Journal of Physiology (1993), **468**, *pp.* 311–324
With 6 figures
Printed in Great Britain

PURINE NUCLEOSIDE TRANSPORT AND METABOLISM IN ISOLATED RAT JEJUNUM

BY RUTH A. STOW AND J. R. BRONK
From the Department of Biology, University of York, York YO1 5DD

(*Received 2 March 1992*)

SUMMARY

1. The absorption and metabolism of purine nucleosides and their constituent bases has been investigated by perfusion through the lumen of isolated loops of rat jejunum. In control perfusions and those with luminal purines or purine nucleosides, high-performance liquid chromatography (HPLC) revealed uric acid as the only detectable purine in the mucosal epithelial layer and the serosal secretions unless the xanthine oxidase inhibitor allopurinol was present.

2. Adenosine (0·5 mM) was quantitatively deaminated to inosine in the lumen after perfusion for 30 min.

3. Luminal inosine and hypoxanthine (0·15–1·0 mM) increased the serosal uric acid concentration significantly ($P < 0·001$); at 0·5 and 1·0 mM the nucleoside gave a significantly greater ($P < 0·01$) rate of serosal uric acid appearance than the base.

4. Luminal guanosine (0·05–0·50 mM) and guanine (0·05–0·15 mM) increased the serosal uric acid concentration significantly ($P < 0·001$); with 0·15 mM nucleoside the serosal uric acid appeared significantly faster ($P < 0·01$) than it did from the base.

5. Luminal allopurinol (0·3 mM) inhibited xanthine oxidase by 80% and reduced serosal purine appearance significantly ($P < 0·01$) from luminal guanine, hypoxanthine and inosine. With allopurinol, guanosine (0·1 and 0·15 mM) and inosine (0·1–1·0 mM) gave significantly higher ($P < 0·01$) total serosal purine concentrations than their respective bases.

6. Inosine and guanosine were cleaved to their respective bases plus ribose phosphate by the action of a cytoplasmic nucleoside phosphorylase, which was found to have widely different Michaelis constants (K_m; 318 ± 45 and $41·4 \pm 3·6$ μM for inosine and guanosine, respectively) and maximum velocities (V_{max}; $79·3 \pm 4·0$ and $20·5 \pm 0·05$ μmol min^{-1} (mg protein)$^{-1}$ for inosine and guanosine, respectively).

7. We conclude that hypoxanthine and guanine absorbed by rat small intestine are oxidized to uric acid which is released in the serosa. The corresponding nucleosides are split by phosphorolysis after absorption and the resulting purine bases are converted to uric acid which appears on the serosal side with similar quantities of ribose phosphate.

INTRODUCTION

Wilson & Wilson (1962) observed that radiolabelled purine and pyrimidine bases were taken up by everted sacs of both the rat and hamster intestine but were not

concentrated within the sacs, and that nucleotides placed in the medium did not enter the serosal solution. It was found that studies of purine transport were complicated because the purines, unlike the pyrimidines, are rapidly oxidized to uric acid in the intestinal epithelium by xanthine oxidase. It had previously been demonstrated with the everted sac technique that the pyrimidines are absorbed from the small intestine of rats (Schanker & Tocco, 1960, 1962), hamsters (Schanker & Tocco, 1962) and frogs (Czaky, 1961; Schanker & Tocco, 1962) by an active transport process. Competition experiments by Schanker, Jeffery & Tocco, (1963) suggested a common transport mechanism for purines and pyrimidines. Berlin & Hawkins (1968) showed that hypoxanthine, xanthine and uric acid are secreted by the intestine. The transport of uric acid by everted sacs of rat small intestine was reported to be passive (Oh, Dossetor & Beck, 1967), and, although Kahn, Wilson & Crawhall (1975) were able to confirm the oxidation of hypoxanthine and xanthine to uric acid in both rat and hamster intestine they could find no evidence for concentration gradients between the tissue fluid and the medium on either side of the everted sacs. Harms & Stirling (1977) demonstrated that purine nucleosides were absorbed by rabbit ileum.

Recent work on the uptake of pyrimidines into tissue rings and isolated loops of rat jejunum employing high-performance liquid chromatography (HPLC) techniques demonstrated that the free bases were not metabolized to any great extent (Bronk & Hastewell, 1987; Bronk, Lister & Lynch, 1987). However, studies of the transport and metabolism of the pyrimidine nucleosides using the isolated-loop preparation revealed considerable differences in the metabolism of the various pyrimidine nucleosides, (Bronk & Hastewell, 1988). The nucleosides thymidine and uridine were found to be split by the cytoplasmically located pyrimidine nucleoside phosphorylase leading to the appearance of thymine or uracil in the serosal secretions. Cytidine was not split by the phosphorylase. Comparisons of nucleoside and base transport revealed that in the case of uridine, the rate of serosal uracil appearance was significantly greater from the nucleoside than from the equivalent luminal concentration of base. Such an advantage was not observed when perfusions with luminal thymidine were compared with those in which thymine was present in the lumen.

Earlier purine transport work was dependent on the use of radioisotopes and metabolic studies were limited to the use of specific enzymes and paper partition chromatography to analyse the nucleic acid derivatives in the biological fluid. Parsons & Shaw (1983*a*) investigated the translocation of the purine bases hypoxanthine, guanine, adenine and xanthine across the rat jejunum using the isolated-loop preparation in conjunction with HPLC analysis and determined that significant metabolism to uric acid occurred.

With regard to the transport of nucleosides in other tissues, Wheeler & Yudilevich (1988) studied the transport of adenosine across guinea-pig placenta and found evidence for carrier-mediated uptake followed by rapid metabolic transformation, with extensive conversion to uric acid.

From the previous studies of the absorption of purine bases by isolated loops of rat small intestine (Parsons & Shaw, 1983*b*), it seemed likely that metabolism is important in purine transport across the intact cell from luminal purine nucleosides. The aim of this study was to use HPLC analytical techniques to compare the

transport and metabolism of purine nucleosides and bases across the epithelial layer of isolated loops of rat jejunum in the presence and absence of allopurinol, which inhibits xanthine oxidase.

