found in the lumen of the small intestine. Endogenous nucleic acids and their components are released by the cellular turn-over of the intestinal mucosa. This can yield up to 30 mg of nucleic acid per day in the rat (see Parsons & Shaw, 1983). Dietary intake of nucleic acids and their components will vary from a minimal amount in standard laboratory diets to amounts similar to or in excess of the endogenous supply depending on the composition of the diet.

In view of this it is of interest to study the absorption of the pyrimidines in the small intestine. The work of Schanker and colleagues (Schanker & Tocco, 1960; Schanker & Tocco, 1962; Schanker, Jeffery & Tocco, 1963) using everted sacs of small intestine suggested that there are pyrimidine transporters in the small intestine of rat, hamster and frog, and that there was competition between the various pyrimidines for the process. In addition, Csáky (1963) showed a Na^+-dependent active

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uracil transport in anuran small intestine. More recently uracil transport has been studied in sheep (Scharrer & Amann, 1979). This study also indicates that uracil transport is an active process.

The previous observations on the transport of pyrimidines in rat intestine were based on results obtained using radioactive tracer to follow the transport of the pyrimidines and therefore might be complicated by other events, such as metabolism (Bronk & Shaw, 1986) or counter transport (Levine & Levine, 1969), which could result in the accumulation of labelled compounds within the tissue. Such accumulation cannot be differentiated from a true active transport process on the basis of the tracer technique alone.

In addition, the earlier results did not indicate the nature of the transport process or the region of the small intestine primarily responsible for the process. The latter point is significant in view of the fact that most of the pyrimidines may be derived from endogenous sources and therefore may be relatively scarce at the proximal end of the small intestine.

In the present study we have attempted to characterize pyrimidine transport in the small intestine using the tissue accumulation method (Agar, Hird & Sidhu, 1954; Crane & Mandelstam, 1960; Alvarado, Lherminier & Phan, 1984). We have concentrated on 5-fluorouracil, a synthetic pyrimidine which does not naturally occur in the intestinal mucosa. Both radioactive tracer and high-performance liquid chromatography (h.p.l.c.) have been used to analyse the tissue levels of the pyrimidines being studied. Therefore, the uncertainties caused by metabolism and the possibility of counter transport, which can occur when the substrate under study is found in the mucosal cells of the small intestine, have been avoided.

METHODS

Animals. Male Wistar rats (200–250 g) were used for all experiments. They were fed *ad libitum* (modified BIO 41B diet, Oxoid, Basingstoke, U.K.) until the day of the experiment, unless they were being used to determine the effect of diet on pyrimidine absorption along the intestine. In that case either the water was supplemented with 1·0 mM-uracil and 0·5 mM-thymine or the food was removed for the 48 h prior to the experiment. Before each experiment the animal was rapidly anaesthetized by an intraperitoneal injection of 40 mg of sodium pentobarbitone.

Incubation media. The incubation media were all based on a bicarbonate Krebs–Ringer solution containing the relevant pyrimidine. For some experiments the medium was modified by substitution of the NaCl with either KCl or mannitol. Standard (Na^+) bicarbonate Krebs–Ringer solution: 120 mM-NaCl, 4·5 mM-KCl, 1 mM-$MgSO_2$, 1·8 mM-Na_2PO_4, 0·2 mM-NaH_2PO_4, 25 mM-$NaHCO_3$ and 1·25 mM-$CaCl_2$. K^+-substituted bicarbonate Krebs–Ringer solution: as standard bicarbonate Krebs–Ringer solution with 120 mM-KCl in place of 120 mM-NaCl and 25 mM-$KHCO_3$ in place of 25 mM-$NaHCO_3$. Mannitol-substituted bicarbonate Krebs–Ringer solution: as K^+ bicarbonate Krebs–Ringer solution with 240 mM-mannitol in place of 120 mM-KCl. All solutions were gassed for at least 45 min with 95 % O_2–5 % CO_2 before a 20 ml aliquot was transferred to a 40 ml beaker and placed in a water-bath at 37·5 °C. The contents of the beaker were gassed through a fine-tip pipette and allowed to come to temperature. In experiments where radioactive tracer was used this was added at 0·05–0·10 μCi/ml.

Preparation of the intestinal tissue. The intestine was removed from the rat and quickly washed out with ice-cold 150 mM-saline solution. Excess saline was removed by passing a syringe full of air through the intestine before the rings (Agar *et al.* 1954; Crane & Mandelstam, 1960) were cut on a glass plate sitting on ice. When the rings, approximately 1 mm thick (wet weight 12·6 $\pm$ 0·2 mg, n = 500), had been cut they were mixed before being incubated in the appropriate bicarbonate

Krebs–Ringer solution. Two sections of the intestine adjacent to the region from which the rings were cut were removed for the determination of the wet/dry weight ratio for that segment of intestine.

For the study of the relationship between pyrimidine absorption and distance along the intestine, three rings were cut at 10 cm intervals along the small intestine starting either from the pyloric sphincter or the ileo-caecal valve. Sections of the intestine adjacent to where the rings were cut were used to determine the wet/dry weight ratio along the intestine.

