Absorption of 5-fluorouracil and related pyrimidines in rat small intestine

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Summary
1. The transport of 5-fluorouracil, uracil and thymine has been studied with isolated jejunal loops of rat small intestine. High performance liquid chromatography was used to identify the pyrimidines and measure their concentrations.

2. When the lumen of the intestine was perfused with 5-fluorouracil or uracil at 0.1 mmol/l or 0.2 mmol/l, the concentration in the serosal secretions was significantly higher than that in the lumen. For thymine the serosal concentration exceeded that in the lumen only at 0.1 mmol/l.

3. Analysis of the mucosal tissue water at the end of the perfusion demonstrated that when the intestinal lumen was perfused with any one of the three pyrimidines at 0.1 mmol/l or 0.2 mmol/l the concentration within the tissue was significantly above that in the lumen.

4. After an initial lag period linear rates of transport from the lumen to the serosal secretions were obtained for all three pyrimidines over a 10-fold concentration range from 0.1 mmol/l to 1 mmol/l.

5. Uracil and thymine inhibited the transmural transport of 5-fluorouracil.

6. The transport of 5-fluorouracil was also studied with a vascularly perfused preparation of rat small intestine. At 0.1 mmol/l the rate of transmural transport of the drug in this preparation was substantially higher than in the jejunal loops. This difference was eliminated by adding 5-fluorouracil to the vascular perfusate, suggesting that the higher transport rate in the vascularly perfused preparation was due to the lower serosal drug concentrations in the mesenteric circulation of the perfused intestine.

7. At a concentration of 5 mmol/l 5-fluorouracil inhibited water transport in the isolated loops and transmural D-galactose transport in the vascular perfusions.

Key words: absorption, 5-fluorouracil, intestine, jejunum, pyrimidine transport, thymine, uracil.

Abbreviation: 5-FU, 5-fluorouracil.

Introduction
Despite the widespread use of 5-fluorouracil (5-FU) as an orally administered chemotherapeutic drug [1], little is known about its absorption from the small intestine. Early observations by Schanker and his colleagues [2-4] suggested that everted sacs of rat jejunum could accumulate 14C-labelled uracil and 5-FU against a concentration gradient, and we have now made a detailed study of the absorption of 5-FU, uracil and thymine with more sophisticated preparations of rat small intestine.

Natural pyrimidines are incorporated into nucleic acids via a variety of metabolites and the effectiveness of 5-FU as a cytotoxic agent is dependent on the formation and subsequent metabolism of fluorouridine 5-phosphate. Uracil can also be reduced to dihydrouracil by the intestine [5]. Such metabolic transformations could make it difficult to interpret the results of transport studies with labelled pyrimidines in intact intestinal preparations, but we have been able to minimize these uncertainties by the use of high performance liquid chromatography (HPLC) to follow the transport of 5-FU and related pyrimidines in the intestine.

In addition to the work by Schanker and his colleagues [2-4] there have been reports of active uracil transport in the frog [6] and the sheep [7]. By
contrast it appears that the entry of 5-FU into tumour cells is by facilitated diffusion rather than active transport [8]. In order to study transmural transport it is essential to use a preparation of intact intestine. We have used two preparations: isolated loops [9, 10], and vascularity perfused intestine [11–13].

Methods

Animals

Male Wistar rats (220–250 g) were fed on a standard laboratory diet (Oxoid modified BIO 41B diet) in conditions of controlled temperature and light. For the isolated loop experiments the rats were deprived of solid food 24 h before the experiment, but were allowed free access to a 0.5% (w/v) D-glucose solution.

Absorption studies with isolated loops of rat intestine

Isolated loops of rat jejunum were perfused by a recirculation technique based on the modification by Parsons & Shaw [10] of the technique described by Fisher & Gardner [9]. The rats were anaesthetized with 21 mg of sodium pentobarbitone. After the flow of oxygenated perfusate through the lumen was established a 10–12 cm segment of jejunum (immediately distal to the ligament of Treitz) was taken from each anaesthetized animal and suspended in liquid paraffin (sp.gr. 0.850–0.865) in a water-jacketed vessel maintained at 37°C [10]. The initial luminal perfusate was supplied from one of two water-jacketed reservoirs filled with 200 ml of Krebs–bicarbonate Ringer with the following composition (mmol/l): NaCl 118, KCl 4.74, KH₂PO₄ 1.18, MgSO₄ 1.18, CaCl₂ 1.27, NaHCO₃ 24.88 and 28 mmol/l D-glucose. The perfusate, which was previously fully equilibrated with O₂ + CO₂ (95:5, v/v), was recirculated through the lumen at 25 ml/min with a pump and the flow was segmented with bubbles of O₂ + CO₂ (95:5, v/v) to facilitate mixing within the intestine and oxygenation of the tissue [9].