METHODS

Animals. Male Wistar rats were fed *ad libitum* (rat and mouse diet, Bantin & Kingham Ltd, North Humberside) until they weighed 200–250 g and then deprived of food, except for free access to 0·5% (w/v) D-glucose solution for approximately 18 h prior to the experiment. Each animal was anaesthetized with an intraperitoneal injection of sodium pentobarbitone (10 mg (100 g body weight)$^{-1}$).

Perfusion medium. The perfusion medium was bicarbonate Krebs–Ringer solution, containing (mM): 120 NaCl, 4·5 KCl, 1 $MgSO_4$, 1·8 Na_2HPO_4, 0·2 NaH_2PO_4, 25 $NaHCO_3$, 1·25 $CaCl_2$ and 28 D-glucose. It was gassed for at 45 min at 37·5 °C with 95% O_2–5% CO_2 to give a pH of 7·42 and recirculated through the lumen of the intestine at 23 ml min^{-1}. The flow was segmented with bubbles of 95% O_2–5% CO_2 (Fisher & Gardner, 1974) to produce a stirring effect and facilitate oxygenation (Hanson & Parsons, 1976).

Perfusion technique. Isolated jejunal loops 15–20 cm in length were perfused for 2 h by the technique of Fisher & Gardner (1974) modified as described by Bronk & Hastewell (1988). The loops were suspended in liquid paraffin (specific gravity 0·83–0·86) at 37·5 °C. The first 50 min was a control period during which the loop was perfused with 200 ml of perfusate containing 28 mM glucose but no other transport substrate. Serosal secretion samples were collected every 10 min and after the 50 min sample perfusion was switched to a second 200 ml of perfusate containing the relevant transport substrate in addition to glucose for a further 70 min. The viability of the preparation was assessed by its ability to maintain a steady water flow and to transport D-glucose actively. The rate of appearance of the serosal fluid was 0·197 ± 0·002 ml min^{-1} (g dry wt)$^{-1}$ (mean ± S.E.M.; $n = 30$). This was unaffected by the addition of purines or purine nucleosides to the luminal medium, except at the highest concentrations of guanosine (0·5 mM) or inosine (1 mM). The mean serosal D-glucose concentration was 55·2 ± 0·4 mM ($n = 30$), giving a serosal/luminal ratio of approximately 2. When allopurinol, the inhibitor of the enzyme xanthine oxidase, was used it was added at 0·3 mM to both perfusates.

Preparation of serosal and luminal samples for analysis of purine metabolites. The volumes of serosal samples were measured after centrifugation at 1800 *g* for 2 min to enable a meniscus between any paraffin and the secretion to form. Luminal perfusate samples were taken initially and at intervals of 20 min or less during the perfusion. Both serosal secretion and perfusate samples were filtered through 0·45 μm Whatman cellulose nitrate filters before analysis by HPLC.

Preparation of tissue samples for HPLC analysis. A 4 cm portion of intestine was cut open lengthwise, gently blotted, the mucosa removed by scraping with a microscope slide, frozen in liquid nitrogen and weighed before being homogenized in 1 ml of 6% perchloric acid. The resulting homogenate was then centrifuged at 1800 *g* for 2 min. An aliquot of the supernatant was neutralized with potassium hydroxide (0·6 M) and rapidly frozen in liquid nitrogen before being recentrifuged at 1800 *g*. The resulting supernatant, having been filtered through a 0·45 μm cellulose nitrate filter, was analysed by HPLC.

HPLC analysis of samples. The samples were analysed isocratically with 25 mM $NH_4H_2PO_4$ (pH 4·5) in 3% methanol as the mobile phase and a flow rate of 1 ml min^{-1}. For each analysis, 20 μl aliquots of filtered sample were injected into the HPLC apparatus fitted with a 4·6 mm i.d. × 250 mm length Hypersil 5 ODS column (HPLC Technology, Macclesfield, Cheshire). The elution profile was measured using a Beckman 165 dual-wavelength detector set to measure the absorbance of the eluant at 254 and 280 nm.

Determination of the fate of the ribose group of the nucleoside subsequent to phosphorolysis. A Spherogel carbohydrate column (Beckman Instruments Inc., High Wycombe, Bucks) 7·5 mm × 30 cm and a Beckman 156 refractive index detector were used for sugar analysis. Freshly de-gassed double-distilled water was used as the mobile phase with a flow rate of 0·6 ml min^{-1}. The column temperature was maintained at 80 °C by means of a heating block. Lactic acid has strong interactions with the packing material and was removed from samples by a Bond Elut anion-exchange column (Analytichem International, Harbor City, CA, USA) and the column eluate was

subsequently filtered through 0·45 μm filter units. Under these conditions the Spherogel column had the following retention times: glucose, 7·0 min; ribose phosphate, 3·4 min; ribose, 13·2 min.

Preparation of purine nucleoside phosphorylase from jejunal mucosa. The method used for the partial purification of mucosal homogenates was essentially that of Bronk & Hastewell (1988), involving a $CaCl_2$ precipitation step, differential centrifugation and collection of the fraction precipitating between 40 and 70% ammonium sulphate saturation. An additional purification step was incorporated using a Sephadex G200 column to separate purine nucleoside phosphorylase from xanthine oxidase.

Purine nucleoside phosphorylase assays. A 50 μl aliquot of the column eluate with purine nucleoside phosphorylase activity was added to an equal volume of reaction medium (mM): 40 imidazole (pH 7·5), 80 Na_2HPO_4 (pH 7·5), 160 NaCl, 100 mannitol, 1 nucleoside substrate. This reaction mixture was incubated for 1 min at room temperature and the reaction stopped with 100 μl 6% perchloric acid. After centrifugation at 1800 *g* for 2 min a 100 μl aliquot of the resulting supernatant was neutralized in 68 μl of 0·6 M KOH, rapidly frozen in liquid nitrogen, thawed and recentrifuged at 1800 *g* for 2 min. Samples (20 μl) of the final supernatant were analysed by HPLC.

Assay of protein. The protein concentrations in the various fractions were determined using a Bio-rad protein assay kit (Bio-rad Laboratories, Hemel Hempstead, Herts).

