Evidence *in vivo* that most of the intraluminally absorbed glucose is absorbed intact into the portal vein and not metabolized to lactate

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Studies *in vitro* and acute studies *in vivo* have indicated that the intestine may be a significant producer of portal-venous lactate, a major carbon source for liver glycogen synthesis. To determine if a significant portion of intraluminal glucose is converted into lactate by the intestine *in vivo*, we measured the ratio of intraluminal glucose which is absorbed intact into the portal vein to that which is converted into lactate by the intestine in a chronically catheterized rat, in which catheters were surgically placed into the portal vein, aorta and stomach. This ratio was 36–42 when intraluminal [^{14}C]glucose concentrations of 5–200 mM were used, suggesting that the intestine may not be a significant source of portal-venous lactate *in vivo*. Under hypoxic conditions [$\text{PaO}_2 < 40$ Torr (5.3 kPa)] the ratio decreased to 2.1, indicating that the amount of intraluminal glucose converted into lactate had increased significantly.

INTRODUCTION

It has been reported that the intestine metabolizes glucose to lactate under aerobic conditions [1–4]. Boyd *et al.* [5] and Remesy *et al.* [6] have reported elevated portal-venous plasma lactate concentrations after high-carbohydrate feeding, and lactate has been reported to be a major carbohydrate precursor for liver glycogen synthesis [5]. Boyd *et al.* [5] have proposed that the intestine may be a significant source of portal-venous lactate, which is used by the liver for glycogen synthesis.

The extent to which the intestine metabolizes intraluminal glucose to lactate under aerobic conditions has been studied by several investigators using various animal preparations. Windmueller & Spaeth [4], using an intestinal preparation *in vivo*, in which the segment of jejunum had its native arterial vascular supply, nervous innervation and lymphatic drainage intact, and was continuously perfused with donor arterial blood, found that 97% of 70 mM intraluminal glucose was absorbed intact across the intestinal wall of fed rats. Hanson & Parsons [1], using a vascularly perfused jejunal model *in situ*, found that the amount of glucose utilized, and the amount of lactate produced, depended on the concentrations of intraluminal and intravascular glucose, and on the haematocrit of the perfusate. Using a vascular perfusate with a haematocrit of 40% and a luminal glucose concentration of 10–28 mM, they found that 52–69% of glucose was converted into lactate by the intestine. Nicholls *et al.* [3], also using a vascularly perfused preparation *in situ*, in which bovine erythrocytes were added to the perfusate to give a haematocrit of 25%, found that at low concentrations of intraluminal glucose (2 mM) none was absorbed intact, whereas at higher concentrations (5–10 mM) only 25–50% was absorbed intact; the remainder was metabolized to lactate.

We determined the ratio of intraluminal glucose absorbed intact into the portal vein to that metabolized to lactate and then transferred to the portal vein in an animal model *in vivo* using a chronically catheterized rat.

We determined the effect of various intraluminal glucose concentrations and the effect of hypoxia on this ratio.

METHODS

Animals

All experiments were performed with adult male albino Sprague-Dawley rats (250–300 g body wt.) obtained from Simonsen Labs (Gilroy, CA, U.S.A.). The animals were anaesthetized with pentobarbital (10 mg/kg) intraperitoneally and ketamine (20 mg/kg) intramuscularly. Gastric, portal-venous and aortic catheters were surgically placed by using sterile technique in accordance with the method described in detail by Kimura & LaPine. [7]. After surgery, the catheters were flushed daily with 0.35 ml of 0.9% NaCl, containing 500 units of heparin/ml and 2.5 mg of Ampicillin/ml.