The serosal secretions appeared as droplets on the outer surface of the intestinal segment and fell to the bottom of the vessel containing the liquid paraffin in which the intestinal loop was immersed. At 10 min intervals the serosal secretion was collected through a tap at the bottom of the vessel and the secretion volume was measured in calibrated microcentrifuge tubes, after centrifugation to separate it from the small amount of liquid paraffin drawn off with the aqueous layer. A second reservoir allowed the introduction of a second oxygenated perfusate at an appropriate point during a perfusion. For all the experiments described in this paper a switch to the second recirculated luminal perfusate was made directly after the 50 min sample was taken.

In control experiments both reservoirs contained 200 ml of the standard glucose-Ringer, but in the studies of pyrimidine transport the second reservoir contained 200 ml of the glucose-Ringer supplemented with the appropriate concentrations of 5-FU, uracil or thymine. The use of two separate reservoirs in this way ensured that the second perfusate was fully equilibrated with O₂ + CO₂ (95:5, v/v) when it was introduced into the loop. The use of a large volume of recirculated luminal perfusate and the change to fresh perfusate after 50 min helped to minimize any decreases in luminal solute concentrations during the perfusion. This procedure also allowed us to determine that each loop was transporting water at a steady rate before the pyrimidine was introduced. Furthermore, each perfusion had both a control as well as an experimental period so that we could test the comparability of the various groups of perfusions.

The viability of the perfused loops was assessed by the constancy of fluid transport and by the capacity of the loop to transport glucose from the lumen to the serosal secretions against a concentration gradient. Periodic checks were made for gross leaks, and in the rare cases where they occurred the perfusion was abandoned. The volume of the first 10 min sample was small and variable but by 20 min the water flow was established and remained stable in controls for a further 80 min (see control curve in Fig. 3). There was some slowing of the rate of water transport in the last two experimental periods and all perfusions were stopped at 2 h. The mean rate of water transport in the control experiments was 0.317 ± 0.002 (6) ml min⁻¹ g⁻¹ dry wt. and the glucose concentration in the serosal secretions was maintained in the 35–45 mmol/l range throughout the 2 h period. The glucose concentration in the luminal fluid fell from 28 to 27.02 ± 0.36 (6) mmol/l in the first 50 min perfusion period and from 28 to 26.92 ± 0.40 (6) mmol/l in the second (70 min) perfusion period. Glucose was measured with a commercial reagent kit (Boehringer, London). Our observation that the glucose concentration in the serosal secretions remained virtually constant during the perfusion is in agreement with Parsons & Shaw [10], although these authors reported a higher serosal glucose concentration in their experiments.

Vascular perfusion experiments

The technique used for vascular perfusion experiments was essentially that of Hanson & Parsons [11] as modified in this laboratory [12, 13].
A 30–40 cm section of rat jejunum was perfused in situ by recirculating Krebs–bicarbonate Ringer previously fully equilibrated with O₂ + CO₂ (95:5, v/v) and segmented with bubbles of the gas through the lumen at the rate of 7 ml/min. Two separate 20 min perfusion periods were used with 50 ml of fresh fully gassed luminal perfusate being used for each. The vascular bed was perfused through the superior mesenteric artery at the rate of 1.5 ml/min with Krebs–bicarbonate Ringer containing 5 mmol/l D-glucose, 2.5% bovine serum albumin and sufficient bovine erythrocytes to give a 40% packed cell volume. The vascular perfusate was collected from a cannula in the hepatic portal vein and was not recirculated.

Chromatography

Samples of the luminal perfusate and the serosal secretions were analysed directly by HPLC using an Altex 340 isocratic system and an 4.6 mm × 250 mm Ultrasphere ODS column with a mean particle size of 5 μm (Altex, U.K.). The mobile phase was 21 mmol/l KH₂PO₄, pH 3.0, at a flow rate of 1.3 ml/min. Detection was at 254 nm and Fig. 1 shows that the analytical system could be used to estimate separately each of the three pyrimidines we have investigated in this study. Uric acid is normally released into the serosal secretions by the small intestine, but Fig. 1 shows that this purine can easily be distinguished from the pyrimidines. The chromatogram shown in Fig. 1 was obtained with a mixture of uric acid and the three pyrimidines at equal concentrations (0.5 nmol/l). The quantities of the three pyrimidines in the samples which were analysed by HPLC were determined by measurement of peak heights and reference to external standards.