Incubation of the rings. The beaker was shaken at 60 strokes/min and this together with the gassing ensured that the rings were in constant motion throughout the period of the incubation. The medium occasionally frothed after 15 min and therefore the results were only used up to the 15 min value.

In the studies of absorption along the intestine the three rings cut from each section were incubated in separate test-tubes containing 5 ml of incubation medium. All the test-tubes were shaken at 60 strokes/min, and each tube was gassed through a fine-tip pipette.

Measurement of solute uptake into tissue rings by liquid scintillation counting. At the appropriate time two rings were removed from the incubation medium and individually placed on Whatman No. 1 filter paper. They were washed with 10 ml of unlabelled incubation medium using an Amicon (Gloucestershire, U.K.) vacuum filtration manifold (VFM1) and allowed to remain for a further 3 min on the manifold. Each ring was weighed before being dissolved in 4 ml of scintillation fluid. The scintillation vial was immediately whirlimixed and left to stand for approximately 2 h before being whirlimixed again. After this the vials were counted on a LKB (Croydon, U.K.) rack-beta scintillation counter. There was no change in the level of radioactivity when the samples were counted again the next day indicating that all the labelled material had been liberated into the scintillation fluid by the whirlimixing. The incubation medium was also sampled and aliquots assayed by liquid scintillation counting.

Measurement of solute uptake into tissue rings by h.p.l.c. At the appropriate time ten rings were removed from the incubation medium and immediately blotted on Whatman No. 1 filter paper. The rings were weighed before being homogenized in 1 ml of either 6% perchloric acid or 15% trichloroacetic acid at 5000 rev/min in a glass–Teflon homogenizer. The resulting homogenate was centrifuged at 2000 *g* for 2 min. An aliquot of the supernatant was neutralized with potassium hydroxide and the solution was recentrifuged at 2000 *g* for 2 min. The supernatant was filtered through 0·4 μM Whatman cellulose nitrate filters. The filtrate was analysed by h.p.l.c. using a Beckman 341 isocratic liquid chromatograph fitted with a 5 μM 4·6 × 230 mm Ultrasphere-ODS column. The mobile phase used was 21 mM-KH_2PO_4, pH 3·0.

Analysis of carry over of medium on the tissue rings. The carry over of incubation medium on the rings was measured by analysing rings that had been rapidly dipped in the medium (0 min value). Liquid scintillation counting showed that the carry over was only sufficient to give 6·5 ± 0·3% ($n = 350$) of the medium concentration in the water associated with the rings. With h.p.l.c. no 5-fluorouracil could be detected in the 0 min samples after the rings had been dipped in 0·1 mM-5-fluorouracil.

Materials. All chemicals were of analytical grade. [5,6-^{3}H]uracil, 5-fluoro[6-^{14}C]uracil, 5-fluoro[6-^{3}H]uracil, [methyl-^{3}H]thymine, inulin-[^{14}C]carboxylic acid and L-[1-^{14}C]glucose were purchased from Amersham International plc, U.K. Optiphase MP scintillation fluid was purchased from Fisons plc, U.K. Sodium pentobarbitone was purchased from May and Baker Ltd., Dagenham, U.K. Ultra-fine-tip pastette pipettes were purchased from Alpha Laboratories, Eastleigh, U.K.

Expression of results. The volume of total tissue water associated with each ring was determined from the wet weight of the ring and the dry/wet weight ratio of the same intestine. This makes it possible to express the uptake of the substrate into the ring as a concentration (μmol/ml total tissue water) and enables the uptake to be compared with the concentration of substrate in the incubation medium to determine whether or not the tissue was able to accumulate the pyrimidines against a concentration gradient (i.e. whether the tissue/medium concentration ratio was significantly greater than one). For the uptake along the intestine the accumulation after 15 min is expressed as μmol/g dry weight. The small intestine was divided into an upper and lower region at a point 45% along its length. Thus, the upper region includes the duodenum and the jejunum and lower region is considered to include the upper and lower ileum.

No correction has been made for the contribution of the extracellular space to any of the values for the accumulation into the rings. Estimates of the extracellular space were made using

0·1 mM-inulin (assuming the average molecular weight to be 5000) and 1·0 mM-L-glucose. The results obtained after a 15 min incubation are expressed as the percentage of tissue water which is inulin or L-glucose accessible. The values are 9·4±0·8% for 0·1 mM-inulin, 13·5±1·3% for 1·0 mM-L-glucose in K^+-substituted incubation medium and 23·6±1·4% for L-glucose in Na^+ incubation medium.

The initial uptake data derived from the 3 min value (see Results) are expressed in nmol/g dry weight. min.

Except in Fig. 1 the n value for all data obtained using radioactive tracer is 10. This is calculated from five animals being used for each condition with two rings being analysed at each time point. The n value for data obtained using h.p.l.c. is 8 based on four animals being used for each condition with two analyses conducted for each animal. All results are given as the mean ± the standard error of the mean.

