Metabolism and Transport of Glutamine and Glucose in Vascularly Perfused Small Intestine Rat

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1. The metabolism and transport of glutamine and glucose were investigated in a preparation of rat small intestine perfused through the vascular bed *in vitro* and *in situ*.
2. With glucose (7.5 mM) or glutamine (4.5 mM) in the lumen, approx. 40% of the substrate taken up appears unchanged on the vascular side. When glutamine (1.5 mM) is also added to the vascular perfusate, metabolism of glutamine is increased and there is uptake of glutamine from both the vascular bed and lumen. Orientation of substrate (vascular bed or lumen) influences the value of alanine production/glutamine utilization and lactate production/glucose utilization.
3. Deprivation of food and metabolic acidosis have no effect upon the utilization of glutamine by unit length of jejunum. In fed rats, glutamine utilization is 44% of glucose utilization, but in rats deprived of food it is 112% of glucose utilization.
4. Glucose utilization and lactate production are not significantly altered by the presence of glutamine in the vascular bed or lumen.
5. With glucose only in the vascular perfusate, glucose utilization is the same in jejunum and ileum. Glutamine metabolism in the ileum is 28% lower than in the jejunum.
6. Glutamine utilization is dependent on the concentration of glutamine in the vascular perfusate, but is not significantly affected by the absence of glucose.
7. Results are discussed in relation to the role of intestinal glutamine metabolism and with respect to some problems of the transepithelial movement of substrates that are both transported and metabolized.

The small intestine is a major site of glutamine metabolism in the rat and other species (Windmueller & Spaeth, 1974, 1975). Uptake of glutamine by rat intestine, from either the luminal or the vascular side, results in the appearance of ammonia, alanine, citrulline and proline in the vascular medium, and, as more than one-half of the glutamine carbon may be converted into CO₂ (Windmueller & Spaeth, 1974, 1975), metabolism of glutamine may make an important contribution to the energy requirements of the small intestine. However, a definite role has yet to be assigned to the glutamine metabolism of the intestine.

We have used a preparation of rat small intestine perfused through the vascular bed *in situ* (Hanson & Parsons, 1976) to examine inter-relationships between the metabolism of glucose, thought to be an important energy-providing substrate for the small intestine (Parsons, 1975), and the metabolism of glutamine. We have also investigated the effects of deprivation of food and acidosis on glutamine metabolism, for the uptake and output of glutamine by different tissues change under such circumstances (Addae & Lotspeich, 1968; Ishikawa, 1976; Lund & Watford, 1976) and the intestine might also be affected.

With either glutamine or glucose in the vascular bed, the addition of the same substrate to the lumen increases its metabolism (Windmueller & Spaeth, 1975; Hanson & Parsons, 1976). We have now compared the uptake and metabolism of glucose and glutamine when present only in the lumen with that when present only in the vascular bed, and an attempt is made to reconcile the findings with those obtained with substrates on both sides.

Methods

Animals

Male Wistar rats, supplied by OLAC Ltd., Bicester, Oxon., U.K., were fed with Oxoid Breeding diet (supplied by H. C. Styles, Basingstoke, Hants., U.K.). Experiments were performed between 13:00 and 17:00 h on rats of 175–225 g body wt. Rats described in the Tables and text as 'fed' were allowed continuous access to food, but were probably in a post-absorptive state when used, for the small intestine was usually found to be empty of food. Rats were deprived of food by removing it at 12:00 h 2 days before they were required for use. Acidosis, as indicated by a decrease in plasma [HCO₃⁻] to

17±2mm (mean±S.E.M., three experiments), was induced by including 280mm-NH₄Cl in the drinking water for 7–11 days. The few rats that lost weight under such treatment were not used for experimentation.

Materials

Fatty acid-poor bovine serum albumin (fraction V) was supplied by Miles Laboratories, Slough, Berks., U.K. Glutaminase (grade V) was from Sigma, Kingston-upon-Thames, Surrey, U.K.; other enzymes and nucleotides were from Boehringer, Lewes, Sussex, U.K. Benzylpenicillin and streptomycin sulphate were from Glaxo, Greenford, Middx., U.K. 3-Mercaptopicolinic acid was a generous gift from Dr. H. L. Saunders of Smith, Kline and French Laboratories, Philadelphia, PA, U.S.A.

Perfusion fluids

The basic vascular perfusate to which substrates were added was a Krebs–Ringer bicarbonate medium containing: dialysed fatty acid-poor bovine serum albumin (3g/100ml); washed bovine erythrocytes (haematocrit 40%); benzylpenicillin [10000 units (6mg)/100ml]; streptomycin sulphate (5mg/100ml). The medium was gassed with O₂/CO₂ (19:1) by using a rotating thin-film oxygenator. Further details, including the composition of the Krebs–Ringer bicarbonate medium, which was also used as the luminal fluid, are given in Hanson & Parsons (1976).

