Effects of starvation on the maximal activities of some glycolytic and citric acid-cycle enzymes and glutaminase in mucosa of the small intestine of the rat

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(Received 26 March 1982/Accepted 28 April 1982)

Starvation decreases activities of some glycolytic and citric acid-cycle enzymes, and increases those of glucose 6-phosphatase and fructose biphosphatase, whereas that of glutaminase is unchanged. These findings may be of significance for the control of glucose metabolism in the absorptive cells of the intestine.

There is controversy concerning the proportion of glucose, which is absorbed into the absorptive cells of the small intestine from the lumen, that is metabolized to glycolytic end-products in the cell and that which is not metabolized but enters the hepatic portal blood directly. Of the glucose removed from the lumen by the cells of a perfused small-intestinal preparation (perfused both through the lumen and through the normal vasculature), about 50% is converted into lactate (Hanson & Parsons, 1976). Similarly, isolated enterocytes convert a high proportion of the glucose removed from the incubation medium into lactate (see Porteous, 1978). The lactate, produced by the intestine, would enter the hepatic portal blood, be converted into glucose in the liver via gluconeogenesis and hence enter the peripheral circulation as glucose. Support for this process is provided by experiments in which the hepatic portal vein was surgically connected directly to the inferior vena cava, thus bypassing the liver (portocaval shunt); of glucose removed from the intestine of these animals, only 15% entered the circulation as glucose, whereas at least 60% was accounted for as lactate (Shapiro & Shapiro, 1979). On the other hand, in an experiment in which the lumen of the intestine of the rat was perfused with a simulated meal (containing glucose and amino acids), 97% of the glucose absorbed from the lumen was transported across the intestine (Windmueller & Spaeth, 1980). In addition, in man, arteriovenous differences indicate that a large proportion (at least 60%) of an oral glucose load is taken up by the liver (Felig *et al.*, 1976).

To provide more information on intestinal metabolism, it was decided to investigate activities of some enzymes of carbohydrate metabolism along

the length of the small intestine of the rat. It is known that the maximum activities of certain enzymes can be used to indicate quantitatively the maximum rate of certain metabolic pathways; hexokinase (EC 2.7.1.1) indicates the capacity to utilize glucose, 6-phosphofructokinase (EC 2.7.1.11) the capacity to utilize glycogen, and oxoglutarate dehydrogenase (EC 1.2.4.2) the capacity of the citric acid cycle and aerobic metabolism in insect flight muscle and some vertebrate muscles (Newsholme *et al.*, 1980; Cooney *et al.*, 1981). The activities of these enzymes, together with those that are important in the reversal of the early stages of glycolysis and hence which could play a role in regulation of flux [i.e. glucose 6-phosphatase (EC 3.1.3.9) and fructose biphosphatase (EC 3.1.3.11)], have been measured in the mucosa of the small intestine from fed and starved animals, since changes occurring in starvation might provide an indication of the quantitative importance of glycolysis in the fed state. In addition, the activities of glucokinase (EC 2.7.1.2), citrate synthase (EC 4.1.3.7) and glutaminase (EC 3.5.1.2) have been measured. The last enzyme was included since glutamine is an important fuel for the mucosal cells of the small intestine, and its rate of utilization does not appear to change during starvation (Hanson & Parsons, 1980; Windmueller & Spaeth, 1980; Porteous, 1980).

The activities of some of these enzymes in the small intestine, including glucokinase (see Weinhouse, 1976) and glucose 6-phosphatase (Öckerman, 1965), have been reported previously and the effect of starvation has been investigated (Anderson & Zakim, 1970; Anderson, 1974). However, a systematic study of the key enzymes indicating the maximum flux through both glycolysis (hexokinase and 6-phosphofructokinase) and the citric acid cycle (oxoglutarate dehydrogenase) and the effects of

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starvation on activities in different sections along the length of the small intestine have not been previously reported.

Materials and methods

Chemicals, enzymes and animals

Male Wistar rats (180–240 g body wt.) were obtained from Banting and Kingsman, Hull, U.K. Rats were fed on a standard maintenance diet (rat and mouse No. 1 beta diet; B.P. Nutrition Ltd., Witham, Essex, U.K.). Starved rats were deprived of food for 48 h before they were required for use. All chemicals and enzymes were obtained from sources given previously (Newsholme *et al.*, 1972; Crabtree & Newsholme, 1972; Zammit & Newsholme, 1976).