RESULTS

Absorption along the small intestine

The capacity of the different regions of the small intestine for the absorption of 5-fluorouracil was determined by cutting tissue rings at various distances down the

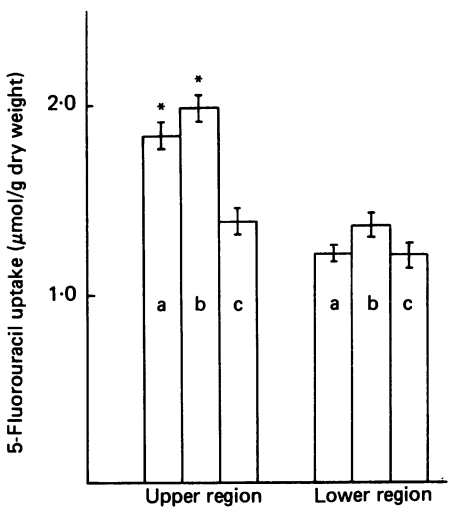

Fig. 1. The accumulation of 5-fluorouracil into tissue rings cut at 10 cm sections along the intestine. The tissue rings were grouped into upper and lower regions of the intestine (see Methods). Normal fed animals (a), 48 h diet supplemented animals (b) and 48 h starved animals (c). *Values significantly different ($P < 0·01$) to the accumulation in the lower intestine ($n > 60$ for each observation).

intestine and incubating them for 15 min with 0·1 mM-labelled 5-fluorouracil in the standard Na^+ bicarbonate Krebs–Ringer solution. The results (Fig. 1) show that in rats fed on a normal laboratory diet (which is low in nucleic acids and their components) there is a significantly greater capacity ($P < 0·01$) in the upper region of the intestine than in the lower region. This difference is unaffected by supplementing the rats diet with 1·0 mM-uracil and 0·5 mM-thymine for 48 h prior to the experiment. However, starvation for 48 h reduced the absorptive capacity of the upper region of the intestine. In both regions of the intestine the concentration of 5-fluorouracil in the rings was significantly ($P < 0·01$) greater than the medium

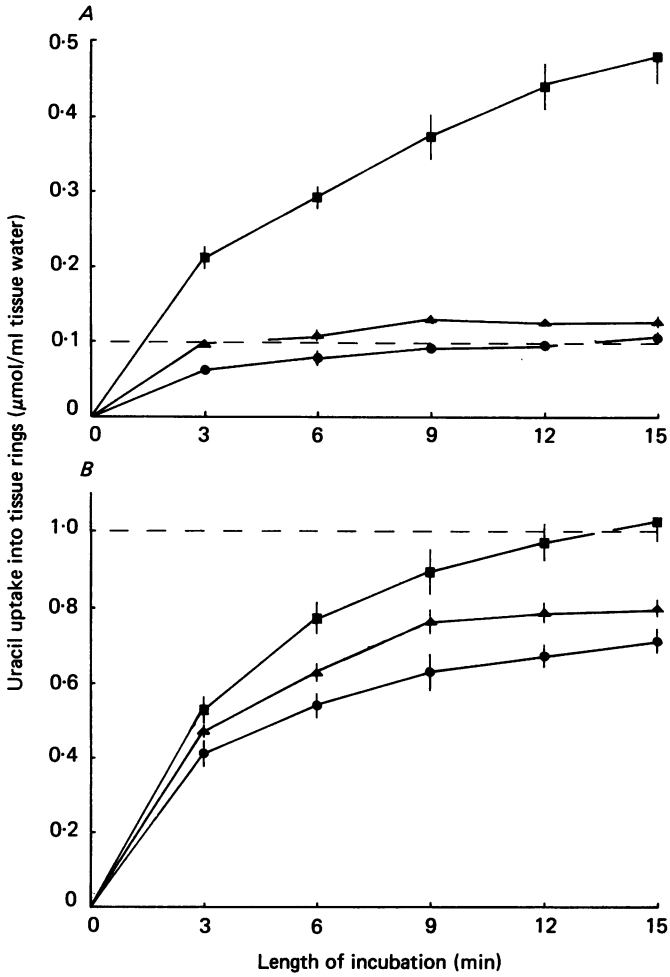

Fig. 2. The accumulation of uracil into tissue rings with time. *A*, 0·1 mM-uracil and *B*, 1·0 mM-uracil in the medium. (■) Na^+ bicarbonate Krebs–Ringer solution. (●) K^+-substituted bicarbonate Krebs–Ringer solution. (▲) Mannitol-substituted bicarbonate Krebs–Ringer solution. -- Medium concentration of uracil.

concentration. There was no significant difference in the dry/wet weight ratio along the small intestine.

As a result of these findings the jejunum was selected for our studies of pyrimidine transport in the small intestine.

