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Transport and metabolism of glucose by rat small intestine

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1. Glucose transport and metabolism by rat small intestine was investigated by using a preparation for the combined perfusion of the lumen and the vascular bed. 2. When 5mM-glucose was present in the lumen, only 29% was transported unchanged to the vascular side. Lactate output into the vascular and luminal fluids accounted for a further 53% and 4% respectively of the glucose taken up from the lumen. 3. Glucose was readily taken up when added at 5mM to the vascular compartment only. Vascular lactate output accounted for approx. 33% of the glucose uptake, and luminal lactate for 6%. 4. When 2mM-glucose was added to the lumen with 5mM-glucose in the vascular perfusate, there was no detectable net transport of glucose to the vascular side. However, of the glucose taken up from the lumen, 31% and 2% could be accounted for by increases in vascular and luminal lactate respectively. 5. When 10mM-glucose was added to the lumen, with 5mM-glucose in the vascular perfusate, 33% of the glucose disappearing from the lumen was transported to the vascular side. Extra lactate output to the vascular and lumen perfusates accounted for 40% and 9% respectively of the glucose uptake from the lumen. 6. These studies indicate that at low luminal glucose concentrations no sugar is transferred to the blood unchanged, and at sugar concentrations of 5–10mM only 25–50% of the glucose leaving the lumen reaches the serosal side intact. Furthermore, the small intestine has a greater propensity to form lactate from luminal glucose than from vascular glucose.

Glucose derived from the digestion of dietary carbohydrates has generally been thought to remain intact during its absorption by the small intestine. Hanson & Parsons (1976, 1977), using a preparation of rat small intestine vascularly perfused *in vitro*, and Shapiro & Shapiro (1979), working with the rat *in vivo*, have presented evidence suggesting that the amount of glucose absorbed intact is much smaller than has previously been supposed, and that a considerable proportion of the glucose is converted into lactate during absorption. Pritchard & Porteous (1977) reached a similar conclusion, working with everted sacs. In contrast, Windmueller & Spaeth (1980), using an elegant technique *in vivo*, have claimed that 97% of luminal glucose appears unchanged in the intestinal venous blood.

The glucose concentrations used in the above studies differed considerably, and some of the experiments were performed with radioactive glucose, others with unlabelled sugar. In an attempt to resolve these discrepancies, we have performed a series of experiments using a preparation in which both the lumen and the vascular bed of the rat small intestine are perfused. This has enabled us to monitor glucose fluxes and lactate formation continuously, and to examine the effects of orientation and concentration on the transport and metabolism of glucose.

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Materials and methods

Animals

Male Wistar rats (240–260g body wt.) were kept on a 12h-day/12h-night cycle (light from 07:00 to 19:00h) and were allowed free access to food (Oxoid diet 4B) and water until 2h before perfusion. They were taken from the animal house to the laboratory at approx. 11:30h, and all experiments were performed between 13:00 and 15:00h.

Materials

All common chemicals were of analytical-reagent quality. Lactate dehydrogenase (EC 1.1.1.27) and

NAD^+ together with the test combination (GOD-Perid) used in the determination of glucose were from Boehringer. Radiochemicals were supplied by Amersham International. Bovine serum albumin (fraction V) was supplied by Sigma.

Perfusion fluids

The luminal fluid was a modified Krebs–Henseleit bicarbonate medium (Krebs & Henseleit, 1932), with the following composition (mM): NaCl 118, KCl 4.74, KH_2PO_4 1.18, $MgSO_4$ 1.18, $CaCl_2$ 1.27, $NaHCO_3$ 24.88; gassed with O_2/CO_2 (19:1); final pH 7.4. The vascular perfusate consisted of the same medium supplemented with dialysed bovine serum albumin (2.5%, w/v) and washed bovine erythrocytes (25% haematocrit). The use of bovine rather than human erythrocytes probably minimized vasoconstriction in the blood vessels serving the intestine (Hanson & Parsons, 1976; Bronk & Ingham, 1979).

Perfusion apparatus

This was based on the apparatus described by Hanson & Parsons (1976) as modified by Bronk & Ingham (1979).