Preparation of tissue samples

At the conclusion of both the isolated loop experiments and the vascular perfusions, the perfused segment of the small intestine was immediately measured, cut open, blotted and cut into measured lengths. Samples of mucosal tissue (approximately 100 mg) were scraped off two of these intestinal sections with a microscope slide and rapidly weighed. One sample was deproteinized for analysis by HPLC and the other was dried at 105°C to give an estimate of the total tissue water/g dry wt. For the isolated loop experiments deproteinization was either by precipitation with 6% perchloric acid or by ultrafiltration through Centricon 30 filters (Amicon Ltd). HPLC measurements of the pyrimidine content of fresh mucosal tissue were made on measured lengths of unperfused mucosal tissue by the method described above and adjacent measured sections were used for determinations of wet and dry weight. The mucosal tissue from the vascular perfusion experiments was deproteinized with 6% perchloric acid and this technique was also used to deproteinize the samples of both luminal and vascular perfusates before HPLC analysis. The chromatograms of the tissue extracts showed a number of peaks in addition to those in Fig. 1 but these did not interfere with the separation of 5-FU, uracil and thymine. The peaks associated with each pyrimidine in the mucosal extracts were identified by analysing a second sample of the extract to which a known amount of the pyrimidine had been added. We also measured the recovery of pyrimidine added to the tissue before deproteinization and obtained a value of 92%.
Measurement of dry weight

Since some of the perfused intestine in both the isolated loop and vascular perfusion experiments was used for mucosal tissue samples, it was necessary to determine the total dry weight and the water content of the perfused intestinal segments indirectly. At the end of each perfusion one of the measured sections of the perfused intestine was placed in a preweighed vial and reweighed to give the wet weight. The vial was then dried at 105°C and the final weighing allowed calculation of the dry weight/cm and thus the total dry weight of the intestine that was perfused.

Radioisotope measurements

In the vascular perfusion experiments [6-14C]-5-FU (Amersham International) was used to study transport of the drug and D-[1-3H]galactose (Amersham International) was used to measure the transport of D-galactose. Radioisotope estimations were made by adding 0.4 ml samples of the luminal and vascular perfusates, or deproteinized tissue extracts, to 4 ml of Optiphase MP (LKB) scintillant, and counting them in a LKB 1216 Rackbeta liquid scintillation counter.

Materials

All chemicals were of analytical grade. Uracil, 5-FU and thymine were purchased from Sigma Chemical Co. Ltd, Poole, U.K. Sodium pentabarbitalone was purchased from May and Baker Ltd, Dagenham, U.K.

Expression of results

The transport of the pyrimidines across the intestinal wall from the lumen is expressed both in terms of the concentration in the serosal secretions or vascular perfusate and as the rate of appearance per g dry wt. of intestinal tissue (μmol min⁻¹ g⁻¹ dry wt.). Tissue content of the pyrimidines is given as concentration in the total tissue water without correction for extracellular space (with one exception noted in the Results). All values are reported as means ± SEM with n shown in parentheses. Statistical comparisons were made by analysis of variance except for the cumulative plots where Student's t-test was used to test the significance of the differences between the regression lines.

Results

Transport of 5-FU in loops of rat jejunum

Four groups of perfusions were performed in order to study the transport of 5-FU from the lumen to the serosal secretions at luminal 5-FU concentrations from 0.1 to 5.0 mmol/l. Fig. 2 shows that 40 min after the addition of (a) 0.1 mmol/l or (b) 0.2 mmol/l 5-FU to the lumen the concentration of the pyrimidine in the serosal secretions reached a

![Graph](image-url)

Fig. 2. 5-FU transport in isolated loops of rat jejunum perfused with the drug at concentrations (a) 0.1, (b) 0.2, (c) 1.0, (d) 5.0 mmol/l. The 5-FU was added after 50 min to give the initial luminal concentration shown by the broken line. The values are means of the 5-FU concentration in the serosal secretion ± SEM of six perfusions (five at 0.2 mmol/l).
steady level which was significantly \( P < 0.01 \) above that in the lumen indicating that the transmural transport of the drug was occurring against a concentration gradient. However, with 5-FU \((c)\) at 1 mmol/l or \((d)\) at 5 mmol/l in the lumen the serosal concentration remained significantly \( P < 0.01 \) below that in the lumen and at 5 mmol/l a steady level was not achieved in the serosal secretions within the perfusion period. It is important to emphasize that the concentrations reported in Fig. 2 were obtained from HPLC analysis of the serosal secretions and therefore are true values for 5-FU concentration and do not depend on the assumption, as was the case with earlier studies using everted sacs, that there has been no metabolism of radioactive 5-FU. No peaks were detected at the retention time for 5-FU in the 50 min serosal secretions.

**Effect of 5-FU on water transport in isolated loops**

The rate of transmural pyrimidine transport is dependent on the rate of water transport as well as the concentration in the serosal secretions. Fig. 3 shows the cumulative appearance of serosal fluid in the controls throughout the 2 h perfusion period and indicates that the rate was constant except for the last 20 min. However, when 5 mmol/l 5-FU was present in the lumen from the 50 min time point, the rate of water transport was reduced significantly \( P < 0.001 \). Although the decrease in rate of water transport was apparent after the intestine had been in contact with the 5-FU for only 10 min the inhibition appeared to be progressive since it increased from 23% in the 60–100 min period to 36% in the final 20 min. The addition of 5-FU at 1 mmol/l reduced the rate of water transport by 9% to 0.288 ± 0.006 (6) ml min\(^{-1}\) g\(^{-1}\) dry wt. \( P < 0.05 \) when compared with the control, although this result has been omitted from Fig. 3 for the sake of clarity. Lower concentrations of 5-FU had no effect on water transport. The glucose concentration in the serosal secretions was unaffected by the presence of 5-FU in the lumen, but it should be recognized that the lower rates of water transport at 1 and 5 mmol/l 5-FU mean that the cumulative rate of glucose transport will be correspondingly reduced. Previous work by Gardner & Heading [14] has shown that administration of the drug \textit{in vivo} caused a severe inhibition of water transport, although it should be noted that this effect was not observed until the third day.