The absorption of uracil, 5-fluorouracil and thymine

The accumulation of labelled uracil, 5-fluorouracil and thymine into jejunal tissue rings was studied at 0·1 and 1·0 mM concentrations in the three different incubation buffers (Na^+ bicarbonate Krebs–Ringer solution, K^+- and mannitol-substituted bicarbonate Krebs–Ringer solutions). The results (Figs. 2, 3 and 4) show that with 0·1 mM-pyrimidine in the incubation medium active transport was seen in the

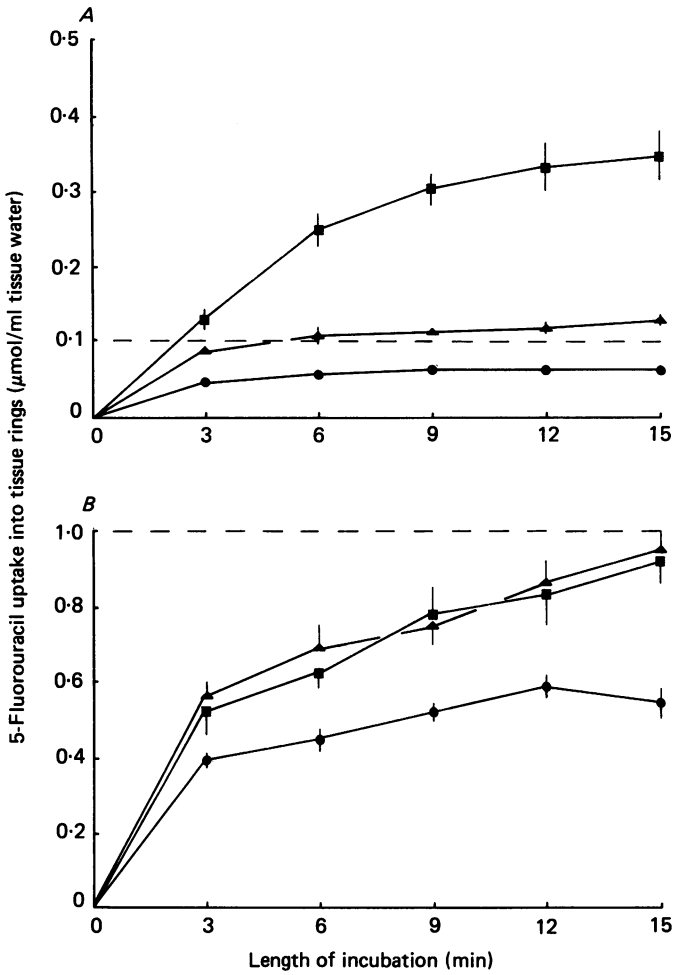

Fig. 3. The accumulation of 5-fluorouracil into tissue rings with time. *A*, 0·1 mM-5-fluorouracil. *B*, 1·0 mM-5-fluorouracil in the medium. (■) Na^+ bicarbonate Krebs–Ringer solution. (●) K^+-substituted bicarbonate Krebs–Ringer solution. (▲) Mannitol-substituted bicarbonate Krebs–Ringer solution. – – Medium concentration of 5-fluorouracil.

standard (Na^+) bicarbonate Krebs–Ringer solution but not in either the K^+- or mannitol-substituted bicarbonate Krebs–Ringer solution. With 1·0 mM concentrations of the three pyrimidines none of the incubation media (standard Na^+ or substituted bicarbonate Krebs–Ringer solutions) showed any active transport, although there were significant differences ($P < 0·01$) between the Na^+ and the K^+-substituted bicarbonate Krebs–Ringer solutions. The accumulation of 0·1 mM-uracil, 5-fluorouracil and uracil at 0 min (see Methods) and 15 min incubation times was analysed by h.p.l.c. (Table 1). The results indicate that the radioactive tracers are reporting the true tissue concentrations of the pyrimidines as there was no significant difference between the net accumulation measured by h.p.l.c. and that found in the experiments in which the accumulation was estimated with radioactive