The luminal perfusate flow was interrupted with bubbles of O_2/CO_2 (19:1) (segmented circulation) to minimize unstirred layers and improve the oxygenation of the epithelial cells (Fisher & Gardner, 1974). The fluid was recirculated through the lumen at a total flow rate of 10 ml/min (perfusate 7 ml/min and gas 3 ml/min). The luminal distension pressure was 15–20 cm of Krebs–Henseleit medium, which minimized peristalsis and facilitated even access of nutrients and oxygen to the mucosa.

The vascular perfusate was introduced into the superior mesenteric artery at a rate of 2 ml/min, and collected from a cannula in the hepatic portal vein. It was not recirculated. The vascular perfusion pressure was 45–65 mmHg, measured with an anaeroid dial gauge (Accoson; Isis Instruments, Oxford, U.K.) inserted into the circuit just before the arterial cannula.

Experimental procedure

Animals were anaesthetized with 12 mg of sodium pentobarbitone (Sagatal; May and Baker, Dagenham, U.K.) intraperitoneally. The section of small intestine used extended 30–40 cm from a point about 5 cm below the Ligament of Treitz. The operative procedure was similar to that of Bronk & Ingham (1979), except that three modifications (two of them used by Hanson & Parsons, 1976) were introduced to minimize vasoconstriction of the blood vessels supplying the segment. Firstly, killing the animals by cutting the heart seemed to promote vasoconstriction, possibly because of nervous stimulation generated when the thorax was cut open. Several alternatives were tried, of which injection of a large amount of pentobarbitone anaesthetic into the heart was the most effective. Secondly, the animals were heparinized by the injection of 300 i.u. of heparin into the femoral vein before cannulation of the aorta. Finally, since vasoconstriction also seemed to occur as a response to unduly high vascular perfusion pressures, the perfused segment was isolated from the animal's own blood supply by tying the upper ligature round the aorta before, rather than after, the vascular perfusate flow was started.

Both perfusates were recirculated for 1–2 h before a perfusion to ensure that they were completely equilibrated and oxygenated. During this period, glucose metabolism and lactate production by erythrocytes were minimal and did not significantly affect the perfusate glucose concentration. The vascular perfusate was filtered during recirculation by insertion of a filter unit, containing a small disc of Whatman 540 paper, into the vascular circuit. This was removed for the experiment, since it caused variations in the vascular flow rate.

The perfused segment was distended by pumping air through it before the perfusate reached the tissue, minimizing non-specific fluxes. The perfusions were timed from the point at which the luminal perfusate entered the jejunal segment. Since vascular perfusion began once the aorta was cannulated, the intestine was exposed to substances in this perfusate before timing of the experiment began. To minimize variation from this, luminal perfusion was always started 7 min after vascular perfusion had begun. This was the time required for reliable cannulation of the portal vein and connection of the luminal circuit.

Samples of vascular effluent were collected in small auto-analyser cups over 1 min intervals, and 0.5 ml of each sample was added to 1 ml of ice-cold 0.6 M-$HClO_4$. After extraction, the tubes were centrifuged for 1 min at 8860 *g*. In experiments involving radioactive sugars, 0.5 ml of supernatant was added to 5 ml of scintillation fluid (Fisofluor, Fisons). A further sample was neutralized with K_2CO_3 and the supernatant from this was used for glucose or lactate determination. At the end of each experiment (usually 45 min), the perfused section of intestine was removed from the animal and its length measured. A standard curve of length versus wet and dry weight was determined to enable the results for glucose fluxes and rates of lactate appearance to be expressed as nmol/min per g dry wt. The regression equation for this was:

$$\text{Dry wt. (g)} = 0.020 \times \text{length (cm)} - 0.029.$$

Analytical methods

Glucose was determined by a glucose method (GOD-Perid) in a Technicon Autoanalyser. Lactate was determined with lactate dehydrogenase by the

automated fluorimetric procedure of Leese & Bronk (1972).

Expression of results

The results are means ± S.E.M., with numbers of values (*n*) in parentheses. The statistical significance of difference between means was determined by Student's *t* test.