Study protocol

We utilized fed, catheterized, conscious rats who had recovered from catheter-placement surgery. Absorption of intraluminal substrates is normal by 48 h, and only rats that were at least 4 days post-operative were used. We infused 10 ml of 5 mM-, 20 mM- or 200 mM-[^{14}C]glucose (1.2 $\mu\text{Ci/ml}$) into the gastrostomy over 2–3 min. Early studies [7] monitoring these bolus infusions to 30 min showed that peak absorption occurred between 9 and 15 min after introduction of the glucose. For the current studies, 0.25 ml blood samples were simultaneously withdrawn from the portal-venous and aortic catheters during the peak absorption period. These blood samples were immediately placed in equal volumes of ice-cold 1 M- HClO_4 , then centrifuged to remove the protein precipitate. The supernatant was neutralized and the precipitate removed by centrifugation. Lactate concentrations were measured on the neutralized supernatant by a fluorimetric adaptation of a spectrophotometric assay for lactate [8], using lactate dehydrogenase (EC 1.1.1.27).

The ^{14}C in the form of lactate and glucose was

separated with Dowex ion-exchange columns by the method of Katz *et al.* [9]. The neutralized sample was pipetted on to Dowex 50 columns (previously activated with HCl), then the eluate was dripped through a similar column containing Dowex 1 (previously activated with acetic acid after treatment with NaOH), and the final eluate was collected. The Dowex 50 binds alanine, the Dowex 1 binds lactate, whereas the glucose, which is not bound to either column, passes through to the collecting tube. Since alanine has been shown to be a significant end product of intestinal glucose metabolism [4], we have removed the alanine produced from [^{14}C]glucose by the intestine. We have also removed any $^{14}\text{CO}_2$ produced by acidification of the blood. The bound lactate in the Dowex 1 column was released by using 1 M-acetic acid. The glucose and lactate solutions were each mixed in EcoLite (WestChem, San Diego, CA, U.S.A.) and counted for ^{14}C radioactivity with a Beckman liquid-scintillation counter (model LS1800). By this method, we recovered 86 % of [$\text{U-}^{14}\text{C}$]glucose in the glucose fraction, and 81 % of [$\text{U-}^{14}\text{C}$]lactate in the lactate fraction.

Hypoxic studies

For the hypoxic studies, the fed rats were placed into an enclosed Plexiglass container. The chamber was then flushed with a gas mixture of 48 % room air and 52 % of N_2/CO_2 (19:1) providing an FiO_2 (fraction of inspired O_2) of 0.07. The rats remained in this hypoxic environment with a continuous inflow (10.0 litres/min) of the gas mixture for 5 min, after which time a zero-time blood sample was drawn. Then 10 ml of 20 mM-[$\text{U-}^{14}\text{C}$]glucose (1.2 $\mu\text{Ci/ml}$) was infused into the gastrostomy. Blood samples were withdrawn at 10 and 12 min after the infusion. The chamber lid was then opened and the rats were allowed to recover in room air. Blood samples were withdrawn at 10 and 12 min after the return to room air. Blood samples drawn were processed in the same manner as in the normoxic rats, and analysed for lactate, [^{14}C]glucose and [^{14}C]lactate.

The adequacy of the hypoxic environment was judged by the development of an aortic lactic acidosis. The actual measurement of PaO_2 (arterial partial pressure of O_2) was not done at the time of each experiment, since the blood was radioactive. However, by using seven separate rats under identical conditions, arterial blood gases were drawn after the rats had been in the hypoxic chamber for 15 min. These data demonstrated that the

mixture we used gave a PaO_2 of 37.5 ± 0.1 Torr and a PCO_2 (CO_2 partial pressure) of 26.2 ± 1.0 Torr, compared with normoxic rats, who had a PaO_2 of 72.9 ± 2.2 Torr and a PCO_2 of 28.5 ± 0.1 Torr (all values mean $\pm$ S.E.M.; 1 Torr = 133 Pa).

Viability of the catheterized rats

After the experiments the rats were returned to their cages and placed into a vented holding area for radioactive animals. The catheters were flushed daily, and the animals were re-used for subsequent experiments as long as catheters remained patent. Animals were transfused at the conclusion of each experiment to restore blood volume. The animals appeared to suffer no ill effects from the infusions, from the radioactive glucose or from the hypoxic environment.