Assay of D-glucose. The D-glucose concentrations in the serosal and luminal samples were determined manually with a commercial reagent kit (Boehringer Corporation, London).

Measurement of dry weight. A measured segment of the intestine was blotted, weighed (to determine the wet weight) and dried to a constant weight in an air oven (105 °C). This gave a dry weight/wet weight ratio which was used to calculate the water content of each tissue sample.

Calculation and expression of results. All results are expressed as mean ± standard error of the mean (S.E.M.); $n = 5$ for each mean except where noted. Transport data are presented either as serosal concentrations or cumulative rates of serosal appearance (μmol (g dry wt jejunal segment)$^{-1}$). Statistical comparisons were carried out using two-way analysis of variance to compare sets of concentration data or covariance analysis for cumulative serosal appearance.

RESULTS

Release of endogenous purines and pyrimidines by perfused jejunal loops

The release of endogenous constituents into the serosal fluid of isolated loops was monitored during 2 h perfusion experiments in the absence of any transport substrate other than D-glucose. The principal nucleic acid metabolites appearing in the serosal secretions were the pyrimidine uracil and uric acid, the end product of purine catabolism. No other purines were detected. During the second perfusion period (51–120 min) the serosal uric acid concentration fell to a mean value of 0·233 ± 0·007 mM ($n = 30$), or about half the initial level, (see Fig. 3 below).

The fate of luminal adenosine

Loops perfused from 51 to 120 min with 0·5 mM adenosine showed an increase in serosal uric acid from control levels to 1·64 ± 0·09 mM with a cumulative rate of 0·381 ± 0·052 μmol min^{-1} (g dry wt)$^{-1}$. No adenosine or adenine could be detected in the serosal secretions. Analysis of the luminal perfusate 10, 20 and 30 min after the introduction of the 0·5 mM adenosine showed concentrations (mM) of 0·486 ± 0·011, 0·306 ± 0·014 and 0, respectively. No luminal adenine could be detected but inosine was found in the lumen at 0·027 ± 0·012, 0·201 ± 0·011 and 0·443 ± 0·011 mM 10, 20 and 30 min, respectively, after the adenosine addition.

The transport and metabolism of luminal inosine and hypoxanthine

In view of the quantitative conversion of adenosine to inosine in the lumen, the transmural transport of purine from luminal inosine was compared with that from its

base, hypoxanthine, at concentrations of 0·15, 0·5 and 1·00 mM. Perfusion of isolated loops of jejunum with either inosine or hypoxanthine gave rise to significant increases in the concentration of uric acid in the serosal fluid (Fig. 1*A*, *B* and *C*). Neither of the luminal substrates nor any of the intermediates in the catabolic pathway between inosine and uric acid could be detected in the serosal fluid.

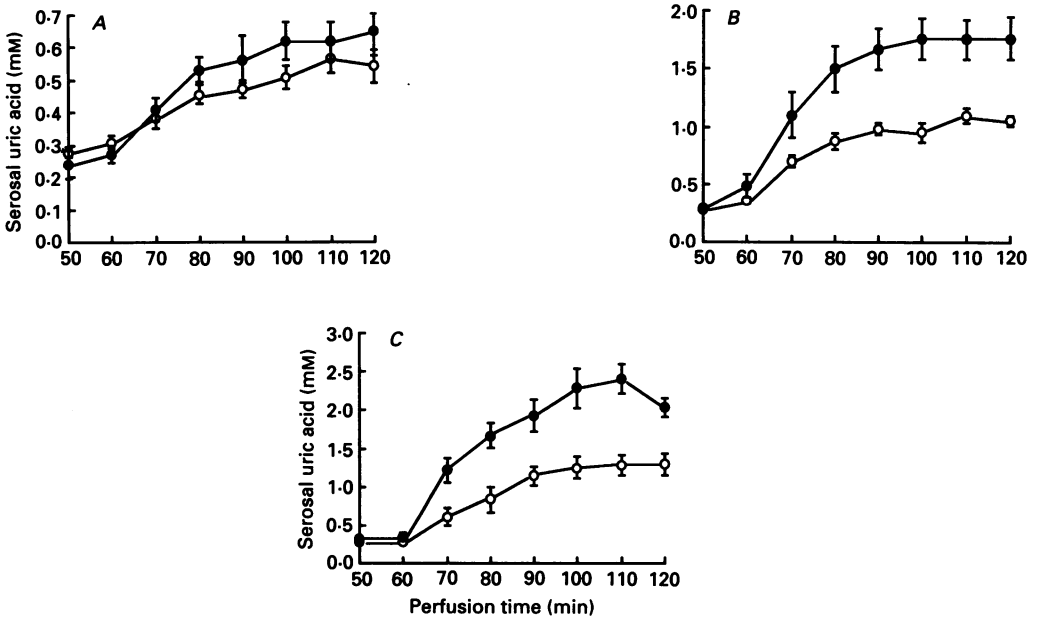

Fig. 1. The serosal concentration of uric acid achieved by perfusing the lumen of the intestine with either inosine (●) or hypoxanthine (○) at 0·15 mM (*A*), 0·50 mM (*B*) and 1·00 mM (*C*). Data presented as mean ± S.E.M. ($n = 5$) at each time point.

Analysis of variance showed no significant difference ($P > 0·1$) between the serosal uric acid from the nucleoside and its base at 0·15 mM (Fig. 1*A*). However, luminal inosine gave rise to significantly ($P < 0·001$) higher levels of uric acid than the equivalent luminal concentration of hypoxanthine at 0·5 and 1·0 mM (Fig. 1*B* and *C*). When the volume of water transported is taken into account, linear cumulative plots of serosal uric acid release were obtained. The rates of uric acid appearance at 0·15, 0·5 and 1·0 mM for luminal hypoxanthine were 0·079 ± 0·008, 0·196 ± 0·008 and 0·158 ± 0·10 μmol min^{-1} (g dry wt)$^{-1}$; respectively, and for luminal inosine they were 0·082 ± 0·006, 0·392 ± 0·041 and 0·310 ± 0·032 μmol min^{-1} (g dry wt)$^{-1}$, respectively. The rates of uric acid appearance from luminal inosine are significantly higher ($P < 0·01$) than those from hypoxanthine at 0·5 and 1·0 mM, showing that the differences between the serosal urate concentrations resulting from the two substrates in Fig. 1*B* and *C* are not the consequences of some indirect effect on water transport. Uric acid was the only purine we could detect within the mucosal tissue at the end of the experiment and it was present at levels which were not significantly different from those of the purine substrates in the lumen except for perfusions with 0·5 mM inosine where the tissue uric acid (0·701 ± 0·078 mM) was higher than that with 0·5 mM

hypoxanthine (0·491 ± 0·050 mM), $P < 0·05$. At all three concentrations of both nucleoside and base the serosal uric acid concentration was significantly ($P < 0·01$) above that in the tissue.