Results

Viability of the preparation

The mucosa of the rat small intestine is notoriously susceptible to anoxia, and this has bedevilled the interpretation of studies on glucose transport and lactate formation by this tissue. For this reason we were particularly concerned to provide evidence that the oxygen supply to our preparation was adequate to support rates of aerobic respiration.

It was difficult to measure the uptake of oxygen from the erythrocyte-containing vascular perfusate, but oxygen was removed from the luminal perfusate presented as a continuous flow of medium rather than a segmented circulation, at a rate of 1.94 μl of O_2/h per mg dry wt. If the vascular perfusate was stopped, the rate of uptake of oxygen from the lumen increased to 4.97 μl/h per mg dry wt. Hence, during vascular perfusion, oxygen uptake from the lumen was occurring at only 40% its potential rate. This suggested that the normal preparation with vascular perfusion and segmented circulation of luminal perfusate was adequately oxygenated. High rates of active galactose transport similar to those reported by Bronk & Ingham (1979) were maintained throughout perfusion. The addition of 5 mM-D-glucose to the vascular perfusate increased the uptake of D-galactose from the lumen and its rate of transfer to the vascular bed. Similar results have been reported by Tzur & Shapiro (1960) and Bronk & Ingham (1979).

Glucose transport and metabolism

Experiments were performed in which glucose was added either to the vascular or to the luminal perfusates or to both solutions. Glucose fluxes and lactate appearance were measured simultaneously, and the results are shown in Tables 1 and 2.

(1) *Glucose present in one perfusate only* (*Table 1*). (*a*) Vascular glucose. With 5 mM-glucose present

Table 1. *Effects of orientation and concentration of glucose added to either the lumen or the vascular compartment on glucose absorption and lactate production by perfused rat jejunum*

For full details see the text. Results are expressed as means ± S.E.M., with numbers of experiments in parentheses. * Indicates values significantly different ($P < 0.05$) from the value obtained when 5 mM-glucose was present in the vascular perfusate only.

Initial glucose concns. (mM)		Rate (nmol/min per g dry wt.)			
Vascular medium	Luminal medium	Average rate of glucose uptake from lumen	Average rate of glucose uptake (−) or appearance (+) in vascular medium	Average rate of lactate output into lumen	Average rate of lactate output into vascular medium
5	0	—	−3661 ± 99 (6)	453 ± 57 (3)	2385 ± 73 (3)
10	0	—	−8417 ± 271 (4)	641 ± 104 (4)	5729 ± 167 (4)*
0	5	−4151 ± 380 (5)	+1219 ± 380 (5)	354 ± 26 (5)*	4411 ± 323 (5)*

Table 2. *Effects of concentration of glucose added to the lumen on glucose absorption and lactate production by perfused rat jejunum*

Glucose was added to the lumen after 10 min preincubation. In each case, glucose (5 mM) was present in the vascular perfusate throughout. For other details see the text. Results are expressed as means ± S.E.M.; *n* = numbers of experiments, except for 0 glucose concentration in luminal medium, where these are given in parentheses. * Indicates values significantly different ($P < 0.05$) from those in the absence of luminal glucose. † Indicates values significantly different ($P < 0.05$) from the corresponding value in the period 0–10 min.

Glucose concn. in luminal medium (mM)	*n*	Rate (nmol/min per g dry wt.)						
		Average rate of glucose uptake from lumen	Average rate of glucose uptake from vascular medium		Average rate of lactate output into lumen		Average rate of lactate output into vascular compartment	
			0–10 min	10–30 min	0–10 min	10–30 min	0–10 min	10–30 min
10	5	4307 ± 792	3604 ± 349	2172 ± 432*†	396 ± 36	1161 ± 198*†	3417 ± 156	6896 ± 797*†
2	4	1406 ± 151	3458 ± 130	3458 ± 130	464 ± 68	531 ± 89	3583 ± 240	4464 ± 464*†
0			3661 ± 99 (6)		453 ± 52 (3)		3151 ± 422 (3)	2297 ± 167 (3)

in the vascular perfusate, the rate of glucose uptake was fairly constant throughout the perfusion. The average rate of uptake was 3661 ± 99 nmol/min per g dry wt. ($n = 6$), of which 39% could be accounted for by lactate output. When the glucose concentration was increased to 10 mM, glucose uptake from the vascular perfusate and lactate output were increased to approximately the same extent. At both concentrations, the rate of lactate output to the vascular perfusate was much greater than to the luminal perfusate.