**Rates of transepithelial transport of 5-FU**

When water transport is taken into account, as shown in Fig. 4 the cumulative appearance of 5-FU with time was linear at all four luminal concentrations after an initial lag period. This is true despite the lower rates of water transport in the presence of 1 and 5 mmol/l 5-FU.

**Tissue concentrations of 5-FU**

The data in Fig. 2 indicate that 5-FU transport across the wall of the intestine occurs against a concentration gradient but provide no indication of whether this is a function of the apical or the basolateral membrane. In order to provide evidence on this point we carried out an HPLC analysis of a mucosal scrape taken from each perfused loop at the end of the perfusion. For each group of perfusions Table 1 compares the 5-FU concentration in the mucosal tissue at the end of the perfusion with the final luminal and serosal 5-FU concentrations. The mucosal tissue concentration of 5-FU at the end of the perfusion is significantly \( P < 0.01 \) higher than the final luminal concentration in perfusions with 5-FU at 0.1 mmol/l or 0.2 mmol/l, and significantly \( P < 0.01 \) lower with a luminal concentration of 5 mmol/l. Although the mucosal
surface was blotted before a sample of mucosal tissue was scraped off for analysis, some carry-over of luminal fluid did occur. No correction was made for this carry-over except for the perfusions with 5-FU at 5 mmol/l. For these perfusions the tissue concentrations of 5-FU have been corrected by subtracting an estimate of carry-over [0.44 ± 0.04 (3) mmol/l] which was obtained by taking tissue samples from loops perfused for 1 min with 5-FU at 5 mmol/l.

The results in Table 1 indicate that it is the movement across the brush border membrane into the cell which is occurring against a concentration gradient. The fact that all the tissue/serosal concentration ratios in Table 1 are above 1 indicate that the movement of 5-FU across the basolateral membrane from the mucosa into the serosal secretions is always down a concentration gradient. For all four groups of perfusions the tissue 5-FU concentration was significantly (P < 0.01) higher than that in the serosal secretions.

Table 1 also shows that the final luminal concentrations of 5-FU were lower than the initial values although none of these differences is statistically significant. If the amounts of 5-FU found in the serosal secretions and in the mucosal tissue are added to that remaining in the luminal perfusate we get an average recovery of 95%, suggesting that some 5-FU may be metabolized.

Transport of uracil and thymine in intestinal loops

Although previous investigators have examined the uptake of uracil in everted sacs of small intestine, this has been done only with radioactively labelled pyrimidine [2-4, 6, 7]. Consequently we considered that it was important to re-examine the absorption of the natural pyrimidines using isolated loops of intestine and HPLC. Groups of six perfusions were carried out at three luminal concentrations of uracil and thymine and Fig. 5 shows the pyrimidine concentrations in serosal secretions collected during the entire perfusion, since endogenous uracil is released during the 50 min control period. When 0.1 mmol/l or 0.2 mmol/l uracil was perfused through the lumen it appeared in the serosal secretions at a significantly (P < 0.01) higher concentration than that in the lumen. With thymine, however, the concentration in the serosal secretions exceeds that in the lumen only when it is perfused at a concentration of 0.1 mmol/l. At a luminal concentration of 1 mmol/l both of the natural pyrimidines appeared in the serosal secretions at concentrations below the luminal level. Cumulative plots of uracil and thymine transport are given in Fig. 6 and indicate that transmural transport of the natural pyrimidines is linear with time over the range of concentrations from 0.1 to 1.0 mmol/l. At the lower concentrations uracil (Fig. 6a) is transported significantly (P < 0.01) faster than thymine (Fig. 6b),

![Fig. 4. Cumulative serosal appearance of 5-FU. The regression lines give the following values for the rate of 5-FU transport (μmol min⁻¹ g⁻¹ dry wt.) at each luminal concentration: O, 5 mmol/l, 0.421 ± 0.010; ■, 1 mmol/l, 0.129 ± 0.003; ▲, 0.2 mmol/l, 0.098 ± 0.001; ●, 0.1 mmol/l, 0.056 ± 0.002; n = 6, except n = 5 at 0.2 mmol/l.]