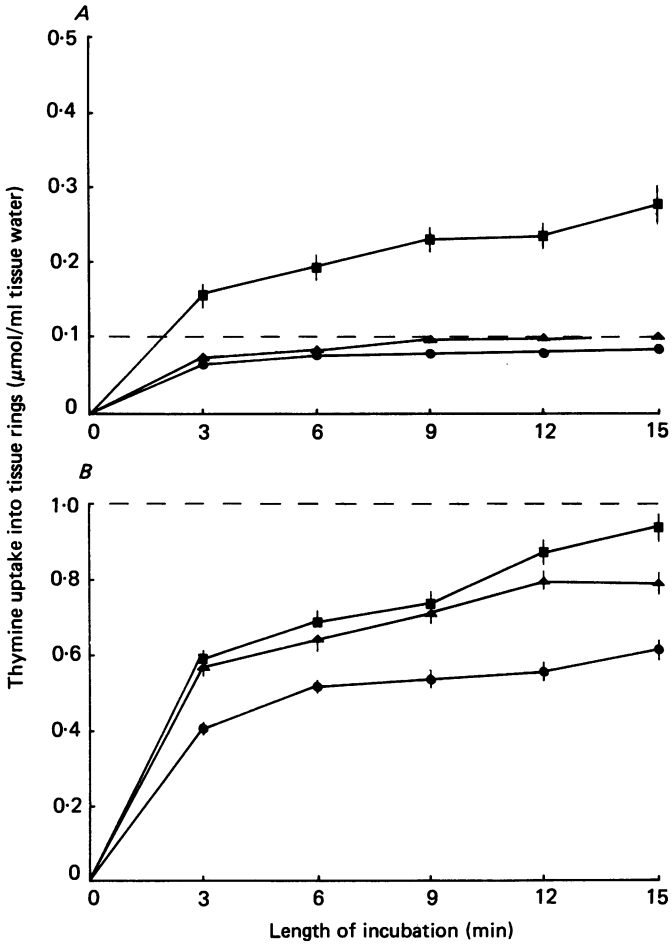

Fig. 4. The accumulation of thymine into tissue rings with time. *A*, 0·1 mM-thymine. *B*, 1·0 mM-thymine in the medium. (■) Na^+ bicarbonate Krebs–Ringer solution. (●) K^+-substituted bicarbonate Krebs–Ringer solution. (▲) Mannitol-substituted bicarbonate Krebs–Ringer solution. -- Medium concentration of thymine.

TABLE 1. Comparison of accumulation of pyrimidines into tissue rings analysed by h.p.l.c. and liquid scintillation counting

	15 min accumulation (μmol/ml tissue water)	
Pyrimidine in incubation medium	Analysed by l.s.c.	Analysed by h.p.l.c.
0·1 mM-5-fluorouracil	0·35 ± 0·03	0·41 ± 0·05
0·1 mM-uracil	0·48 ± 0·03	0·50 ± 0·05
1·0 mM-uracil	1·03 ± 0·06	1·02 ± 0·04

The uracil values determined by h.p.l.c. are the net values obtained by subtracting the 0 min value (see Methods) from the 15 min value. The initial tissue level (0 min value) of uracil was 0·38 ± 0·03 mM. This level was unaffected by the 15 min incubation with 0·1 mM-5-fluorouracil. There is no significant difference ($P > 0·1$) between the tissue levels measured by the two techniques. Liquid scintillation counting, l.s.c.

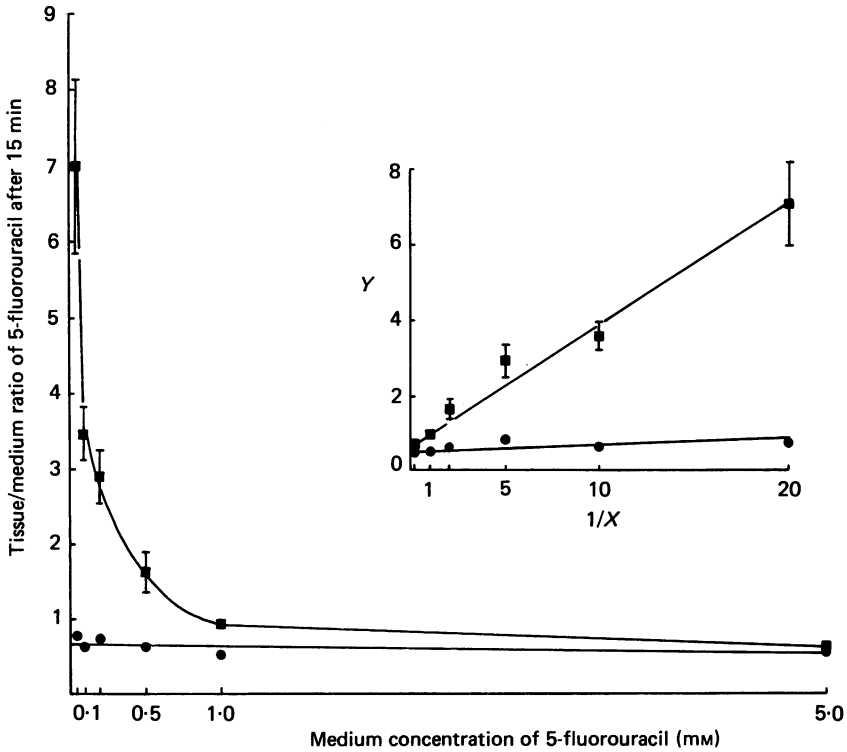

Fig. 5. 15 min tissue/medium ratio in rings incubated at various concentrations of 5-fluorouracil. (■) Na^+ bicarbonate Krebs–Ringer solution. (●) K^+-substituted bicarbonate Krebs–Ringer solution. Inset, 15 min tissue/medium ratio against 1/medium concentration of 5-fluorouracil.

tracers. These data also indicate that the accumulation of 0·1 mM-5-fluorouracil into the rings over 15 min does not alter the initial (0 min) tissue level of uracil.