Experimental procedure

Perfusion of the jejunum (20cm lengths, starting about 5cm below the ligament of Trietz) was carried out as previously described (Hanson & Parsons, 1976). Perfusion of the ileum (20cm segments, ending about 20cm above the ileocaecal valve) also necessitated tying-off those vessels supplying the small intestine proximal to the perfused section.

The flow rate of the vascular perfusate was 1.5ml/min (about 5ml/min per g dry wt.), and that of the luminal fluid was 2ml/min. The luminal distension pressure was 10–14cm of Krebs–Ringer bicarbonate medium. This distension pressure ensured uniform exposure of the intestinal mucosa to the solution passing through the lumen, and inhibited peristalsis.

Samples (0.5ml) were usually taken at 10min intervals from the recirculating vascular perfusate over the period 20–50min, and from the luminal fluid over the period 25–55min, and were deproteinized in 4ml of ice-cold 0.59M-HClO₄. A portion of the supernatant obtained after centrifugation at 3000g for 5min was neutralized with 2.67M-KOH.

Analytical methods

Substrates and metabolites were determined spectrophotometrically by the following methods: L-lactate (Hohorst, 1963); pyruvate (Bücher *et al.*, 1963); L-glutamine (Lund, 1974); L-glutamate (Bernt & Bergmeyer, 1974); L-alanine (Williamson, 1974). Glucose was determined by a method using hexokinase and glucose 6-phosphate dehydrogenase (Boehringer kit).

Tissue weights

Dry weights of perfused tissue were determined as described previously (Hanson & Parsons, 1976), and the proportions of muscle and mucosa in the intestinal wall as described by Bronk & Parsons (1965).

Calculation and expression of results

Rates of the uptake of a substrate by the tissue, or the appearance of a substrate or metabolite in the vascular perfusate or luminal fluid, were calculated

Fig. 1. Time course of glutamine uptake and alanine appearance

●, Uptake of glutamine from the vascular medium of perfused rat jejunum; ■, appearance of alanine in the vascular perfusate. Results are means of four experiments; bars represent ±S.E.M. Perfusion was commenced at zero time, and recirculation of the vascular perfusate initiated at 15min. Initial concentrations of glucose and glutamine in the vascular perfusate plasma were 7.5 and 1.5mm respectively. No substrates were present in the lumen. During the period used for measurement (30–50min), the concentration of glutamine in the vascular perfusate plasma decreased from 1.2±0.08mm (4) to 0.88±0.09mm (4).

by regression analysis from the linear part of the curve relating the amount of uptake or appearance ($\mu\text{mol/g dry wt.}$) to time. In virtually all experiments, as previously reported (Hanson & Parsons, 1976), the uptake of glucose from the vascular perfusate, and the appearance there of lactate, were linearly related to time for the period 20–50 min. However, when glutamine was present only in the vascular perfusate, glutamine uptake and alanine output did not become steady until 30 min after the onset of perfusion (Fig. 1).

If the steady-state rates of uptake or appearance, calculated for the vascular perfusate and for the luminal fluid, are summed, the result is an estimate of the metabolism of the tissue, denoted in the Tables below as a rate of utilization or of production. When glucose, glutamine or glutamate were present only in the vascular perfusate, the concentration of glucose in the luminal fluid was often undetectable (less than 0.03 mM) and was never higher than 0.1 mM, and that of glutamine, glutamate and alanine was always below 0.03 mM. In such cases, rates of metabolism were calculated solely from changes in the composition of the vascular perfusate.

Rates of glucose utilization and lactate production were corrected for the contribution made by the metabolism of the ox erythrocytes. In the absence of glucose, the erythrocytes did not produce lactate. When the vascular perfusate contained glutamine, there was a small amount of spontaneous conversion of glutamine into glutamate on incubation in the absence of an intestine. Correction for this had little effect on glutamine utilization (rate decreased by 2% in experiments with 1.5 mM-glutamine and 7.5 mM-glucose in the vascular perfusate), but had a greater effect on glutamate production (rate decreased by 26% in the same series of experiments). Rates of absorption of substrates from the lumen or of the appearance there of metabolites were little affected by correction for the small changes in volume of the luminal fluid in excess of those produced by sampling.

Glucose, glutamine and glutamate, but not alanine or lactate, appear to be present at very low concen-

trations inside the ox erythrocytes, for in the former group the concentration in the perfusate plasma is approx. 1.5 times that in the whole perfusate. Substrate concentrations are expressed below as mmol/litre of perfusate plasma, as it is from there that these substrates will be taken up.