Calculations

The flux of [^{14}C]glucose into the portal vein is equal to the difference between portal-venous and aortic [^{14}C]glucose concentrations ($[\text{glucose}]_{\text{PV}} - [\text{glucose}]_{\text{A}}$) $\times$ mesenteric blood flow (MBF), and the flux of [^{14}C]lactate into the portal vein is equal to the difference between portal-venous and aortic [^{14}C]lactate concentrations ($[\text{lactate}]_{\text{PV}} - [\text{lactate}]_{\text{A}}$) $\times$ MBF. The ratio of the flux of [^{14}C]glucose into the portal vein to the flux of [^{14}C]lactate into the portal vein is:

$$\frac{[\text{glucose}]_{\text{PV}} - [\text{glucose}]_{\text{A}}}{[\text{lactate}]_{\text{PV}} - [\text{lactate}]_{\text{A}}} \times \frac{\text{MBF}}{\text{MBF}}$$

or

$$[\text{glucose}]_{\text{PV}} - [\text{glucose}]_{\text{A}} : [\text{lactate}]_{\text{PV}} - [\text{lactate}]_{\text{A}}$$

RESULTS

Aerobic data

Experiments were performed in which 10 ml of 5 mM-, 20 mM- or 200 mM-[$\text{U-}^{14}\text{C}$]glucose was infused into the gastrostomy, and portal-venous and aortic blood samples were drawn at peak absorption times and counted for radioactivity (Table 1). With 5 mM-glucose the ratio $[\text{glucose}]_{\text{PV}} - [\text{glucose}]_{\text{A}} : [\text{lactate}]_{\text{PV}} - [\text{lactate}]_{\text{A}}$ was 36 ± 14 (mean $\pm$ S.E.M.). With 20 mM-glucose, the ratio was 37 ± 6 , and with 200 mM-glucose, the ratio was 42 ± 10 (Table 1). The data for these three sets of infusions show no significant difference in the ratios of [^{14}C]glucose

Table 1. Effect of various concentrations of intraluminal glucose on [^{14}C]glucose and [^{14}C]lactate in aortic and portal-venous blood samples

Blood was drawn from the portal vein and aorta during peak absorption times after intraluminal infusion of 5 mM-, 20 mM- or 200 mM-[$\text{U-}^{14}\text{C}$]glucose. Results are c.p.m./g of blood and are means $\pm$ S.E.M. GLUC/LAC refers to the ratio $[\text{glucose}]_{\text{PV}} - [\text{glucose}]_{\text{A}} : [\text{lactate}]_{\text{PV}} - [\text{lactate}]_{\text{A}}$. *indicates values significantly different ($P < 0.05$) when calculated by a Friedman non-parametric ANOVA for related measures.

[Glucose]	[^{14}C]Glucose			[^{14}C]Lactate			GLUC/LAC
	Aorta	Portal vein	[PV - A]	Aorta	Portal vein	[PV - A]	
5 mM ($n = 8$)	381922 $\pm$ 117375	468166 $\pm$ 139600	86244 $\pm$ 24292*	10629 $\pm$ 2877	14057 $\pm$ 4017	3428 $\pm$ 1268*	36 $\pm$ 14
20 mM ($n = 12$)	106637 $\pm$ 13560	130581 $\pm$ 16058	23945 $\pm$ 4341*	3540 $\pm$ 510	4406 $\pm$ 610	956 $\pm$ 218*	37 $\pm$ 6
200 mM ($n = 7$)	55953 $\pm$ 9087	66935 $\pm$ 9474	10982 $\pm$ 1653*	1335 $\pm$ 209	1733 $\pm$ 264	399 $\pm$ 105*	42 $\pm$ 10

Table 2. Effect of hypoxia on [^{14}C]glucose and [^{14}C]lactate in aortic and portal-venous blood samples

Blood was drawn from the portal vein and aorta during peak absorption times after intraluminal infusion of 20 mM-[^{14}C]glucose under normoxic and hypoxic conditions and after return to room air (Recovery). Results are expressed as c.p.m./g of blood and are means $\pm$ S.E.M. GLUC/LAC refers to the ratio [^{14}C]glucose [PV-A]:[^{14}C]lactate [PV-A]. *indicates values significantly different ($P < 0.05$) when calculated by a Friedman non-parametric ANOVA for related measures.