Concentration dependence of 5-fluorouracil active transport

The time-dependent transport of 5-fluorouracil into jejunal tissue rings was studied at various concentrations (0·05, 0·1, 0·2, 0·5, 1·0 and 5·0 mM) in both the Na^+ and the K^+-substituted bicarbonate Krebs–Ringer solutions. The 15 min tissue/medium ratio at each concentration and in both incubation media are shown in Fig. 5. A value for the tissue/medium ratio of greater than one represents an active accumulation into the rings and our results indicate that active accumulation of 5-fluorouracil into the tissue occurred at concentrations of 0·5 mM-5-fluorouracil and below in the (Na^+) incubation medium. The 15 min tissue/medium ratios for 5 mM-5-fluorouracil in the Na^+ bicarbonate Krebs–Ringer solution and all 5-fluorouracil concentrations in the K^+-substituted bicarbonate Krebs–Ringer solution were found to be less than one. This is probably due to the contribution of the muscle found in the tissue rings to the total tissue water. The proportion of muscle to mucosa is 1 to 1·86 (Bronk & Parsons, 1965). This suggests that during the 15 min incubation period the pyrimidines do not equilibrate into the muscle tissue present in the rings.

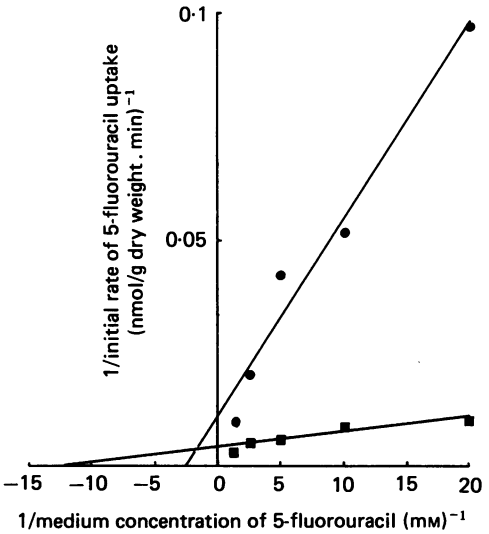

Fig. 6. Lineweaver–Burke plot of initial rate (see text) of 5-fluorouracil accumulation into rings. (■) Na^+ bicarbonate Krebs–Ringer solution. (●) K^+-substituted bicarbonate Krebs–Ringer solution.

Determination of kinetic parameters for 5-fluorouracil transport

There was no significant difference ($P > 0{\cdot}1$) over the time range 0–15 min for the accumulation of 5 mM-5-fluorouracil into jejunal tissue rings from either the Na^+ or the K^+-substituted incubation media (see Fig. 5 for comparison of the 15 min point). It was therefore assumed that the 5·0 mM uptake closely resembled the passive diffusion of 5-fluorouracil into the rings. Using the 3 min point for the uptake of 5-fluorouracil from the 5·0 mM medium the diffusion potential (K_d) for 5-fluorouracil into the rings was calculated. The K_d was used to calculate the diffusion component of the accumulation at each concentration of 5-fluorouracil. These values were then subtracted from the 3 min points obtained at the various concentrations of 5-fluorouracil in both the Na^+ and the K^+-substituted bicarbonate Krebs–Ringer solution. This adjustment removed both the passive-diffusion component and uptake due to the equilibration into the extracellular space, leaving a net transport rate that is attributable to the ion-dependent transport process. A Lineweaver–Burke plot of the initial rate of influx into the rings is given in Fig. 6. The apparent K_m for the transport process in the Na^+ bicarbonate Krebs–Ringer solution is 0·074 mM whereas in the K^+-substituted bicarbonate Krebs–Ringer solution the apparent K_m is more than 5 times as high at 0·394 mM.

The inhibition of 0·1 mM-5-fluorouracil accumulation by other pyrimidines

The inhibition of the uptake of 0·1 mM-labelled 5-fluorouracil into jejunal tissue rings was investigated in the presence of cytosine, orotic acid, uracil or thymine added to the Na^+ incubation medium at a concentration of either 0·05 or 0·1 mM. The time course of the uptake of 0·1 mM-5-fluorouracil in the presence of 0·1 mM concentrations

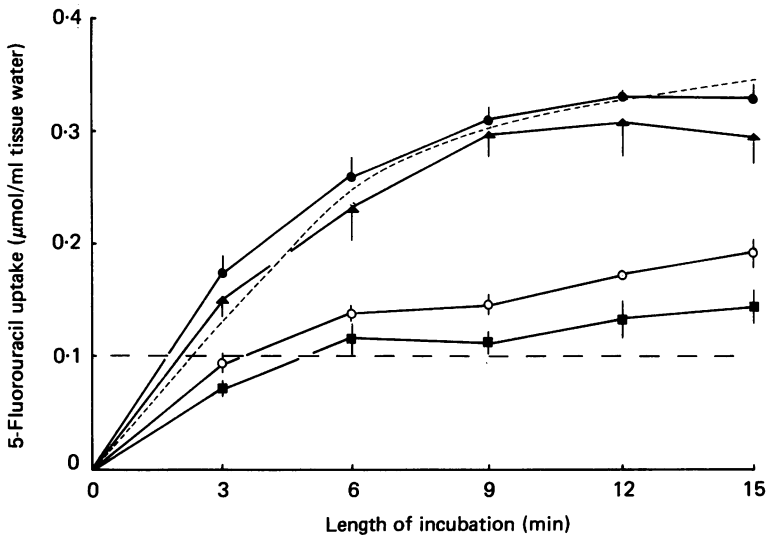

Fig. 7. Accumulation of 0·1 mM-5-fluorouracil into rings in the presence of 0·1 mM-cytosine (●), 0·1 mM-orotic acid (▲), 0·1 mM-uracil (○) or 0·1 mM-thymine (■). ----- Standard accumulation of 0·1 mM-5-fluorouracil into tissue rings. --- Medium concentration of 5-fluorouracil.