If it is assumed that glucose is the only source of lactate, then the expression

$$\frac{(\text{Lactate production} \times 0.5)}{\text{glucose utilization}}$$

can be taken as a measure of the proportion of glucose converted into lactate.

Unless otherwise stated, glucose was added only to the vascular perfusate.

The statistical significance of differences between means, which are presented $\pm$ S.E.M., was determined by Student's *t* test.

Results

Influence of orientation of glutamine on its metabolism

When 4.5 mM-glutamine was added to the luminal fluid, but none was present in the vascular perfusate, 62% of the glutamine taken up from the lumen was metabolized, and the remainder passed into the vascular perfusate. Although glutamine utilization was the same as that found with 1.5 mM-glutamine in the vascular perfusate, alanine production, and alanine production expressed as a percentage of glutamine utilization, were significantly increased ($P < 0.01$; Table 1).

In agreement with the results of Windmueller & Spaeth (1975) *in vivo*, when glutamine was in both the lumen (4.5 mM) and the vascular bed (1.5 mM), glutamine utilization was higher than that found with glutamine (1.5 mM) only in the vascular bed ($P < 0.001$). Alanine production was also increased under these circumstances ($P < 0.001$), but alanine production expressed as a percentage of glutamine utilization was unchanged. When glutamine was present on both sides, the uptake of glutamine from the vascular

Table 1. *Effect of orientation of glutamine on its metabolism by vascularly perfused rat jejunum*

Results are expressed as means $\pm$ S.E.M. Rats were fed, and in all cases glucose (7.5 mM) was present in the vascular perfusate. A negative uptake represents an appearance.

[Glutamine] (mm)		No. of animals	Rate (μmol/h per g dry wt.)				$\frac{\text{Alanine production}}{\text{glutamine utilization}} \times 100$ (%)
Vascular perfusate plasma	Luminal fluid		Glutamine uptake		Glutamine utilization	Alanine production	
			Vascular side	Luminal side			
1.5	0	4	75 ± 7	0	75 ± 7	30 ± 3	41 ± 5
0	4.5	3	-47 ± 10	123 ± 6	76 ± 3	51 ± 1	67 ± 1
1.5	4.5	3	23 ± 8	134 ± 10	157 ± 9	71 ± 2	46 ± 4

Table 3. *Effect of the concentration of glucose in the vascular perfusate on re-uptake of glucose from the lumen*

The Krebs–Ringer bicarbonate medium issuing from the lumen of the jejunal segment was collected in 5 min fractions. During the first period (20–35 min), nothing was added to the Krebs–Ringer bicarbonate medium, but during the second period (35–55 min), 50 μ M-phlorrhizin was present. The values for the appearance and uptake of glucose represent the slope ($\pm$ s.e.) of the line relating the accumulated uptake or appearance of glucose to time. For the comparison of the slopes obtained over the two periods, *P*, where significant, is denoted as follows: **P*<0.05; *****P*<0.001. Values in parentheses represent the concentration of glucose in the whole vascular perfusate. n.d., Not determined.

Expt. no.	[Glucose] in vascular perfusate plasma (mM)	Rate (μ mol/h per g dry wt.)				
		Appearance of glucose in luminal fluid		Re-uptake	Uptake of glucose from the vascular perfusate	
		No addition	+50 μ M-Phlorrhizin		No addition	+50 μ M-Phlorrhizin
1	22 (15)	3 $\pm$ 0.3	4 $\pm$ 0.7	1	330 $\pm$ 38	300 $\pm$ 38
2	22 (15)	1 $\pm$ 0.2	2 $\pm$ 0.3*	1	445 $\pm$ 62	467 $\pm$ 46
3	42 (28)	8 $\pm$ 0.1	48 $\pm$ 1.1****	40	n.d.	n.d.
4	42 (28)	5 $\pm$ 0.5	24 $\pm$ 0.6****	19	n.d.	n.d.

utilization and increased glutamine utilization and alanine production. Acidosis did not affect glucose utilization or alanine production, but glutamine utilization was increased (Table 5).

Results expressed per cm of jejunal length. Depriving rats of food for 48 h markedly decreased glucose utilization and lactate production, but did not affect glutamine utilization or alanine production. Acidosis did not significantly affect metabolism of either glucose or glutamine (Table 5).

Effects of deprivation of food and acidosis on concentrations of glutamine, glutamate and alanine in plasma

In agreement with Ishikawa (1976), deprivation of food decreased the concentrations of glutamate and alanine below those found in the arterial plasma of fed rats, but the concentration of glutamine was unchanged (Table 6). In contrast, acidosis considerably lowered the concentration of glutamine in the arterial plasma, as found by Squires *et al.* (1976) with whole blood, but not by Welbourne (1974) using plasma. Acidosis lowered the plasma concentration of alanine a little, but did not affect that of glutamate (Table 6).