Preparation of homogenates and assay of enzyme activities

Rats were killed by cervical fracture, and most of the small intestine (from about 10 cm below the pylorus to the distal end of the ileum) was removed and washed with 3 vol. (50 ml) of ice-cold 0.9% NaCl. The intestine was then divided into four equal sections (about 5 cm between each section being discarded). Each section was again rapidly rinsed by forcing ice-cold saline through the lumen, and then cut longitudinally. Mucosal tissue was separated from the underlying muscle by scraping with a microscope slide, weighed and homogenized in 8–10 vol. of extraction medium by using a Polytron set at position 4, for 20–30 s at 0°C. For hexokinase, glucose 6-phosphatase and fructose biphosphatase, the extraction medium consisted of 50 mM-triethanolamine, 5 mM-MgCl₂, 1 mM-EDTA and 2 mM-mercaptoethanol, pH 7.5; for 6-phosphofructokinase and citrate synthase the extraction medium consisted of 50 mM-Tris/HCl, 5 mM-MgCl₂, 1 mM-EDTA and 20 mM-mercaptoethanol, pH 8.2. Preliminary experiments established that these extraction media provide maximal activities compared with those obtained after extraction in media specifically developed for each enzyme (see Crabtree *et al.*, 1972; Alp *et al.*, 1976; Zammit & Newsholme, 1976; Surholt & Newsholme, 1981). The extraction media for oxoglutarate dehydrogenase, glutaminase and glucokinase were as described by Cooney *et al.* (1981), Curthoys & Lowry (1973) and Walker & Holland (1965) respectively.

Hexokinase was assayed as described by Zammit & Newsholme (1976); glucokinase was assayed as described by Stanley (1980), by a radiochemical assay in which hexokinase activity was inhibited by the presence of 10 mM-glucose 6-phosphate; it is, however, possible that the activity reported here is residual hexokinase activity not inhibited by glucose 6-phosphate. 6-Phosphofructokinase was assayed as

described by Opie & Newsholme (1967), fructose biphosphatase as described by Crabtree *et al.* (1972), citrate synthase as described by Alp *et al.* (1976) and 2-oxoglutarate dehydrogenase as described by Cooney *et al.* (1981). Glutaminase was assayed as described by Curthoys & Lowry (1973); preliminary experiments established that the activity was linear with time for 20 min (at 37°C), so that the incubation was stopped after this period by addition of HClO₄, and glutamate was assayed spectrophotometrically. Glucose 6-phosphatase was assayed by the method of Kitcher *et al.* (1978) with the modifications described by Surholt & Newsholme (1981).

All spectrophotometric assays were performed at 25°C with a Gilford recording spectrophotometer model 240. Protein was measured by the method of Lowry *et al.* (1951). Enzyme activities are expressed as $\mu\text{mol/min per g wet wt.}$ and $\text{nmol/min per mg of protein.}$

Results and discussion

The small intestine was divided into four sections (A–D) from the duodenum to the colon, and enzyme activities were measured in these sections. With the possible exception of glutaminase, there was a decrease in the activities of all enzymes (whether presented on the basis of g fresh wt. or mg of protein) along the intestine from the duodenum to the colon. The activities of hexokinase and 6-phosphofructokinase decreased by about 50% from portion A and D, whereas there was almost a 10-fold decrease in that of glucose 6-phosphatase (Table 1). Glucokinase activity (or a glucose-phosphorylation enzyme that was insensitive to glucose 6-phosphate inhibition; see the Materials and methods section) was present in the intestine, but the activities were only 10% of that of hexokinase (in contrast with the liver) and decreased about 5-fold along the intestine.

Starvation decreased the activities of hexokinase, glucokinase and 6-phosphofructokinase, whereas it increased the activities of glucose 6-phosphatase and fructose biphosphatase (Table 1), which confirms the work of Anderson (1974). In contrast with the results of Anderson (1974), maximal activities of fructose biphosphatase were well below those of hexokinase, so that the activity of the former enzyme could not seriously decrease maximal glycolytic flux from glucose. However, although the temperature of assay of the two enzymes was different, the results indicate that the activity of glucose 6-phosphatase in portion A of the intestine is very similar to that of hexokinase, and indeed it is greater after starvation. Consequently, it is suggested that, in cells of this portion of the intestine, maximum glycolytic flux could be markedly inhibited by glucose 6-phos-

Table 1. *Enzyme activities in mucosal cells of sections of the small intestine*

Results are presented as means $\pm$ S.E.M., with numbers of separate animals used shown in parentheses in column A. The temperature of assay was 25°C, except for glucokinase and glutaminase (37°C) and glucose 6-phosphatase (30°C). The statistical significance (Student's *t* test) of the difference in activity between fed and starved animals is indicated by * ($P < 0.05$) and ** ($P < 0.005$). The portions of the intestine designated A, B, C and D correspond approximately to jejunum proximal to duodenum (A), jejunum and anterior ileum (B), ileum (C) and ileum proximal to the caecum (D).