TABLE 2. The influence of pyrimidines on the accumulation of 0·1 mM-5-fluorouracil into tissue rings

Additional pyrimidine	Concentration of additional pyrimidine where added	
	0·05 mM	0·10 mM
None	100·0 ± 0·0	100·0 ± 0·0
Cytosine	96·3 ± 8·2	107·9 ± 10·8
Orotic acid	120·5 ± 15·2	102·0 ± 9·3
Uracil	80·5 ± 11·7	51·8 ± 6·1*
Thymine	58·3 ± 5·2*	39·3 ± 5·5*

The 9 min accumulation of 0·1 mM-5-fluorouracil into tissue rings with either 0·1 or 0·05 mM-cytosine, orotic acid, uracil or thymine added to the incubation medium. The results are expressed as a percentage of the uptake of 5-fluorouracil in the absence of added pyrimidine (100 %). * Values where there is a significant ($P < 0·01$) inhibition of the 9 min uptake of 5-fluorouracil into the tissue rings.

of each of the pyrimidines is given in Fig. 7 and a comparison of the inhibitory effects of each of the pyrimidines at both concentrations on the 9 min accumulation of 5-fluorouracil is given in Table 2. The data indicate that of the pyrimidines tested only uracil and thymine inhibited the transport process and that at 0·05 mM where they were less effective only thymine caused a significant ($P < 0·01$) reduction in 5-fluorouracil accumulation into the rings.

DISCUSSION

The upper region of the small intestine has the greatest absorptive capacity for 5-fluorouracil both in animals which were fed normally or those on a diet supplemented with uracil and thymine. This suggests that in the digestive tract of the rat a transport system has evolved to absorb dietary pyrimidines rather than the endogenously derived ones, since the latter would be expected to be found at low concentrations in the upper part of the intestine. However, the over-all decline in the absorptive capacity of the intestine from duodenum to ileum does not rule out the reabsorption of the endogenous pyrimidines released by mucosal-cell turn-over since the lower ileum is still able to absorb 5-fluorouracil actively even though the capacity is only 40 % of that in the duodenum. The over-all pattern of the absorption of pyrimidines along the intestine is similar to that found for the carbohydrates (Crane, 1960; Leese & Mansford, 1971; Hopfer, Sigrist-Nelson & Groseclose, 1976) as is the starvation-induced reduction in duodenal and jejunal transport capacity (Karasov & Diamond, 1983).

The results shown in Figs. 2, 3 and 4 indicate that the transport processes for uracil, 5-fluorouracil and thymine are sensitive to the ionic composition of the incubation medium. At the lower concentration (0·1 mM) the three pyrimidines are all actively transported into the rings in the Na^+ bicarbonate Krebs–Ringer solution. However, the three pyrimidines are not accumulated to the same extent; the order being uracil > 5-fluorouracil > thymine on the basis of 15 min tissue/medium ratios which are $4{\cdot}8 \pm 0{\cdot}3$, $3{\cdot}5 \pm 0{\cdot}3$ and $2{\cdot}7 \pm 0{\cdot}3$, respectively. The effects of replacing the standard (Na^+) bicarbonate Krebs–Ringer solution with a K^+- or mannitol-substituted bicarbonate Krebs–Ringer solution is striking since either substitution prevents active accumulation with 0·1 mM-pyrimidine. These results indicate that the active transport of these pyrimidines is Na^+ dependent. The similarity between the effects of K^+- and mannitol-substitution suggests that it is the absence of Na^+ that blocks the transport process rather than a modification of the electrical properties of the membrane which would result from clamping the membrane potential by replacing Na^+ with K^+. However, small but significant differences ($P < 0{\cdot}01$) were observed between the uptake of the pyrimidines in the K^+- and mannitol-substituted bicarbonate Krebs–Ringer solutions.

At the higher concentration of the three pyrimidines (1·0 mM) no accumulation above the medium concentration was seen suggesting that diffusion was the major component in the uptake of the pyrimidines. However, small but significant ($P < 0{\cdot}01$) Na^+–K^+ differences were observed at 1·0 mM in all three cases (Figs. 2, 3 and 4). Therefore, it is apparent that although at this concentration the diffusion process is predominant there is still a small contribution by the Na^+-dependent transport process.