Transport and metabolism of luminal guanosine and guanine

Perfusion of loops with guanosine or guanine, (0·05–0·15 mM), led to a significant increase in uric acid concentration in the serosal secretions, but neither the substrates

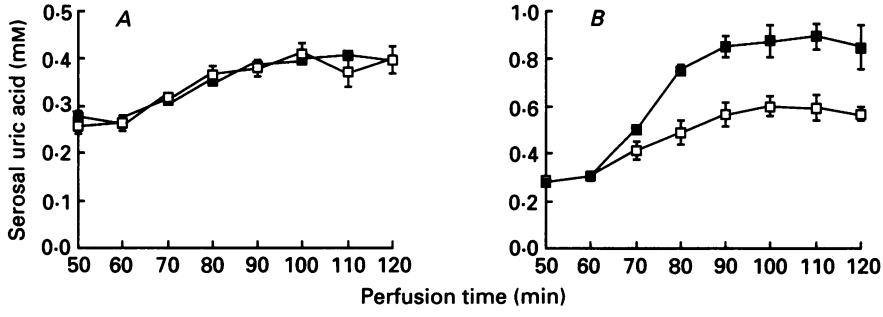

Fig. 2. The serosal concentration of uric acid achieved by perfusing the lumen of the intestine with guanosine (■) or guanine (□) at 0·05 mM (*A*) and 0·15 mM (*B*). Data presented as means ± S.E.M.

nor any intermediates of purine catabolism appeared in the serosal fluid. At the lower concentrations, 0·05 mM (Fig. 2*A*) and 0·10 mM (data not shown) there was no significant difference ($P > 0·1$) between the concentrations of serosal uric acid appearing from the two substrates. However, at 0·15 mM, a significantly ($P < 0·001$) higher serosal uric acid concentration was produced from the nucleoside than from the base (Fig. 2*B*). Cumulative plots of serosal uric acid appearance from the two substrates were linear and at luminal concentrations of 0·05, 0·10 and 0·15 mM, respectively, gave rates of 0·047 ± 0·005, 0·072 ± 0·004 and 0·104 ± 0·008 μmol min^{-1} (g dry wt)$^{-1}$ for guanine and 0·052 ± 0·003, 0·078 ± 0·004 and 0·150 ± 0·013 μmol min^{-1} (g dry wt)$^{-1}$ for guanosine. No purines other than uric acid could be detected within the mucosal tissue at the end of the experiment and the uric acid levels did not differ significantly from those of the purine substrates in the lumen except with 0·15 mM guanosine where the tissue uric acid (0·232 ± 0·021 mM) was higher than that with 0·15 mM guanine (0·158 ± 0·016 mM), $P < 0·05$. At all concentrations of both substrates the mucosal tissue uric acid was significantly lower ($P < 0·01$) than that in the serosal fluid.

Release of endogenous purines in the presence of allopurinol

To investigate the effects of metabolism on the translocation of purine nucleosides and bases across the jejunum, transport was studied in the presence of the xanthine oxidase inhibitor, allopurinol. Figure 3 shows the serosal purine appearance with 0·3 mM allopurinol present in the luminal perfusate for the full 120 min. Under these conditions, two intermediates of purine catabolism, hypoxanthine and xanthine,

were detected in the serosal fluid in addition to uric acid. This concentration of allopurinol reduced serosal uric acid appearance by about 80%. Allopurinol, a structural analogue of hypoxanthine, is metabolized by xanthine oxidase to oxypurinol, which is an analogue of xanthine and also an inhibitor of the enzyme.

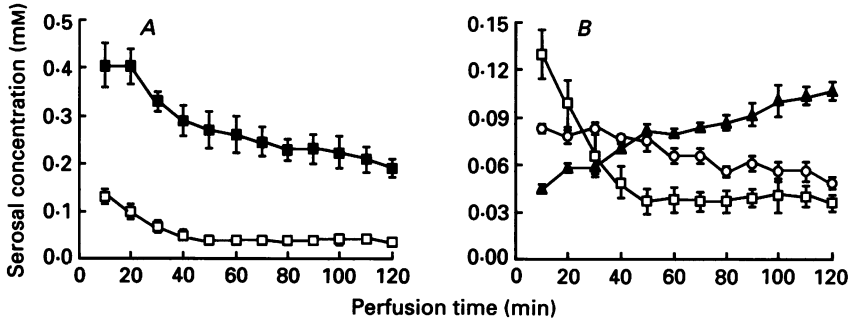

Fig. 3. The appearance of serosal uric acid in control perfusions of isolated jejunal loops with bicarbonate Krebs–Ringer solution containing 28 mM D-glucose in the absence (■) and presence (□) of 0·3 mM allopurinol (*A*). In *B* the serosal appearances of xanthine (▲) and hypoxanthine (○) as well as uric acid (□) are shown in the presence of 0·3 mM allopurinol.

Analysis of tissue extracts at the end of the perfusions with allopurinol showed no significant difference between the final serosal concentration of allopurinol ($0{\cdot}48 \pm 0{\cdot}002$ mM, $n = 30$) and that in the tissue ($0{\cdot}500 \pm 0{\cdot}003$ mM, $n = 30$). This contrasts strongly with the data for oxypurinol which had a tissue concentration of $0{\cdot}515 \pm 0{\cdot}055$ mM ($n = 30$) as compared to a final serosal concentration of $0{\cdot}055 \pm 0{\cdot}005$ mM ($n = 30$), and uric acid which was undetectable in the tissue under these conditions.