Effect of glutamine on the metabolism of glucose

The addition of glutamine (1.5 mM) to the vascular perfusate did not significantly alter glucose utilization or lactate production in either jejunum or ileum (Table 7) (the difference between lactate production by ileum with and without glutamine, although large, was not significant; $0.1 > P > 0.05$). Glucose utilization and lactate production were also unaffected by the presence of glutamine (4.5 mM) in the lumen, or of glutamine in the lumen and vascular bed (Table 8). When glutamine was present only in the luminal fluid, lactate production (in glucose equivalents)

expressed as a percentage of glucose utilization was significantly higher than in the absence of glutamine (*P*<0.01, Tables 7 and 8).

Location of segment and metabolism of glucose and glutamine

Glucose utilization was the same in proximal jejunum and mid-ileum irrespective of whether glutamine was present in the vascular perfusate (Table 7). Lactate production was higher (*P*<0.02) in the jejunum than in the ileum only when glutamine was absent from the vascular perfusate (Table 7). Glutamine utilization was 28% lower in the ileum than in the jejunum (*P*<0.05), but alanine production was the same in the two regions.

Glutamine metabolism: effects of glutamine concentration and glucose

In both jejunum and ileum, the addition of glutamine to the vascular perfusate resulted in an increased production of alanine (*P*<0.01 in both cases; Table 7). Production of glutamate from glutamine was low, ranging from 5 to 9% of the rate of glutamine utilization (Tables 7 and 9). Amino acid analysis of vascular perfusate sampled at the beginning and end of perfusions in the presence and absence of glutamine demonstrated that ammonia and citrulline were also products of glutamine metabolism (P. J. Hanson & D. S. Parsons, unpublished work). There was essentially no production of glutamine when glucose was the only substrate in the vascular perfusate.

Utilization of glutamine and production of alanine and glutamate were not significantly affected by the absence of glucose from the vascular perfusate (Table 9). Under such conditions there appeared to be no net formation of glucose from glutamine

Table 4. *Factors affecting the composition of the intestinal wall*

Results are presented as means $\pm$ s.e.m. Measurements were made on two 5 cm lengths of jejunum taken from a 10 cm region which started 10 cm below the ligament of Trietz. Similar samples of ileum were taken from a region which ended about 25 cm above the ileocaecal valve. The total dry wt./cm and the ratio dry wt./wet wt. were measured with one piece, and the wet wt./cm and the dry wt. of muscle/cm were obtained with the other. The dry wt. of mucosa/cm was calculated from: [(total wet wt./cm) $\times$ (dry wt./wet wt.)] - (dry wt. of intestine was taken as the distance from the ligament of Trietz to the ileocaecal valve. For the comparison with measurements from fed rat jejunum, *P*, where significant, is denoted as follows: **P* < 0.05; ***P* < 0.02; ****P* < 0.01; *****P* < 0.001.

Dietary status of rat	No. of animals	Location of segment	Hydration (g of water/g dry wt.)	Dry wt. (mg)/cm			Dry wt. of mucosa dry wt. of wall (%) ×100	Length (cm)
				Total	Muscle			
					Mucosa	Muscle		
Fed	4	Jejunum	3.5 ± 0.1	14.4 ± 0.2	10.9 ± 0.3	3.7 ± 0.2	74 ± 2	99 ± 2
Fed	4	Ileum	3.5 ± 0.1	12.1 ± 0.4***	8.2 ± 0.2****	3.9 ± 0.2		
Deprived of food for 48 h	4	Jejunum	3.6 ± 0.1	8.8 ± 0.2****	5.6 ± 0.2****	3.1 ± 0.4	68 ± 1**	95 ± 1
Fed; 7-11 days drinking 280 mm-NH ₄ Cl	5	Jejunum	3.7 ± 0.1	11.7 ± 0.7**	8.6 ± 0.6*	3.1 ± 0.3	65 ± 3*	101 ± 2
							74 ± 1	

Table 5. *Effects of deprivation of food and acidosis on metabolism of glucose and glutamine by vascularly perfused rat jejunum*

The initial concentrations of glucose and glutamine in the plasma passing through the vascular bed were 7.5 and 1.5 mm respectively. No substrates were present in the lumen. In (a) results $\pm$ s.e.m. are expressed as μ mol/h per g dry wt.; in (b) the same results are recalculated with respect to intestinal length and are expressed as μ mol/h per cm. For comparisons with control values for fed rats, *P*, where significant, is denoted as follows: **P* < 0.05; ***P* < 0.02; ****P* < 0.01; *****P* < 0.001.