The results discussed above have all been based on data obtained using radioactive tracers. It was therefore important to check that these reported tissue concentrations were genuine and not, at least partly, masking the effects of cellular metabolism which would reduce the true concentration of pyrimidine in the tissue. It was also possible that the pyrimidine accumulation could be due to a counter-transport process, in which an endogenous pyrimidine exchanged with the labelled substrate in the

incubation medium. The analysis by h.p.l.c. of the tissue levels of the pyrimidines, after the rings were incubated in 0·1 or 1·0 mM-uracil for 15 min (Table 1), indicates that estimates of the transport of the pyrimidines into the tissue by direct analysis were identifical with those reported by liquid scintillation counting. Table 1 also shows that uracil from the medium is being transported into the tissue against a concentration gradient rather than being exchanged with the intracellular pool of uracil initially present in the ring. It should also be noted that the intracellular level of uracil was not affected by the accumulation of 5-fluorouracil showing that the latter is accumulated by an active transport process, rather than a 5-fluorouracil–uracil counter transport.

The agreement between the results obtained by liquid scintillation counting and by h.p.l.c. for 5-fluorouracil transport is supported by the report of Yamamoto & Kawasaki (1981), who showed that 97% of the 5-fluorouracil transported in Erlich ascites tumour cells, which would be expected to have high rates of nucleic acid metabolism, remained unmetabolized over a 10 min period.

The possibility that labelled substrate could be accumulated in the tissue rings by a pyrimidine–thymine counter-exchange process can be ignored since no thymine can be detected in the tissue by h.p.l.c.

From Fig. 5 it is possible to conclude that the concentration of 5-fluorouracil at which the Na^+-dependent transport process becomes insignificant is approximately 0·8 mM. At concentrations below this value the transport, in the presence of physiological levels of Na^+, would be expected to be active. If it is assumed that the daily supply of nucleic acids and their components in the diet of the rat is about 50 mg, then this would produce about 8 mg of pyrimidines after total digestion. Thus the concentration of free pyrimidines in the lumen of the jejunum after feeding is likely to be well below 1 mM which may explain why the active transport process for these compounds operates at low concentrations.

To determine the apparent K_m for 5-fluorouracil transport it is necessary to estimate the diffusional component of the transport process (see for example, Alvarado *et al.* 1984). This can be estimated either by using a large enough concentration of the substrate to make the contribution of the mediated transport process insignificant or by using an inhibitor of the transport process and assaying the difference between the inhibited and non-inhibited transport. No fully effective inhibitors for pyrimidine transport in the small intestine are known, therefore either the difference between the Na^+ and the K^+ accumulation at various concentrations could be used to produce diffusion-free transport data or the K_d of the diffusion process could be calculated from the accumulation of 5-fluorouracil at a high (5 mM) medium concentration. The latter method was used as this enabled the kinetic parameters for the transport process operating in both the Na^+- and the K^+-substituted bicarbonate Krebs–Ringer solutions to be evaluated. The results (Fig. 6) indicate that the apparent K_m for 5-fluorouracil transport under physiological (Na^+) conditions was 0·074 mM. The effect of K^+ substitution on this process was to increase significantly ($P < 0·01$) the apparent K_m for the process to 0·394 mM and to reduce the maximum rate of transport. As the apparent K_m for the Na^+-dependent transport process is approximately 70-fold less than the concentration used to determine the K_d for the diffusion process, the error involved in assuming the

accumulation at 5 mM-5-fluorouracil is entirely the result of diffusion will be minimal. The apparent K_m measured for D-glucose transport using the tissue accumulation method (rings) was 1·3 mM (Alvarado *et al.* 1984).

The inhibitory effects of cytosine, orotic acid, uracil and thymine on the accumulation of 5-fluorouracil were investigated at two concentrations (0·05 and 0·1 mM) chosen to be above and below the apparent K_m of the transport process. The results (Fig. 7 and Table 2) indicate that the pyrimidines fall into two clear groups. Cytosine and orotic acid have no influence on the transport process, whereas uracil and thymine are effective inhibitors of 5-fluorouracil transport. This pattern of inhibition was also found for 5-fluorouracil transport into Erlich ascites tumour cells (Yamamoto & Kawasaki, 1981). It is interesting that the inhibitory pyrimidines (uracil and thymine) are both structurally similar to 5-fluorouracil and are both actively transported by the intestine, although thymine is the better inhibitor and uracil is the better substrate for transport. Orotic acid is known to be poorly transported into Novikoff hepatoma cells (Wohlheuter, McIvor & Plagemann, 1980), which is supported by our finding that 0·1 mM-orotic acid is not actively accumulated in either the standard (Na^+) or the K^+-substituted bicarbonate Krebs–Ringer solutions (data not shown).

Our study suggests that there is a high affinity (apparent K_m = 0·074 mM), Na^+-dependent active transport process for pyrimidines structurally related to uracil in rat small intestine. This process has several features in common with pyrimidine transporters reported in other systems (Wohlheuter *et al.* 1980; Yamamoto & Kawasaki, 1981). We conclude that the pyrimidine transporter in the small intestine is similar in both distribution and mode of action to the intestinal D-glucose transporter and fits into the general class of Na^+-dependent active transport processes seen to operate in many epithelial tissues (Schultz & Curran, 1970).

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