The transport and metabolism of luminal inosine and hypoxanthine in the presence of allopurinol

In the presence of 0·3 mM allopurinol, luminal hypoxanthine or inosine gave rise to a significant increase in the serosal appearance of hypoxanthine, together with a smaller increase in xanthine and uric acid. No inosine could be detected in the serosal fluid. Luminal inosine, over the concentration range 0·15–1·00 mM, gave rise to higher purine levels than the free base ($P < 0{\cdot}01$). Figure 4*A* shows the total serosal purine concentrations from perfusions with 0·3 mM allopurinol plus either 0·15 mM inosine or 0·15 mM hypoxanthine. For both the nucleoside and the base approximately 50% of the serosal purine was hypoxanthine, 30% xanthine and 20% uric acid. Figure 5 shows that rates of serosal purine appearance were significantly reduced ($P < 0{\cdot}001$) by allopurinol in perfusions with inosine (0·5–1·0 mM) and hypoxanthine (0·15–1·0 mM), and that the rate with inosine was always significantly higher ($P < 0{\cdot}01$) than that with the same amount of hypoxanthine. Analysis of tissue purines in the presence of allopurinol showed no significant differences between the mucosal tissue concentrations of hypoxanthine and xanthine and those in the serosal secretions. For the perfusions shown in Fig. 4*A* the tissue hypoxanthine and

xanthine concentrations were significantly higher ($P < 0.05$) with the nucleoside (0·278 ± 0·030 and 0·192 ± 0·021 mM, respectively) than with the base (0·190 ± 0·020 and 0·128 ± 0·013 mM, respectively) and there was no detectable uric acid in the tissue.

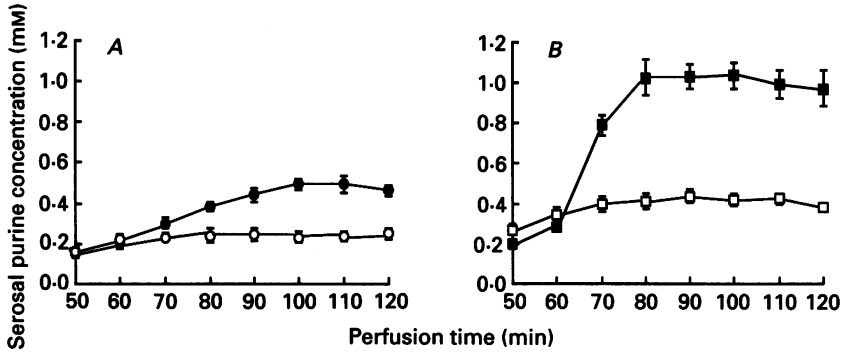

Fig. 4. The influence of allopurinol on serosal purine appearance: *A*, serosal purine concentration (xanthine + hypoxanthine + uric acid) in isolated loops perfused with 0·15 mM inosine (●) and 0·15 mM hypoxanthine (○) plus 0·3 mM allopurinol; and *B*, the serosal purine concentration (xanthine + uric acid) in isolated loops perfused with 0·15 mM guanosine (■) or 0·15 mM guanine (□) plus 0·3 mM allopurinol. All data presented as means ± S.E.M. ($n = 5$).

The transport and metabolism of luminal guanosine and guanine in the presence of allopurinol

Luminal guanosine or guanine (0·05–0·15 mM) in the presence of 0·3 mM allopurinol produced a significant increase in the serosal xanthine and uric acid concentrations. Neither luminal substrate could be detected in the serosal secretions. At the lowest concentration tested, 0·05 mM, there was no significant difference ($P > 0.1$) between the serosal xanthine plus uric acid with these two substrates. However, Fig. 4*B* shows that at 0·15 mM the serosal purine released from loops perfused with guanosine is several times that from those perfused with guanine. The final tissue xanthine concentration with the nucleoside (0·872 ± 0·073 mM) was significantly higher ($P < 0.01$) than that with the base (0·343 ± 0·41 mM). The cumulative rate of serosal purine release from the nucleoside was significantly greater ($P < 0.001$) than that from the base at both 0·1 and at 0·15 mM (Fig. 5). Allopurinol significantly reduced ($P < 0.01$) the rate of serosal purine appearance in the perfusions at all concentrations of guanine, but only at the lowest guanosine concentration (0·05 mM).

Comparison of transmural purine transport from inosine and guanosine

With 0·15 mM luminal nucleoside, both the serosal uric acid concentration (Fig. 1*A* *vs.* Fig. 2*B*) and the cumulative rate of serosal uric acid appearance (Fig. 5) were significantly higher from guanosine than inosine ($P < 0.01$). However, at 0·5 mM the serosal uric acid concentration from luminal guanosine (data not shown) did not differ significantly from that with luminal inosine (Fig. 1*B*), but with 0·5 mM

guanosine there was a significant reduction ($P < 0.01$) in the rates of glucose and water transport by 42 and 39% respectively. Therefore the rate of serosal uric acid appearance, the product of concentration and water flow, was significantly lower ($P < 0.01$) for guanosine than for inosine at 0·5 mM (Fig. 5). Figure 5 also shows that