	[^{14}C]Glucose			[^{14}C]Lactate			GLUC/LAC
	Aorta	Portal vein	[PV-A]	Aorta	Portal vein	[PV-A]	
Control ($n = 12$)	106637 $\pm$ 13560	130581 $\pm$ 16058	23945 $\pm$ 4341*	3540 $\pm$ 510	4406 $\pm$ 610	956 $\pm$ 218*	37 $\pm$ 6
Hypoxic ($n = 4$)	42903 $\pm$ 16368	59321 $\pm$ 15770	16417 $\pm$ 4189*	6883 $\pm$ 1951	19702 $\pm$ 7170	12819 $\pm$ 5315*	2.1 $\pm$ 0.9
Recovery ($n = 4$)	59468 $\pm$ 19677	63822 $\pm$ 20236	4354 $\pm$ 919*	7039 $\pm$ 1721	7916 $\pm$ 1668	876 $\pm$ 273*	7.7 $\pm$ 3.4

[PV-A]:[^{14}C]lactate [PV-A] when 5 mM-, 20 mM- or 200 mM- or 200 mM-glucose is infused intraluminally.

Hypoxic studies

The hypoxic studies were done with 20 mM-[^{14}C]glucose. The adequacy of the hypoxic environment was judged by the development of an aortic lactic acidosis, but full recovery of the rat after return to room air. Rats that did not demonstrate an increase in blood lactate to at least 3.4 $\mu\text{mol/g}$ of blood from the average baseline value of 1.0 $\mu\text{mol/g}$ of blood were excluded. Also, data from those rats that had an excessive increase in blood lactate greater than 8 $\mu\text{mol/g}$ of blood were excluded, in that these animals had an excessive metabolic acidosis and did not recover fully. With the four rats that demonstrated an adequate increase in blood lactate and who showed no ill effects from the hypoxic environment, the peak aortic lactate concentration was 6.1 $\mu\text{mol/g}$ of blood and the ratio [^{14}C]glucose [PV-A]:[^{14}C]lactate [PV-A] was 2.1 ± 0.9 (Table 2). After the 12 min recovery period, the ratio rose to 7.7 ± 3.4 . Comparison of the aerobic data with the hypoxic data shows that under hypoxic conditions the [^{14}C]lactate [PV-A] was 10-fold greater than under aerobic conditions (Table 2).

DISCUSSION

The question of glucose absorption has been addressed numerous times, but none in a mode truly *in vivo*. We have shown that the absorption of intraluminal glucose is significantly different in catheterized rats at the time of surgery, compared with 48 h post-operatively and beyond [7], which is in agreement with suggestions in the literature that gastrointestinal function is significantly different just after surgery [10]. Our chronically catheterized rat model allows the issue of glucose absorption *in vivo* to be investigated.

In our work we were concerned with the flux of glucose and lactate into the portal vein. Flux, by definition, is the difference between the portal-venous and aortic concentrations ([PV-A]) of a substrate, multiplied by the mesenteric blood flow (MBF). We were not measuring MBF, but by using the ratio of [^{14}C]glucose [PV-A]:[^{14}C]lactate [PV-A] we were able to eliminate the need to measure it.