<table>
<thead>
<tr>
<th>Initial luminal concn. of 5-FU (mmol/l)</th>
<th>n</th>
<th>Tissue 5-FU at 120 min (mmol/l)</th>
<th>Final luminal concn. of 5-FU (mmol/l)</th>
<th>Tissue/serosal ratio</th>
<th>Serosal 5-FU at 120 min (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>6</td>
<td>0.506 ± 0.124</td>
<td>0.093 ± 0.004</td>
<td>5.39 ± 0.13</td>
<td>0.186 ± 0.014</td>
</tr>
<tr>
<td>0.2</td>
<td>5</td>
<td>0.632 ± 0.098</td>
<td>0.189 ± 0.005</td>
<td>3.34 ± 0.48</td>
<td>0.286 ± 0.023</td>
</tr>
<tr>
<td>1.0</td>
<td>6</td>
<td>0.993 ± 0.147</td>
<td>0.940 ± 0.026</td>
<td>1.06 ± 0.17</td>
<td>0.634 ± 0.037</td>
</tr>
<tr>
<td>5.0</td>
<td>6</td>
<td>3.03 ± 0.27</td>
<td>4.91 ± 0.087</td>
<td>0.62 ± 0.05</td>
<td>2.47 ± 0.21</td>
</tr>
</tbody>
</table>
although at 1 mmol/l the rates of serosal appearance are the same.

Table 2 gives the concentrations of uracil and thymine in fresh mucosal tissue and in the mucosal tissue scrapes made at the ends of the perfusions with the natural pyrimidines. The Table shows that the transmural transport against a concentration gradient at the lower luminal concentrations was a consequence of accumulation within the mucosal tissue. For thymine the tissue concentration was significantly above that in the serosal secretions \((P < 0.05)\) at a luminal concentration of 0.1 mmol/l; however, with 1 mmol/l luminal thymine the tissue concentration remained below that in the lumen. Table 2 gives the final luminal concentrations of uracil and thymine and indicates that there was no significant decrease in luminal concentrations under the conditions used for these experiments. The data in Table 2 also show that perfusion with 5-FU does not lead to an exchange of luminal 5-FU for cellular uracil since the final tissue level of the natural pyrimidine was unaffected by 5-FU uptake.
TABLE 2. Mucosal tissue concentrations of uracil and thymine

<table>
<thead>
<tr>
<th>Initial luminal concn. of pyrimidine (mmol/l)</th>
<th>n</th>
<th>Tissue at Final luminal concn. of pyrimidine (mmol/l)</th>
<th>Tissue/lumen ratio</th>
<th>Serosal secretion at 120 min (mmol/l)</th>
<th>Tissue/serosal ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Uracil [concn. in fresh mucosal tissue = 0.555 ± 0.068(18)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>0.453 ± 0.114</td>
<td>0.094 ± 0.004</td>
<td>4.70 ± 1.03</td>
<td>0.400 ± 0.037</td>
</tr>
<tr>
<td>0.2</td>
<td>6</td>
<td>0.612 ± 0.087</td>
<td>0.189 ± 0.006</td>
<td>3.24 ± 0.52</td>
<td>0.478 ± 0.027</td>
</tr>
<tr>
<td>1.0</td>
<td>6</td>
<td>0.901 ± 0.019</td>
<td>0.898 ± 0.055</td>
<td>1.00 ± 0.13</td>
<td>0.815 ± 0.101</td>
</tr>
<tr>
<td>0.2 (00.2 mmol/l 5-FU)</td>
<td>6</td>
<td>0.582 ± 0.088</td>
<td>0.205 ± 0.009</td>
<td>2.90 ± 0.53</td>
<td>0.289 ± 0.024</td>
</tr>
<tr>
<td>(b) Thymine [concn. in fresh mucosal tissue = 0.076 ± 0.018(6)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>6</td>
<td>0.327 ± 0.058</td>
<td>0.092 ± 0.003</td>
<td>3.58 ± 0.70</td>
<td>0.173 ± 0.016</td>
</tr>
<tr>
<td>1.0</td>
<td>6</td>
<td>0.635 ± 0.068</td>
<td>0.989 ± 0.027</td>
<td>0.64 ± 0.06</td>
<td>0.784 ± 0.026</td>
</tr>
</tbody>
</table>

Fig. 7. Effects of luminal uracil and thymine on the serosal appearance of 5-FU. All pyrimidines added after 50 min; n = 6 except for 5-FU at 0.2 mmol/l alone, where n = 5. The regression lines give the following values for the rate of 5-FU transport (μmol min⁻¹ g⁻¹ dry wt.): ○, 0.2 mmol/l 5-FU, 0.098 ± 0.001; ■, 0.2 mmol/l 5-FU + 0.2 mmol/l uracil, 0.069 ± 0.001; ▲, 0.2 mmol/l 5-FU + 0.2 mmol/l thymine, 0.037 ± 0.001; ●, 0.2 mmol/l 5-FU + 0.2 mmol/l thymine + 1.0 mmol/l uracil, 0.032 ± 0.001. Although the data are omitted for the sake of clarity, the rate of 5-FU transport with 1.0 mmol/l thymine was the same as that with 1.0 mmol/l uracil.