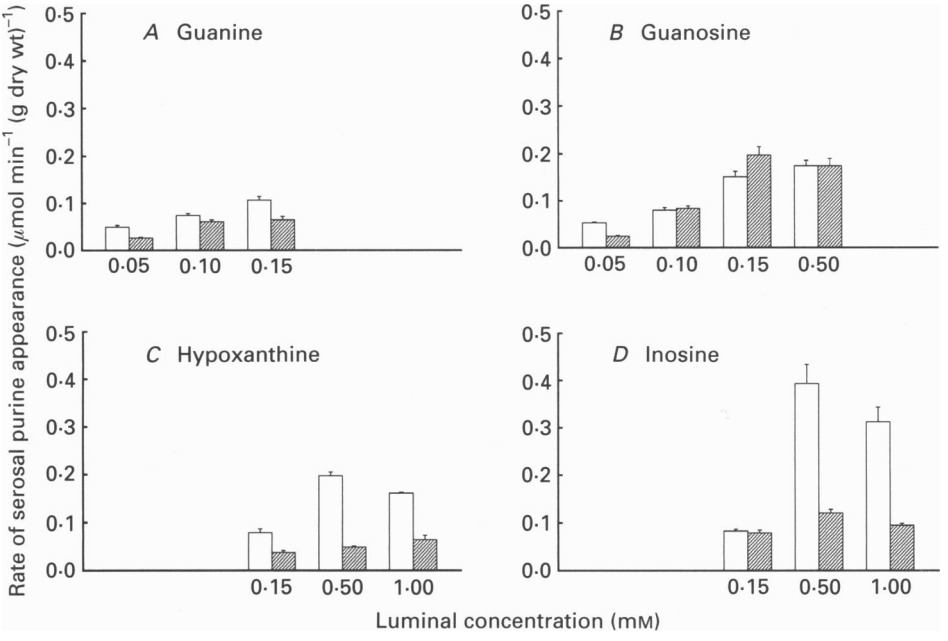

Fig. 5. A histogram showing a summary of the rates of serosal purine appearance (μmol min^{-1} (g dry wt)$^{-1}$) from guanine (*A*), guanosine (*B*), hypoxanthine (*C*) and inosine (*D*) in the presence and absence of allopurinol. The shaded blocks represent the rates in the presence of 0·3 mM allopurinol. All data presented as means ± S.E.M. ($n = 5$).

the rate of purine transport from luminal guanosine was unaffected by the presence of 0·3 mM allopurinol whereas that from 0·5 and 1·0 mM inosine was significantly reduced ($P < 0.001$). Although neither inosine, nor its base hypoxanthine, affected the rates of glucose and water transport at 0·5 mM, both compounds reduced the rate of glucose and water transport by 41 and 37%, respectively, when they were present in the lumen at 1·0 mM. This reduction in fluid flow probably explains why the rates of serosal uric acid appearance in Fig. 5 at 1·0 mM are lower than those at 0·5 mM.

Partial purification of jejunal purine nucleoside phosphorylase

The homogenate of jejunal mucosa was fractionated to determine the cellular location of the purine nucleoside phosphorylase activity and to enable us to determine its kinetic parameters. Centrifugation at 3000 *g* caused only approximately 7% of the purine nucleoside phosphorylase activity to be precipitated with the cellular organelles and recentrifugation at 27000 *g* of the first supernatant sedimented a further 1% leaving 90% of the original activity in the final supernatant. The pellet from the second centrifugation contained the brush border

membranes, as indicated by the 25-fold enhancement of sucrase activity, a brush border marker. The final supernatant containing the purine nucleoside phosphorylase activity also converted the resulting purines to uric acid. Sequential additions of ammonium sulphate revealed that the purine nucleoside phosphorylase was precipitated in the 40–70% fraction, but this also contained the maximum xanthine oxidase activity. A successful separation of purine nucleoside phosphorylase from xanthine oxidase was achieved using a Sephadex G 200 column and the fractions which contained the phosphorylase but were devoid of xanthine oxidase activity were combined for the enzyme assays.

The purine nucleoside phosphorylase did not catalyse the reaction in the absence of phosphate. The K_m and V_{max} for each of the nucleosides were determined by varying the concentrations of guanosine and inosine at a constant phosphate concentration of 40 mM. The K_m values for inosine and guanosine are $317{\cdot}6 \pm 45{\cdot}4$ and $41{\cdot}37 \pm 3{\cdot}55$ μM respectively, as determined using Marquandt non-linear least-squares fitting. The corresponding maximal velocities (V_{max}) are $79{\cdot}32 \pm 4{\cdot}01$ and $20{\cdot}54 \pm 0{\cdot}05$ μmol min^{-1} (mg protein)$^{-1}$. The K_m values for phosphate in the presence of 1 mM inosine or guanosine were $1{\cdot}82 \pm 0{\cdot}24$ and $0{\cdot}91 \pm 0{\cdot}09$ μM respectively and the V_{max} values were $98{\cdot}16 \pm 5{\cdot}05$ and $38{\cdot}08 \pm 0{\cdot}09$ μmol min^{-1} (mg protein)$^{-1}$, respectively.

Serosal release of ribose phosphate

Serosal samples from loop perfusions with 0·5 mM luminal inosine were treated to remove lactic acid and then analysed on the Spherogel column. Only two principal carbohydrate peaks were found and these were identified as glucose and ribose phosphate by retention time and co-chromatography with standards added to the samples. There was a small free ribose peak in the serosal secretions, and the ribose phosphate peak was identified as distinct from it by treatment of the serosal samples with alkaline phosphatase. This caused a sharp reduction in the ribose phosphate peak and a corresponding increase in the free ribose peak. Comparison of the serosal ribose phosphate peak height with that of ribose phosphate standards gave a serosal concentration of $1{\cdot}879 \pm 0{\cdot}190$ mM after perfusion with inosine for 70 min and the free ribose peak corresponded to less than 0·08 mM. The mean uric acid concentration in the same experiments was $2{\cdot}042 \pm 0{\cdot}124$ mM.

DISCUSSION

Fate of luminal nucleosides in the rat small intestine

The results presented in this paper provide evidence for four principal conclusions concerning the fate of purine nucleosides perfused through the lumen of isolated loops of rat small intestine. Firstly, it is clear that none of the three purine nucleosides studied, adenosine, guanosine and inosine, cross the epithelial layer intact. Secondly, analysis of mucosal tissue extracts showed that the only purine metabolite which changed in concentration during the period when the loops were perfused with the nucleosides was uric acid, which increased significantly in concentration in the mucosa as well as in the serosal secretions. These results indicated that the nucleosides are following the degradation pathway shown in Fig.

6, which has been identified in mammalian liver and a number of other tissues (Fox, 1978). Our observation that adenosine was deaminated to inosine within the lumen of the small intestine is in agreement with the evidence from Le Hir & Dubach (1984) who reported that adenosine was deaminated by brush border membrane vesicles.