Dietary status of rat	No. of animals	Glucose utilization	Lactate production	Glutamine utilization	Glutamate production	Alanine production	(Lactate production $\times 0.5$) glucose utilization $\times 100$ (%)	
(a) Fed	4	171 $\pm$ 17	130 $\pm$ 19	75 $\pm$ 7	7 $\pm$ 2	30 $\pm$ 3		38 $\pm$ 4
Deprived of food for 48 h	6	95 $\pm$ 10***	71 $\pm$ 16	107 $\pm$ 8*	7 $\pm$ 3	55 $\pm$ 4***		36 $\pm$ 8
Fed; 7-11 days drinking 280 mm-NH ₄ Cl	3	156 $\pm$ 18	91 $\pm$ 15	109 $\pm$ 5**	4 $\pm$ 1	39 $\pm$ 11		29 $\pm$ 2
(b) Fed	4	2.46 $\pm$ 0.24	1.87 $\pm$ 0.28	1.07 $\pm$ 0.10	0.10 $\pm$ 0.03	0.43 $\pm$ 0.04		38 $\pm$ 4
Deprived of food for 48 h	6	0.84 $\pm$ 0.09****	0.63 $\pm$ 0.14***	0.94 $\pm$ 0.07	0.06 $\pm$ 0.02	0.48 $\pm$ 0.03		36 $\pm$ 8
Fed; 7-11 days drinking 280 mm-NH ₄ Cl	3	1.82 $\pm$ 0.21	1.06 $\pm$ 0.18	1.28 $\pm$ 0.06	0.04 $\pm$ 0.01	0.46 $\pm$ 0.13		29 $\pm$ 2

Table 6. Factors affecting the concentrations of glutamine, glutamate and alanine in the arterial plasma

During the period 15:00–17:00h rats were anaesthetized with intramuscular Nembutal (60mg of pentobarbitone sodium/kg body wt.), and blood was withdrawn from the aorta with a heparinized syringe. The fed rats were probably in a post-absorptive state, as the small intestine was empty of food. Results are presented as means $\pm$ s.e.m. Four rats were used in each case. For comparisons with values for fed rats, *P*, where significant, is denoted as follows: **P* < 0.05; ****P* < 0.01; *****P* < 0.001.

Dietary status	Plasma concentration (μ M) of:		
	Glutamine	Glutamate	Alanine
Fed	562 $\pm$ 33	95 $\pm$ 3	446 $\pm$ 26
Deprived of food for 48 h	486 $\pm$ 25	48 $\pm$ 4****	257 $\pm$ 29***
Fed; 7–11 days drinking 280 mM-NH ₄ Cl	344 $\pm$ 12****	79 $\pm$ 13	367 $\pm$ 15*

(concentration of glucose often below 0.03 mM, and never greater than 0.1 mM), and little uptake of lactate (Table 9) or of pyruvate [$0.16 \pm 3.9 \mu$ mol/h per dry wt. (3) when glutamine concentration 1.5 mM]. Increasing the metabolism of glucose by raising its concentration in the vascular perfusate did not appear to alter glutamine utilization (Table 9). However, raising the concentration of glutamine in the vascular perfusate considerably increased its utilization (*P* < 0.01), and the production of alanine (*P* < 0.01) (Table 9). 3-Mercaptopicolinic acid, an inhibitor of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) (Kostos *et al.*, 1975) did not appear to affect the production of alanine from glutamine in the absence of glucose.

Glutamate metabolism

Glutamate was taken up from the vascular perfusate at a rate that was 17% of that obtained with the same concentration of glutamine (1.5 mM) (Table 9). The presence of glutamate did not raise alanine production above that found with only glucose in the vascular perfusate (Tables 7 and 9).

Discussion

Metabolism of glutamine by the perfused jejunum

In contrast with non-vascularly perfused preparations of small intestine incubated *in vitro* (Matsutaka *et al.*, 1973; MacFarlane-Anderson *et al.*, 1976), metabolism of glutamine by the vascularly perfused preparation seems to resemble that found *in vivo* (Aikawa *et al.*, 1973; Windmueller & Spaeth, 1975), as little glutamate is formed from glutamine.

Glutamine metabolism by jejunum *in vivo* was 45μ mol/h per g dry wt. (Windmueller & Spaeth, 1975), which is somewhat lower than that found with the perfused preparation (75μ mol/h per g dry wt.), possibly because the concentration of glutamine was lower *in vivo* (0.6 mM) than *in vitro* (Fig. 1). Glutamine metabolism by the kidney from fed rats perfused

in vitro with glutamine (1 mM) as the only exogenous substrate is 68μ mol/h per g dry wt. (Ross, 1976), but the kidney differs from the intestine in that glutamine is converted into glucose, and little glutamine nitrogen appears in alanine *in vivo* (Squires *et al.*, 1976), and at near-physiological concentrations of glutamine *in vitro* (Ross, 1976).