Inhibition of 5-FU transport by uracil and thymine

In order to explore the extent to which 5-FU is transported by the transport process responsible for uptake of the natural pyrimidines we have investigated the effects on 5-FU transport of adding uracil or thymine at concentrations of 0.2 mmol/l and 1.0 mmol/l. Fig. 7 shows the cumulative appearance of 5-FU in the serosal secretions when the lumen was perfused with the drug at 0.2 mmol/l and indicates that both uracil and thymine inhibited the rate of 5-FU transport. At 0.2 mmol/l, thymine was a more effective inhibitor than uracil although at 1.0 mmol/l there was no significant difference between the inhibitory effects of the two natural pyrimidines. (The data for 5-FU transport in the presence of 1.0 mmol/l thymine are not shown since the cumulative plot does not differ significantly from the line with 1.0 mmol/l uracil.) We have also studied the transport of uracil and thymine when intestinal loops are perfused with mixtures of the two natural pyrimidines and the results show that each pyrimidine inhibits the transport of the other (data not shown).

Table 3 shows the concentration of 5-FU in the mucosal tissue at the end of the inhibition experiments and reveals another difference between the inhibitory actions of thymine and uracil. When the natural pyrimidine is present at a concentration of 0.2 mmol/l, uracil causes a significant reduction in the tissue 5-FU concentration whereas thymine does not despite the fact that thymine is the more effective inhibitor of 5-FU appearance in the serosal secretions. 5-FU also inhibited the transport of uracil (see Table 2) and thymine (data not shown). Again the final luminal concentrations in Table 3 show no significant changes from the initial values.

Transport of 5-FU during vascular perfusion

In the loop experiments it was possible that the pyrimidine transport was influenced by the presence of α-glucose in the lumen or by solvent drag effects due to the water transport [15, 16]. Consequently we have employed the vascular perfusion technique to study 5-FU transport under conditions...
5-Fluorouracil intestinal absorption

Table 3. Mucosal tissue concentrations of 5-fluorouracil (5-FU) after perfusion with 5-FU at 0.2 mmol/l together with either uracil or thymine

<table>
<thead>
<tr>
<th>Additional pyrimidine</th>
<th>n</th>
<th>Tissue 5-FU at 120 min (mmol/l)</th>
<th>Final luminal concn. of 5-FU (mmol/l)</th>
<th>Tissue/lumen ratio</th>
<th>Serosal concn. of 5-FU at 120 min (mmol/l)</th>
<th>Tissue/serosal ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5</td>
<td>0.632±0.098</td>
<td>0.189±0.005</td>
<td>3.34±0.48</td>
<td>0.286±0.023</td>
<td>2.19±0.22</td>
</tr>
<tr>
<td>Uracil (0.2 mmol/l)</td>
<td>6</td>
<td>0.307±0.015</td>
<td>0.198±0.009</td>
<td>1.56±0.10</td>
<td>0.207±0.014</td>
<td>1.50±0.05</td>
</tr>
<tr>
<td>Uracil (1.0 mmol/l)</td>
<td>6</td>
<td>0.208±0.024</td>
<td>0.187±0.009</td>
<td>1.13±0.14</td>
<td>0.148±0.005</td>
<td>1.39±0.15</td>
</tr>
<tr>
<td>Thymine (0.2 mmol/l)</td>
<td>6</td>
<td>0.713±0.100</td>
<td>0.206±0.007</td>
<td>3.53±0.53</td>
<td>0.167±0.014</td>
<td>4.55±0.84</td>
</tr>
<tr>
<td>Thymine (1.0 mmol/l)</td>
<td>6</td>
<td>0.167±0.014</td>
<td>0.182±0.009</td>
<td>0.94±0.11</td>
<td>0.130±0.005</td>
<td>1.30±0.12</td>
</tr>
</tbody>
</table>

Fig. 8. Transport of 5-FU in vascularly perfused intestine. 5-FU added to the luminal perfusate after a 20 min control period (not shown). (a) Vascular appearance of 5-FU with 1 mmol/l luminal 5-FU. (b) Cumulative appearance of 5-FU in the vascular perfusate during the second 20 min of perfusion. The regression lines give the following values for the rate of 5-FU transport (μmol min⁻¹ g⁻¹ dry wt.): ■, 1 mmol/l 5-FU, 0.111±0.001; ▲, 0.1 mmol/l 5-FU, 0.091±0.001; ●, 0.1 mmol/l, 5-FU with 0.1 mmol/l 5-FU added to the vascular perfusate, 0.057±0.001. For 5-FU at 1 mmol/l n=7; for 5-FU at 0.1 mmol/l n=4 in each group.

in which there is no D-glucose in the lumen and water transport is minimal. A group of perfusions was performed in which the lumen of 30–40 cm sections of rat jejunum was perfused for a 20 min control period with pyrimidine-free Krebs-bicarbonate Ringer, after which the luminal perfusate was replaced with an identical solution containing 1 mmol/l ¹⁴C-labelled 5-FU. Fig. 8(a) shows the time course of radiolabelled 5-FU appearance in the vascular effluent during the second 20 min perfusion period. Rapid clearance of the submucosal spaces is a feature of the vascularly perfused preparation and this kept the 5-FU concentration at only 10% of the serosal concentration found in loops perfused with 5-FU at 1 mmol/l. The top line in Fig. 8(b) shows the cumulative rate of radio-
of 5-FU at 0.1 mmol/l to the vascular perfusate reduced the transport rate to that found in the experiments with isolated loops.