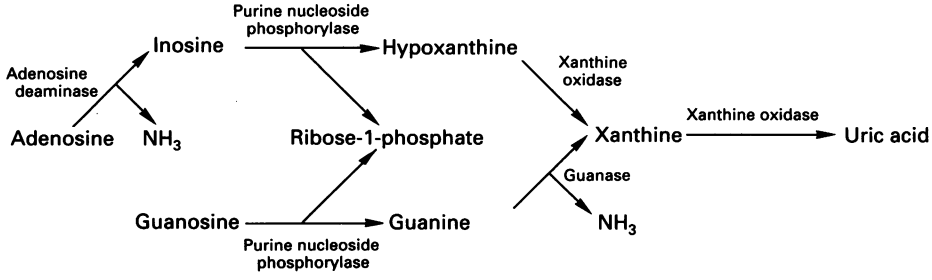

Fig. 6. Catabolism of purine nucleosides in rat small intestine.

Adenosine deaminase was found to have relatively high activity in the intestine compared to other organs (Centelles, Franco & Bozal, 1987). The enzyme had been thought to be largely cytosolic but Franco & Centelles (1990) demonstrated that it was localized on the brush border near the ectoenzyme 5′-nucleotidase with its active site located towards the exterior of the cell.

The third conclusion we can draw from our study is that the measurements of tissue and serosal uric acid concentrations demonstrate clearly that urate is transported across the basolateral membrane against a concentration gradient, in agreement with our earlier work with mouse small intestine (Bronk & Shaw, 1986). Uric acid is a weak acid which will exist almost exclusively as the monovalent anion at physiological pH (Bergmann & Dikstein, 1955). Since the electrical potential across the small intestine is in the range 5–10 mV, with the serosa positive (Clarkson, Cross & Toole, 1961), this would favour the movement of negatively charged urate from mucosa to serosa.

Our fourth point is that at the higher luminal concentrations tested the nucleosides guanosine and inosine gave rise to significantly higher rates of serosal uric acid appearance than did equal concentrations of their constituent purine bases. Our observation that the nucleosides confer an advantage over the free bases as substrates for transmural purine transport is similar to the results of earlier studies (Bronk & Hastewell, 1988) on the comparative effectiveness of uridine and uracil as substrates for transepithelial pyrimidine transport, although in the present case the purine bases are oxidized to uric acid after release from the nucleoside whereas uracil is not metabolized within the enterocyte.

Intestinal purine nucleoside phosphorylase

Fractionation of the mucosal homogenates shows that the mucosal epithelium of the small intestine contains an active purine nucleoside phosphorylase which is localized almost exclusively in the cytoplasm rather than in the membrane fraction. The K_m of the nucleoside phosphorylase for inosine is about 8 times that for

guanosine and this is consistent with our finding (Fig. 5) that, at a 0·15 mM concentration, guanosine gives higher rates of serosal uric acid appearance than inosine. The two phosphorylase activities co-purified during the fractionation procedure so that we cannot say whether two separate enzymes are involved. The fact that the K_m for inosine is 0·32 mM explains the large increase in rate of serosal uric acid release when the inosine concentration is increased from 0·15 to 0·5 mM (Fig. 5). The inhibition of glucose and water transport by guanosine at 0·5 mM makes it a less effective substrate than inosine at that concentration. Our phosphorolysis data for guanosine and inosine agree broadly with the results obtained earlier (Bronk & Hastewell, 1988) for the phosphorolysis of the pyrimidine nucleosides uridine and thymidine, except that the K_m for inosine is significantly higher ($P < 0·01$) than that for the other three nucleosides.

Effects of inhibiting xanthine oxidase with allopurinol

In the experiments with allopurinol, the appearance of both oxypurinol and allopurinol in the serosal fluid confirmed that in the rat, as in humans (Reiter, Simmonds, Webster & Watson, 1983), allopurinol is metabolized to oxypurinol. In this respect rats differ from mice since allopurinol is not oxidized to any great extent in the latter (Bronk & Shaw, 1986). In humans some 60–70% of allopurinol is metabolized to oxypurinol (Simmonds, Cameron, Morris & Davies, 1986) which is similar to our value of 80%.

The results obtained with luminal guanosine in the presence of allopurinol differ only slightly from those in the absence of the inhibitor (Fig. 5). In contrast, the rates of serosal purine release from inosine and the two bases, guanine and hypoxanthine, are substantially reduced by allopurinol. In the perfusions with luminal hypoxanthine plus allopurinol the increase in tissue hypoxanthine probably inhibits uptake at the brush border and in the case of inosine the hypoxanthine may inhibit the phosphorolysis step. It also appears that the increase in tissue xanthine in the presence of allopurinol reduces guanine uptake, although we could not detect any guanine in the tissue extracts. Simmonds, Cadenhead, Jones, Hatfield & Cameron (1973) found that allopurinol inhibited [^{14}C]guanine absorption in the pig.

Implications for the fate of dietary nucleic acids in the small intestine

Pancreatic ribonuclease splits dietary nucleic acids into mononucleotides which previous work (Bronk & Hastewell, 1989*a*) has shown are hydrolysed to nucleosides plus inorganic phosphate in the lumen by brush border phosphatases in the jejunum (Holt & Miller, 1962). The results presented in this paper show that the adenosine released in this way will be rapidly deaminated to inosine which, together with the guanosine, will be absorbed into the mucosa across the brush border membrane. Once inside the enterocytes the nucleosides are degraded by the purine nucleoside phosphorylase to ribose-1-phosphate plus hypoxanthine and guanine. Our data show that the mucosal xanthine oxidase is sufficiently active to convert both purines to uric acid, thus effectively preventing the operation of the salvage pathway effected by hypoxanthine–guanine phosphoribosyltransferase (Fox, 1978). This agrees with the fate of labelled guanine (Simmonds *et al.* 1973) in the pig.

Taken together with our earlier work on pyrimidine nucleosides (Bronk &

Hastewell, 1988, 1989*a*, *b*), the results in this paper make it clear that, at least in the rat, the principal constituents which will enter the vascular system following the intake of nucleic acids will be ribose-1-phosphate, uracil and uric acid.

We wish to thank The Wellcome Trust for their generous support for this work and the MRC for providing a studentship for R.A.S.