Influence of orientation of substrate on metabolism of glucose and glutamine

When glucose is presented to the perfused intestine solely from the lumen there is a greater conversion into lactate, most of the increase appearing in the lumen (Table 2). When glutamine is presented to the tissue from the lumen there is a greater conversion into alanine (Table 1), although in this case alanine appearance in both lumen and vascular bed is increased. Windmueller & Spaeth (1975) found little effect of orientation of [U-¹⁴C]glutamine on the distribution of ¹⁴C among the products of its metabolism, but did not examine the distribution of glutamine nitrogen. Also effects of orientation on intestinal fatty acid metabolism have been demonstrated (Gangl & Ockner, 1975).

The finding that glucose utilization does not become maximal until very high concentrations of glucose are present in the vascular bed (Hanson & Parsons, 1976) may be a consequence of the system by which glucose crosses the basolateral membrane of the epithelial cells having a high capacity but low affinity for glucose (Kimmich & Randles, 1975) thereby limiting glucose metabolism when the plasma concentration is low. An alternative possibility, that glucose may pass through intercellular spaces into the lumen (Boyd *et al.*, 1975) and from there be taken up into a separate metabolic compartment does not seem to be quantitatively very important, for when the concentration of plasma glucose is 42 mM, re-uptake (Table 3) is a relatively small fraction of glucose utilization at this concentration (420μ mol/h per g dry wt.; Hanson & Parsons, 1976).

Table 7. *Metabolism of glucose and glutamine by vascularly perfused rat jejunum and ileum*
 Results are presented as means $\pm$ s.e.m. for four animals. All rats were fed and no substrates were present in the lumen of the intestine. The 20 cm segments of jejunum started about 5 cm below the ligament of Trietz, and the 20 cm segments of ileum ended about 20 cm above the ileocaecal valve. For the comparison of the corresponding measurements for jejunum and ileum, P , where significant, is denoted as follows: * $P < 0.05$; ** $P < 0.02$.

Plasma concentration of additions to basic vascular perfusate (mM)	Location of segment	Rate ($\mu\text{mol/h}$ per g dry wt.)				(Lactate production $\times 0.5$) glucose utilization (%) $\times 100$
		Glucose utilization	Lactate production	Glutamine utilization	Glutamate production	Alanine production
Glucose (7.5)	Jejunum	193 $\pm$ 25	123 $\pm$ 6	0	-0.7 $\pm$ 0.4	13 $\pm$ 1
Glucose (7.5)	Ileum	155 $\pm$ 7	70 $\pm$ 14**	0	2 $\pm$ 2	10 $\pm$ 2
Glucose (7.5) + glutamine (1.5)	Jejunum	171 $\pm$ 17	130 $\pm$ 19	75 $\pm$ 7	7 $\pm$ 2	30 $\pm$ 3
Glucose (7.5) + glutamine (1.5)	Ileum	175 $\pm$ 25	136 $\pm$ 29	54 $\pm$ 2*	4 $\pm$ 1	31 $\pm$ 5
						33 $\pm$ 2
						23 $\pm$ 5
						38 $\pm$ 4
						38 $\pm$ 5

With both glutamine and glucose, when substrate is added only to the lumen, about 40% of the substrate taken up appears in the vascular bed at the concentrations used here. Yet when substrate is present in the vascular bed as well, there may be net uptake from the vascular bed as well as the lumen, and an increase in metabolism (Table 1; Hanson & Parsons, 1976; P. J. Hanson & D. S. Parsons, unpublished work).

One explanation is that the presence of substrate in both the vascular bed and the lumen causes an increase in the intracellular concentration of substrate above that found with substrate in either compartment alone. Such an increase could increase the entry of glutamine into the mitochondrion and thus possibly increase glutamine metabolism. However, when glucose is only in the lumen, it is somewhat difficult to believe that the intracellular concentration of substrate limits metabolism and allows 44% to pass through, unless hexokinase is compartmentalized, for the K_m of this enzyme is low, 0.1 mM (Srivastava *et al.*, 1968).

A second explanation is that, at the concentrations used here, substrate taken up from the lumen may not have access to all of the enzyme activity associated with its metabolism. Thus glutamine utilization is increased above that found with glutamine only in the lumen if glutamine is also added to the vascular bed. It is possible that some of the substrate taken up from the lumen crosses to the vascular bed by a route which avoids metabolism. Movement via the intercellular spaces is unlikely to be important in this context, for glucose does not readily enter the lumen from the vascular bed by this route (Table 3).