**Tissue concentrations of 5-FU during vascular perfusion**

In another group of experiments the vascular perfusion technique and 5-FU estimation by HPLC were used to compare the uptake of 5-FU into the mucosal tissue from the vascular perfusate with that from the lumen. After perfusion for 20 min with 5-FU at 5 mmol/l in the vascular perfusate the concentration in the mucosal tissue was 0.71 ± 0.09 (4) mmol/l and when perfusions were performed with the same concentration of 5-FU in the lumen rather than the vascular perfusate the tissue 5-FU concentration was significantly (P<0.01) higher at 2.88 ± 0.35 (3) mmol/l. This value agrees closely with that found in the isolated loop experiments with 5-FU at 5 mmol/l (Table 1).

**Influence of 5-FU on D-galactose transport during vascular perfusion**

A series of vascular perfusion experiments was carried out as described previously [12, 13] to examine the effect of 5 mmol/l 5-FU on the rate of transport of 5 mmol/l D-[1-3H]galactose from the lumen to the vascular bed. The 5-FU was added to either the luminal or vascular perfusate at the end of the first perfusion period and in both cases the rate of D-galactose transport 10 min after introduction of the drug was significantly (P<0.01) below that in the control group, despite the fact that there was no net water transport in the controls or in either of the experimental groups under these conditions. In the last 10 min of the perfusion the control rate of D-galactose transport was 1.54 ± 0.02 (7) µmol min⁻¹ g⁻¹ dry wt. and this was reduced to 1.25 ± 0.02 (5) µmol min⁻¹ g⁻¹ dry wt. with luminal 5-FU and 1.26 ± 0.01 (4) µmol min⁻¹ g⁻¹ dry wt. with vascular 5-FU.

**Discussion**

The results in Figs. 2 and 4 show that 5-FU and the related natural pyrimidines are transported across the wall of the rat small intestine against a concentration gradient when they are present in the lumen at concentrations in the 0.1–0.2 mmol/l range. The previous work by Schanker and his colleagues [2–4], which suggested that both uracil and 5-FU were transported actively by everted sacs of rat small intestine, had two crucial limitations. First of all, since the earlier workers studied pyrimidine transport only with low concentrations of labelled compounds, it was unclear what part metabolism played in the apparent accumulation of the pyrimidines. Secondly, the experiments with the everted sac preparation did not indicate whether the apparent movement of solute against a concentration gradient occurred at the luminal or basolateral membranes of the epithelial cells. In our experiments with isolated loops, pyrimidine transport was measured by HPLC, so that it was clear that the concentration of 5-FU or uracil in the serosal secretions was significantly higher than that in the lumen once a steady rate of transport was attained at luminal concentrations of 0.1 or 0.2 mmol/l. Furthermore, when we analysed the pyrimidine content of the mucosal tissue at the end of the perfusion experiments, we found that the tissue concentrations of all three pyrimidines were higher than those in the serosal secretions (Tables 1 and 2). This shows that it is the brush border of the enterocyte rather than the basolateral membrane that is the site at which pyrimidine transport occurs against a concentration gradient at luminal concentrations of 0.2 mmol/l or below. Furthermore, the final tissue/lumen ratios for 5-FU and uracil are significantly (P<0.01) greater than one in loops perfused at a concentration of 0.2 mmol/l and at a luminal concentration of 0.1 mmol/l all three pyrimidines had final tissue/lumen ratios significantly (P<0.01) greater than one.

Another ambiguity which arose from the earlier work was the possibility that the apparent accumulation of labelled pyrimidines at low concentrations could have been due to an exchange of the labelled compounds in the luminal solution with unlabelled endogenous pyrimidines, particularly uracil. We have found (Table 2) that the transmural transport of low concentrations of both uracil and thymine is associated with tissue concentrations which are significantly above those in both the lumen and the serosal secretions. With 5-FU we might have expected to see some exchange of the drug in the lumen with intracellular uracil but Table 2 shows that the uracil concentration in the tissue at the end of the experiment was unaffected by the presence of 5-FU in the lumen.

Once the concentration of a solute in the serosal secretion has been determined, the rate of transmural transport can be estimated provided the volume of the serosal secretion is known. The most reliable measure of the rate of transport can be obtained by plotting the cumulative serosal appearance against time. Figs. 4 and 6 show this type of plot for 5-FU, uracil and thymine, and indicate that after an initial lag period the rates of transport became linear at all concentrations tested. At luminal concentrations of 0.1 or 0.2 mmol/l, where pyrimidine transport occurs against a concentration
gradient, uracil is transported most rapidly, followed by 5-FU and thymine. At a luminal concentration of 1 mmol/l, on the other hand, uracil and thymine are transported at the same rate and this is significantly \( P < 0.01 \) greater than the rate for 5-FU. It is important to emphasize that at 1 mmol/l none of the pyrimidines is transported against a concentration gradient, and consequently at this concentration any differences in transport rates may merely reflect different rates of passive diffusion.