(*b*) Luminal glucose. When 5 mM-glucose was present in the luminal perfusate, net transfer of glucose to the vascular bed was observed, and on average 29.4% of the glucose taken up appeared unchanged in the vascular effluent.

The output of lactate was significantly greater ($P < 0.05$) than the rate observed with 5 mM-glucose in the vascular perfusate, but lower than that with 10 mM vascular glucose. The average rate of lactate output into the lumen was significantly lower ($P < 0.05$) with luminal glucose than with vascular glucose.

Lactate output into the vascular and luminal perfusates represented 53% and 4% respectively of the glucose taken from the lumen, and together with the glucose transferred unchanged accounted for a total of 86% of the absorbed sugar. CO_2 production and tissue glucose and lactate contents, which were not measured, would probably account for the remaining glucose.

(2) *Glucose present in both perfusates* (*Table 2*). Glucose was added to the luminal perfusate after 10 min perfusion while 5 mM-glucose was present in the vascular perfusate throughout. In the first series of experiments, the initial luminal glucose was 10 mM so that the lumen-to-vascular concentration gradient was downhill. After addition of glucose to the lumen, vascular glucose uptake fell, by about 28%, to an average value of 2172 ± 432 nmol/min per g dry wt. ($n = 5$). After addition of glucose to the luminal perfusate, lactate output to the vascular bed was increased by more than 100% ($P < 0.05$). The rate of lactate output to the luminal perfusate was also significantly greater when 10 mM-glucose was present in the lumen.

In the second series of experiments, the luminal glucose concentration was 2 mM, so that the gradient from lumen to vascular side was uphill.

When the 2 mM-glucose was added to the lumen after the preincubation period (0–10 min), there was no detectable change in the rate of vascular glucose uptake. Thus the transfer of glucose from the lumen to the vascular bed and any change in the utilization of vascular glucose were not measurable under these conditions.

However, lactate output to the vascular perfusate was significantly increased after addition of glucose to the lumen. Luminal lactate output was also slightly greater than in the absence of luminal glucose, although this difference was not significant.

Fate of glucose absorbed from the lumen

When 10 mM-glucose was added to the lumen, the rate of glucose uptake from the vascular perfusate was decreased. The total decrease in vascular glucose uptake was calculated relative to the average rate of glucose uptake from the vascular perfusate during the 10 min preincubation period. This seemed reasonable, since vascular glucose uptake remained fairly constant in the absence of luminal glucose. A decrease in vascular glucose uptake was equivalent to an increase in glucose appearance in the vascular effluent.

The average rates of lactate output into the vascular and luminal perfusates during the 10 min preincubation period were similar for all the perfusions performed, and rose after addition of glucose to the lumen. Therefore the extra lactate output in the presence of luminal glucose was calculated relative to the rates of output observed in perfusions where there was no glucose in the lumen.

For 10 mM luminal glucose, the average rate of glucose uptake from the lumen was 4307 ± 797 nmol/min per g dry wt. ($n = 5$). The increased glucose appearance in the vascular effluent could account for 33.2% of this uptake. The extra lactate output to the vascular and luminal perfusates accounted for 40% and 8.9% respectively of the glucose taken up from the lumen.

When 2 mM-glucose was added to the lumen, the average rate of glucose uptake from this perfusate was 1406 ± 151 nmol/min per g dry wt. ($n = 4$), which was significantly lower ($P < 0.05$) than the rate with 10 mM-glucose. There was no detectable increase in glucose appearance in the vascular effluent, but extra vascular lactate output accounted for 31.3% of the luminal glucose uptake. Extra lactate output to the lumen accounted for a further 2.4% of the uptake.