It appears that the utilization with glutamine in the lumen and vascular bed may be the sum of that when glutamine is in either compartment alone (Table 1, adding the values in rows 1 and 2). This suggests that the addition of glutamine to the lumen has little effect on metabolism of glutamine from the vascular bed, which agrees with the findings *in vivo* of Windmueller & Spaeth (1975).

Metabolism of glutamine and glucose in acidotic rats and rats deprived of food

Both the deprivation of food and the induction of acidosis increase the rate at which unit dry weight of jejunum utilizes glutamine; deprivation of food also increases alanine production (Table 5). MacFarlane-Anderson *et al.* (1976) found that metabolic acidosis had no effect on ammonia production by rings (transverse slices) of rat jejunum. Also, the activity of glutaminase in the intestinal mucosa was unchanged by the induction of acidosis, but this does not necessarily contradict our findings, because factors such as the access of glutamine to glutaminase may also affect glutamine utilization.

If allowance is made for changes in the amount of mucosa, by expressing results per unit length (Tables

Table 8. *Effect of glutamine in the lumen on metabolism of glucose by vascularly perfused rat jejunum*

Results, from three fed rats in each case, are expressed as means $\pm$ S.E.M. In both cases glucose (7.5 mM) was present in the vascular perfusate, but no glucose was present in the lumen.

[Glutamine]		Glucose utilization ($\mu\text{mol/h per g dry wt.}$)	Lactate production ($\mu\text{mol/h per g dry wt.}$)	$\frac{(\text{Lactate production} \times 0.5)}{\text{glucose utilization}} \times 100$ (%)
Vascular perfusate plasma (mM)	Luminal fluid (mM)			
0	4.5	150 $\pm$ 6	154 $\pm$ 16	51 $\pm$ 3
1.5	4.5	162 $\pm$ 8	110 $\pm$ 23	34 $\pm$ 7

4 and 5), then it appears that deprivation of food for 48 h is unlikely to alter the metabolism of glutamine by the jejunum *in vivo*. Deprivation of food, however, markedly decreases glucose utilization by the perfused preparation (Table 5) as it does by non-vascularly perfused jejunum incubated *in vitro* (Newey *et al.*, 1970; P. J. Hanson & D. S. Parsons, unpublished work). In jejunum from fed rats, glutamine utilization is 44% of glucose utilization, but in jejunum from rats deprived of food it is 112% of glucose utilization, which suggests that glutamine may be an important metabolic fuel in the intestine of rats deprived of food. Moreover, although no glutamine is directly converted into glucose, the alanine that is produced could be used by the liver for gluconeogenesis.

There is no effect of acidosis on glutamine utilization per cm of jejunum, which suggests that there may not be any decrease in intestinal glutamine metabolism to compensate for the increase found in the acidotic rat kidney (Hems, 1972; Welbourn, 1974; Ross, 1976). Indeed, Addae & Lotspeich (1968) found that the uptake of glutamine by the non-hepatic splanchnic organs of the dog was increased by the induction of acidosis. However, interpretation of our results is complicated by the fall in the plasma concentration of glutamine that takes place *in vivo* and which may affect glutamine utilization by the intestine (see below). Further, the uptake of glutamine by the non-hepatic splanchnic bed of the rat may decrease under acidotic conditions *in vivo* (Lund & Watford, 1976).

Effect of glutamine on glucose metabolism

Glutamine metabolism by vascularly perfused jejunum from fed rats occurs at a considerable rate, and, since more than half of the glutamine may be oxidized (Windmueller & Spaeth, 1974), it might be expected that glutamine by acting as an alternative substrate would inhibit the utilization of glucose, but, as with fatty acids (Hülsmann, 1971), this is not the case (Tables 7 and 8). A possible explanation is that an important factor limiting metabolism of glucose from the vascular perfusate is the entry of glucose into the epithelial cells (see above), and glutamine may have no influence on this process.

In virtually all cases the addition of glutamine does not significantly alter lactate production, or lactate production expressed as a percentage of glucose utilization, a result which is in contrast with that found with fatty acids (Hülsmann, 1971).

Influence of location of segment on metabolism

Glucose. The absence of an aborally decreasing gradient of glucose utilization (i.e. decreasing from the part of the small intestine closest to the mouth) when glucose, at a concentration close to physiological values, is present only in the vascular perfusate, suggests that in this instance there is little difference in the capacity of jejunal and ileal mucosa to take up and metabolize glucose from the vascular bed. With non-vascularly perfused preparations of intestine incubated *in vitro*, a gradient of glucose utilization is evident (Fisher & Parsons, 1950; Hanson & Parsons, 1976), and may possibly be related to the uptake and metabolism of glucose via the luminal pole of the intestinal epithelial cells, for in these preparations there is a gradient of glucose uptake from the lumen (Fisher & Parsons, 1950).