Our data show that when various amounts of glucose were given intragastrically, under aerobic conditions, most was absorbed intact, with minimal amounts being converted into lactate. By using the same amount of

[^{14}C]glucose with increasing unlabelled glucose concentrations, the specific radioactivity decreased with the higher glucose concentrations, thereby giving lower c.p.m. values. In comparing our work with that of Windmueller & Spaeth [4], our data are fairly comparable with theirs, perhaps owing to the elegance of their model, which acknowledges that the intestine is not merely a hollow tube, and perhaps their model avoided local areas of hypoxia. Nicholls *et al.* [3] concluded that the reason why Windmueller & Spaeth [4] had such a low percentage of glucose metabolized to lactate was because of the relatively high intraluminal glucose concentration (70 mM) used. We found that glucose concentrations from 5 to 200 mM did not alter our results. Nicholls *et al.* [3] and Hanson & Parsons [1] found similar results; less than half of the intraluminal glucose was absorbed intact in their vascularly perfused models. Those authors acknowledge that the oxygen supply to the tissues is important. The discrepancy between our data *in vivo* and published data obtained *in vivo* and *in situ* by those earlier authors may be related to actual delivery of oxygen to the enterocyte.

Early data with perfused intestine and isolated enterocytes showed that substantial quantities of lactate were formed from glucose [11]. Hanson & Parsons [1], using rings of jejunum, failed to demonstrate a Pasteur effect. Lamers & Hulsmann [12], however, showed a decrease in the amount of lactate produced by isolated small intestine, by increasing the oxygen-carrying capacity of the perfusate by using fluorocarbons. Our hypoxic data show that *in vivo*, when oxygen is limited, the intestine metabolizes its glucose to lactate to a significant degree, rather than absorbing it intact, demonstrating the presence of a Pasteur effect *in vivo*.

One problem encountered in the use of radioactive labels for metabolic studies is the dilution of the label through metabolism of the labelled substrate. This has been detailed by Katz [13], for gluconeogenesis from ^{14}C -labelled substrates. We acknowledge that, with the use of [^{14}C]glucose, the estimate of lactate production may be slightly high, owing to the contribution of intravascular [^{14}C]glucose, which was initially absorbed intact, and then on subsequent circulation through the intestine was metabolized to lactate.

Despite this potential for overestimation of lactate production, we conclude that, *in vivo*, most intraluminal glucose is absorbed across the intestinal wall and presented to the liver intact, with minimal amounts being metabolized to lactate under aerobic conditions, and that, under hypoxic conditions, a significant amount of

glucose is converted into lactate and then presented to the liver.

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REFERENCES

1. Hanson, P. J. & Parsons, D. S. (1976) *J. Physiol. (London)* **255**, 775–795
2. Hanson, P. J. & Parsons, D. S. (1978) *J. Physiol. (London)* **278**, 55–67
3. Nicholls, T. J., Leese, H. S. & Bronk, J. R. (1983) *Biochem. J.* **212**, 183–187
4. Windmueller, H. G. & Spaeth, A. E. (1980) *J. Biol. Chem.* **255**, 107–122
5. Boyd, M. E., Albright, E. B., Foster, D. W. & McGarry, J. D. (1981) *J. Clin. Invest.* **68**, 142–152
6. Remesy, C., Demigne, C. & Aufrere, J. (1978) *Biochem. J.* **170**, 321–329
7. Kimura, R. E. & LaPine, T. R. (1988) *Pediatr. Res.* **23**, 235–240
8. Hohorst, H. J. (1965) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.), pp. 266–270, Academic Press, New York
9. Katz, J., Okajima, F., Chenoweth, M. & Dunn, A. (1981) *Biochem. J.* **194**, 513–524
10. Gelman, S. I. (1976) *Arch. Surg. (Chicago)* **111**, 881–883
11. Porteous, J. W. (1978) *Biochem. Soc. Trans.* **6**, 534–539
12. Lamers, J. M. J. & Hulsmann, W. C. (1972) *Biochim. Biophys. Acta* **275**, 491–495
13. Katz, J. (1985) *Am. J. Physiol.* **248**, R391–R399

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