Discussion

The two main findings to emerge from this work are (1) that there is a glucose concentration below which the rat small intestine will not translocate the sugar intact from the lumen to the vascular bed and (2) that the main product of glucose metabolism is lactate. Thus, although glucose at 2 mM in the lumen (with 5 mM-glucose on the vascular side) was taken up by the mucosal cells, net transport to the serosal compartment was not observed. At the higher concentration of 5 mM in the lumen (and the absence of glucose on the serosal side), 29% appeared unchanged in the vascular effluent. This value was slightly higher than that reported by Pritchard &

Porteous (1977), i.e. 25% with 10mM-glucose present in both the mucosal and serosal compartments, and somewhat lower than that of Hanson & Parsons (1977), i.e. 44% with 7.5mM-glucose present in only the luminal fluid. Of the luminal glucose uptake, 57% could be accounted for by increased lactate in the vascular and luminal fluids. This value is fairly close to those reported by Pritchard & Porteous (1977) and Hanson & Parsons (1977) (50% and 29% respectively). These two studies and our own favour the view that at sugar concentrations of 5–10mM only 25–50% of the glucose leaving the lumen reaches the serosal side intact.

Glucose was readily taken up when presented to the tissue via the vascular perfusate. The rates of uptake and lactate output were roughly doubled when the vascular concentration was raised from 5 to 10mM, suggesting that the glycolytic pathway accessible to glucose from the blood was far from saturated at these glucose concentrations. The lactate output accounted for about 39% of the glucose uptake. This was virtually identical with that obtained in equivalent experiments by Hanson & Parsons (1977) (40%). It was noteworthy that total lactate output was significantly greater with 5mM-glucose in the lumen than with the same concentration in the vascular fluid (4765 rather than 2838nmol/min per g dry wt.). This suggested that the intestine had a greater propensity to form lactate from luminal glucose than from vascular glucose, in agreement with the findings of Hanson & Parsons (1976, 1977).

When 10mM-glucose was added to the lumen with 5mM-glucose in the vascular perfusate, the overall picture as regards glucose translocation was similar to that observed when 5mM-glucose was added to the lumen only, i.e. glucose appearance in the vascular fluid accounted for about 33% of lumen disappearance, and approx. 50% was accounted for by lactate formation.

Although these results are in general agreement with those of Hestrin-Lerner & Shapiro (1954), Tzur & Shapiro (1960), Hanson & Parsons (1976, 1977) and Pritchard & Porteous (1977), in demonstrating that a considerable proportion of luminal glucose is absorbed as lactate, they contradict other reports [Kiyasu *et al.* (1956), Taylor & Langdon (1956), Atkinson *et al.* (1957), Windmueller & Spaeth (1980)], which have claimed that there is very little conversion of glucose into lactate during absorption from loops of intestine *in vivo*. Some of these discrepancies may be accounted for by the high luminal glucose concentrations (20–70mM) used by these workers, where lactate formation from luminal glucose would be saturated and most of the absorbed glucose would be translocated unchanged. However, this cannot apply to the study of Windmueller & Spaeth (1980), in which in two experiments trace amounts of radioactive glucose were added to the lumen and 97% and 81% of the label appeared as unchanged glucose in the blood. The significance of these experiments may lie in their use of radioactive glucose. A rapid exchange of radioactive glucose in the mucosal cells for unlabelled glucose in the blood could give the impression of a high rate of net radioactive glucose translocation across the intestine. The existence of possible discrepancies between chemical and isotopic sugar determinations has also been referred to by Tzur & Shapiro (1960).

Our results, together with those of Hestrin-Lerner & Shapiro (1954), Tzur & Shapiro (1960), Hanson & Parsons (1976, 1977) and Pritchard & Porteous (1977), underline the importance of the metabolic role of the small intestine in carbohydrate metabolism. By metabolizing a substantial amount of the available glucose to lactate, the intestine can obtain most of the energy it requires and at the same time release most of the glucose carbon into the blood as lactate, which can be reconverted into glucose by the liver. The fact that the formation of lactate from glucose is more pronounced when the sugar is presented to the intestine from the lumen rather than the vascular bed suggests that this conversion is particularly associated with the uptake of dietary glucose.

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