With non-vascularly perfused preparations of rat small intestine incubated *in vitro*, lactate production is lower in the ileum than in the jejunum (Wilson & Wiseman, 1954; Sherratt, 1968; Hanson & Parsons, 1976). This is also found with the perfused intestine when glucose is the only substrate in the vascular perfusate, but not if glutamine is also present (Table 7).

Glutamine. The ileum appears to be somewhat less important than the jejunum as a site of glutamine utilization (Table 7), particularly if the lower weight of mucosa per unit length is taken into account (Table 4). There is an increased proportion of muscle, which may not metabolize glutamine, in the ileal wall, but this cannot account for the difference between jejunum and ileum.

Influence of concentration, and presence of glucose, on metabolism of glutamine

Metabolism of glutamine is dependent on the concentration of glutamine in the vascular perfusate

Table 9. *Some factors affecting the metabolism of glutamine and glutamate by vascularly perfused rat jejunum*

Results are presented as means $\pm$ S.E.M., or, where only two experiments were performed, the two results and their mean are shown. All rats were fed and no substrates were present in the lumen of the intestine. A negative production represents a utilization.

Plasma concentration of additions to basic vascular perfusate (mM)	No. of animals	Rate ($\mu\text{mol/h}$ per g dry wt.)					(Lactate production $\times 0.5$) glucose utilization (%) $\times 100$
		Glucose utilization	Lactate production	Glutamine utilization	Glutamate production	Alanine production	
Glucose (7.5) + glutamine (1.5)	4	171 $\pm$ 17	130 $\pm$ 19	75 $\pm$ 7	7 $\pm$ 2	30 $\pm$ 3	38 $\pm$ 4
Glucose (1.5) + glutamine (1.5)	2	297 (269)	152 (134)	72 (59)	5 (3)	25 (21)	25 (25)
Glucose (7.5) + glutamate (1.5)	3	166 $\pm$ 12	95 $\pm$ 28	0	-12 $\pm$ 2	13 $\pm$ 2	28 $\pm$ 6
Glutamine (1.5)	4	0	0.04 $\pm$ 6	104 $\pm$ 13	7 $\pm$ 3	30 $\pm$ 4	—
Glutamine (4.5)	3	0	-14 $\pm$ 6	185 $\pm$ 3	9 $\pm$ 3	66 $\pm$ 1	—
Glutamine (4.5) + 3-mercaptopicolinic acid (0.1)	2	0	-15 (-5)	188 (191)	13 (13)	81 (91)	—
† 3-Mercaptopicolinic acid (0.1 mM) also present in the luminal fluid.			(-26)†	(185)†	(14)†	(72)†	—

(Table 9), and, in contrast with liver and skeletal muscle, intestinal mucosa does not appear to accumulate glutamine (Windmeuller & Spaeth, 1974), which suggests that the entry of glutamine into the epithelial cells may be a factor limiting its metabolism.

Glutamine seems to be metabolized in a similar manner, and at the same rate, irrespective of the presence of exogenous glucose (Table 9), which necessitates an alternative source of pyruvate for transamination to alanine with the glutamate derived from glutamine. It is possible that glutamate itself might be converted into pyruvate. Such a conversion would involve either phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) or 'malic' enzyme (EC 1.1.1.40), both of which are present in the intestinal mucosa (Anderson, 1970; Tyrrell & Anderson, 1971). Indeed the conversion of glutamate into pyruvate is essential if glutamate carbon is to be completely oxidized in the tricarboxylic acid cycle (Goldstein & Newsholme, 1976). However, an attempt to demonstrate the involvement of phosphoenolpyruvate carboxykinase by the use of the inhibitor 3-mercaptopicolinic acid (Kostos *et al.*, 1975), which at a concentration of 0.1 and 0.15 mM respectively markedly inhibits gluconeogenesis in perfused liver (DiTullio *et al.*, 1974) and kidney (Ross, 1976), was unsuccessful. However this may have been because the inhibitor was unable to penetrate to the site of phosphoenolpyruvate carboxykinase.

Uptake of glutamate from vascular perfusate

Glutamate uptake from the vascular infusate is much slower than that of glutamine. The metabolism and hence the uptake of glutamate is unlikely to be limited by the activity of glutamate-pyruvate aminotransferase (EC 2.6.1.2), for this enzyme has considerable activity in homogenates of jejunal mucosa (Volman-Mitchell & Parsons, 1974). However, it is possible that glutamate is poorly transported across the basolateral membrane of the intestinal epithelial cells and that this limits its uptake from the vascular bed.

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