REFERENCES

BERGMANN, F. & DIKSTEIN, S. (1955). The relationship between spectral and structural changes in uric acid and related compounds. *Journal of the American Chemical Society* **77**, 691–696.

BERLIN, R. D. & HAWKINS, R. A. (1968). Secretions of purines by the small intestine: general characteristics. *American Journal of Physiology* **215**, 932–941.

BRONK, J. R. & HASTEWELL, J. G. (1987). The transport of pyrimidines into tissue rings cut from small intestine. *Journal of Physiology* **382**, 475–488.

BRONK, J. R. & HASTEWELL, J. G. (1988). The transport and metabolism of naturally occurring pyrimidine nucleosides by isolated rat jejunum. *Journal of Physiology* **395**, 349–361.

BRONK, J. R. & HASTEWELL, J. G. (1989*a*). The transport and metabolism of uridine mononucleotides by rat jejunum *in vitro*. *Journal of Physiology* **408**, 129–135.

BRONK, J. R. & HASTEWELL, J. G. (1989*b*). The specificity of pyrimidine nucleoside transport and metabolism by rat jejunum *in vitro*. *Journal of Physiology* **408**, 405–411.

BRONK, J. R., LISTER, N. & LYNCH, S. (1987). Absorption of 5-fluorouracil and related pyrimidines in rat small intestine. *Clinical Science* **72**, 891–895.

BRONK, J. R. & SHAW, M. I. (1986). The transport of uric acid across mouse small intestine *in vitro*. *Journal of Physiology* **378**, 229–239.

CENTELLES, J. J., FRANCO, R. & BOZAL, J. (1987). Distribution of adenosine deaminase in some rat tissues. Inhibition by ethanol and dimethyl sulphoxide. *Comparative Biochemistry and Physiology* **86**, 95–98.

CLARKSON, T. W., CROSS, A. C. & TOOLE, S. R. (1961). Electrical potentials across isolated small intestine of the rat. *American Journal of Physiology* **200**, 1233–1235.

CSAKY, T. Z. (1961). Significance of sodium ions in active intestinal transport of nonelectrolytes. *American Journal of Physiology* **201**, 999–1001.

FISHER, R. B. & GARDNER, M. L. G. (1974). A kinetic approach to the study of absorption of solutes by isolated perfused small intestine. *Journal of Physiology* **241**, 211–234.

FOX, I. H. (1978). Degradation of purine nucleotides. *Handbook of Experimental Pharmacology*, vol. 51, ed. KELLY, W. N. & WEINER, I. M., chap. 5, pp. 93–124. Springer-Verlag, New York.

FRANCO, R. & CENTELLES, J. J. (1990). Release of adenosine deaminase from rat intestinal mucosa. *Biochemical Society Transactions* **18**, 641–642.

HANSON, P. J. & PARSONS, D. S. (1976). The utilization of glucose and production of lactate by *in vitro* preparations of small intestine: effects of vascular perfusion. *Journal of Physiology* **255**, 775–795.

HARMS, V. & STIRLING, C. E. (1977). Transport of purine nucleotides and nucleosides by *in vitro* rabbit ileum. *American Journal of Physiology* **233**, E47–55.

HOLT, J. H. & MILLER, D. (1962). The localisation of phosphomonoesterase and amino peptidase in brush borders isolated from intestinal epithelial cells. *Biochimica et Biophysica Acta* **58**, 239–243.

KAHN, A. H., WILSON, S. & CRAWHALL, J. C. (1975). The influx of uric acid and other purines into everted jejunal sacs of the rat and hamster. *Canadian Journal of Physiology and Pharmacology* **53**, 113–119.

LE HIR, M. & DUBACH, U. C. (1984). Sodium-gradient energized concentrative transport of adenosine in renal brush border membrane vesicles. *Pflügers Archiv* **401**, 58–63.

OH, J. H., DOSSETOR, J. B. & BECK, I. T. (1967). Kinetics of uric acid transport and its production in rat small intestine. *Canadian Journal of Physiology and Pharmacology* **45**, 121–127.

PARSONS, D. S. & SHAW, M. I. (1983*a*). Application of high performance liquid chromatography to study transport and metabolism of nucleic acid derivatives *in vitro*: endogenous washout. *Quarterly Journal of Experimental Physiology* **68**, 39–51.

PARSONS, D. S. & SHAW, M. I. (1983*b*). Use of high pressure liquid chromatography to study absorption and metabolism of purines by rat jejunum *in vitro*. *Quarterly Journal of Experimental Physiology* **68**, 53–57.

REITER, S., SIMMONDS, H. A., WEBSTER, D. R. & WATSON, A. R. (1983). On the metabolism of allopurinol. Formation of allopurinol-1-riboside in purine nucleoside phosphorylase deficiency. *Biochemical Pharmacology* **32**, 2167–2174.

SCHANKER, L. S., JEFFERY, J. J. & TOCCO, D. J. (1963). Interaction of purines with pyrimidine transport process of the small intestine. *Biochemical Pharmacology* **12**, 1047–1053.

SCHANKER, L. W. & TOCCO, D. J. (1960). Active transport of some pyrimidines across the rat intestinal epithelium. *Journal of Pharmacology and Experimental Therapeutics* **128**, 115–121.

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

SIMMONDS, H. A., CADENHEAD, A., JONES, A. S., HATFIELD, P. J. & CAMERON, J. S. (1973). The effect of allopurinol on oral purine absorption and excretion in the pig. *Advances in Experimental Medicine and Biology* **41**, 653–655.

SIMMONDS, H. A., CAMERON, J. S., MORRIS, G. S. & DAVIES, P. M. (1986). Allopurinol in renal failure and tumour lysis syndrome. *Clinica Chimica Acta* **160**, 189–195.

WHEELER, C. P. D. & YUDILEVICH, D. L. (1988). Transport and metabolism of adenosine in the perfused guinea-pig placenta. *Journal of Physiology* **405**, 511–526.

WILSON, D. W. & WILSON, H. C. (1962). Studies *in vitro* of the digestion and absorption of purine ribonucleosides by the intestine. *Journal of Biological Chemistry* **237**, 1643–1647.