Since 5-FU does not occur naturally it is reasonable to expect that it is transported in the intestine by means of the transport system for uracil or thymine. The data shown in Fig. 7 indicate that both of the natural pyrimidines inhibit 5-FU transport and suggest that all three pyrimidines share a common transport system. However, the tissue concentrations reported in Table 3 indicate that the principal effect of uracil is on 5-FU uptake, since it reduces the final tissue 5-FU concentration, whereas thymine may also influence basolateral exit since 0.2 mmol/l thymine inhibits transmural transport without affecting the tissue 5-FU concentration.

Transmural transport studies with intestinal loops suffer from two main disadvantages: it is necessary to have relatively high luminal glucose to drive water transport, and the absence of the mesenteric circulation means that the submucosal spaces are not cleared of transported solutes in the normal way. Our measurements of the rate of pyrimidine transport in isolated loops could have been influenced by both of these factors and we investigated this possibility by studying 5-FU transport in vascularly perfused intestine under conditions in which there was no \( \alpha \)-glucose in the lumen and the rate of water transport was negligible. When the data presented in Fig. 8(b) are compared with those in Fig. 4, it is clear that there are some significant differences in the rate of 5-FU transport by these two preparations. If they are important for pyrimidine transport, solvent drag effects [15, 16] should be particularly noticeable at a luminal concentration of 1 mmol/l, where a substantial fraction of the 5-FU transport is by passive diffusion and a significant reduction (14\%) in the transport rate was observed in the vascular perfusions. On the other hand, the presence of luminal \( \alpha \)-glucose in the isolated loops might be expected to influence 5-FU most clearly at the low concentrations, where transport occurs against a concentration gradient, and we did find at 0.1 mmol/l that the 5-FU transport rate was 62\% higher in the vascular perfusions where no luminal \( \alpha \)-glucose was present. However, it seems likely that this difference was due to the low 5-FU concentration at the serosal face of the epithelial cells in the vascularly perfused intestine, since increasing the vascular 5-FU concentration by the addition of 5-FU at 0.1 mmol/l to the vascular perfusate reduced the transport rate to that found in the loops.

Thus, our vascular perfusion data indicate that there may be a small solvent drag effect at high 5-FU concentrations where diffusion predominates, but they provide no evidence for inhibition of pyrimidine transport by sugars in agreement with Schanker & Tocco [3]. On the other hand, our data do show that the higher concentrations of 5-FU did reduce the rate of water transport and caused a corresponding reduction in the transmural transport of \( \alpha \)-glucose. This inhibition of sugar transport was confirmed by the vascular perfusion experiments in which the transport of \( \alpha \)-galactose was inhibited by the addition of 5-FU at 5 mmol/l to either the luminal or vascular perfusate.

Our data on 5-FU transport provide some suggestions for the clinical use of orally administered 5-FU, if one is prepared to extrapolate from the rat to man. First of all, in order to take advantage of the capacity of the intestine to accumulate pyrimidines it seems advisable to use low doses, repeatedly if necessary, rather than to rely on a smaller number of high doses, where uptake will be mainly by diffusion. The use of low doses would maximize uptake while at the same time minimizing the exposure of the gastrointestinal tract to the drug. The inhibitory effects of uracil and thymine also indicate the value of minimizing the dietary intake of nucleic acids during 5-FU treatment, although it is impossible to prevent the appearance of some nucleic acid derivatives in the intestinal lumen owing to the endogenous release of nucleic acids as a result of the normal cellular turnover in the small intestine [17].

Finally, our vascular perfusion experiments with 5-FU added to the vascular perfusate indicate that the injection of the drug into the circulatory system would not prevent its uptake by the intestinal mucosa, although the amount entering the epithelial cells across the basolateral membranes is likely to be smaller than the uptake across the brush border membranes when the same concentration is present in the lumen. However, it is interesting to note that 5-FU at 5 mmol/l was as inhibitory for \( \alpha \)-galactose transport when it was added to the vascular perfusate as it was when it was present in the lumen, despite the observation that the tissue concentration was four times larger in the latter case.

The intestinal preparations used in this study are not suitable for an investigation of the kinetic characteristics of pyrimidine transport. This problem has been tackled in a separate study of pyrimi-
dine accumulation by everted rings of rat intestine [18], which makes it clear that the transport of 5-FU, uracil and thymine is Na⁺-dependent. The work with intestinal rings also shows that the capacity of the intestine for tissue accumulation shows the same pyrimidine specificity that we have found for transmural transport in this study.

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References