Enzyme	Condition	A				B				C				D			
		$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein	$\mu\text{mol/min per g fresh wt.}$	nmol/min per mg of protein
Hexokinase	Fed	7.2 $\pm$ 0.24 (7)	46.2 $\pm$ 4.1 (7)	5.5 $\pm$ 0.31	39.6 $\pm$ 3.1	4.5 $\pm$ 0.40	31.9 $\pm$ 1.9	4.5 $\pm$ 0.40	31.9 $\pm$ 1.9	4.5 $\pm$ 0.40	31.9 $\pm$ 1.9	4.5 $\pm$ 0.40	31.9 $\pm$ 1.9	4.5 $\pm$ 0.40	31.9 $\pm$ 1.9	4.5 $\pm$ 0.40	31.9 $\pm$ 1.9
	48 h starved	5.7 $\pm$ 0.25 (7)**	32.7 $\pm$ 2.5 (7)*	4.6 $\pm$ 0.17*	24.1 $\pm$ 2.5**	3.8 $\pm$ 0.22	17.7 $\pm$ 1.2**	3.8 $\pm$ 0.22	17.7 $\pm$ 1.2**	3.8 $\pm$ 0.22	17.7 $\pm$ 1.2**	3.8 $\pm$ 0.22	17.7 $\pm$ 1.2**	3.8 $\pm$ 0.22	17.7 $\pm$ 1.2**	3.8 $\pm$ 0.22	17.7 $\pm$ 1.2**
Glucokinase	Fed	0.76 $\pm$ 0.01 (7)	6.9 $\pm$ 0.34 (8)	0.54 $\pm$ 0.02	5.2 $\pm$ 0.50	0.35 $\pm$ 0.04	3.4 $\pm$ 0.41	0.35 $\pm$ 0.04	3.4 $\pm$ 0.41	0.35 $\pm$ 0.04	3.4 $\pm$ 0.41	0.35 $\pm$ 0.04	3.4 $\pm$ 0.41	0.35 $\pm$ 0.04	3.4 $\pm$ 0.41	0.35 $\pm$ 0.04	3.4 $\pm$ 0.41
	48 h starved	0.47 $\pm$ 0.05 (8)**	3.8 $\pm$ 0.36 (7)**	0.31 $\pm$ 0.03**	2.7 $\pm$ 0.23**	0.19 $\pm$ 0.03**	1.8 $\pm$ 0.23*	0.19 $\pm$ 0.03**	1.8 $\pm$ 0.23*	0.19 $\pm$ 0.03**	1.8 $\pm$ 0.23*	0.19 $\pm$ 0.03**	1.8 $\pm$ 0.23*	0.19 $\pm$ 0.03**	1.8 $\pm$ 0.23*	0.19 $\pm$ 0.03**	1.8 $\pm$ 0.23*
Glucose 6-phosphatase	Fed	6.1 $\pm$ 0.29 (7)	31.7 $\pm$ 0.84 (7)	1.4 $\pm$ 0.19	7.1 $\pm$ 0.75	0.70 $\pm$ 0.09	4.2 $\pm$ 0.25	0.70 $\pm$ 0.09	4.2 $\pm$ 0.25	0.70 $\pm$ 0.09	4.2 $\pm$ 0.25	0.70 $\pm$ 0.09	4.2 $\pm$ 0.25	0.70 $\pm$ 0.09	4.2 $\pm$ 0.25	0.70 $\pm$ 0.09	4.2 $\pm$ 0.25
	48 h starved	8.8 $\pm$ 0.96 (6)*	45.5 $\pm$ 4.47 (6)*	3.6 $\pm$ 0.68**	16.1 $\pm$ 2.34**	1.9 $\pm$ 0.33**	6.7 $\pm$ 0.53**	1.9 $\pm$ 0.33**	6.7 $\pm$ 0.53**	1.9 $\pm$ 0.33**	6.7 $\pm$ 0.53**	1.9 $\pm$ 0.33**	6.7 $\pm$ 0.53**	1.9 $\pm$ 0.33**	6.7 $\pm$ 0.53**	1.9 $\pm$ 0.33**	6.7 $\pm$ 0.53**
6-Phospho-fructokinase	Fed	13.0 $\pm$ 1.01 (6)	68.9 $\pm$ 4.07 (6)	11.4 $\pm$ 0.62	62.6 $\pm$ 3.88	7.9 $\pm$ 0.38	39.4 $\pm$ 3.22	7.9 $\pm$ 0.38	39.4 $\pm$ 3.22	7.9 $\pm$ 0.38	39.4 $\pm$ 3.22	7.9 $\pm$ 0.38	39.4 $\pm$ 3.22	7.9 $\pm$ 0.38	39.4 $\pm$ 3.22	7.9 $\pm$ 0.38	39.4 $\pm$ 3.22
	48 h starved	10.1 $\pm$ 0.95 (6)*	50.9 $\pm$ 3.37 (6)*	8.2 $\pm$ 0.78*	40.0 $\pm$ 3.11**	7.1 $\pm$ 0.47	34.9 $\pm$ 3.84	7.1 $\pm$ 0.47	34.9 $\pm$ 3.84	7.1 $\pm$ 0.47	34.9 $\pm$ 3.84	7.1 $\pm$ 0.47	34.9 $\pm$ 3.84	7.1 $\pm$ 0.47	34.9 $\pm$ 3.84	7.1 $\pm$ 0.47	34.9 $\pm$ 3.84
Fructose biphosphatase	Fed	1.2 $\pm$ 0.13 (7)	6.9 $\pm$ 0.57 (7)	0.71 $\pm$ 0.09	4.4 $\pm$ 0.70	0.38 $\pm$ 0.04	2.4 $\pm$ 0.15	0.38 $\pm$ 0.04	2.4 $\pm$ 0.15	0.38 $\pm$ 0.04	2.4 $\pm$ 0.15	0.38 $\pm$ 0.04	2.4 $\pm$ 0.15	0.38 $\pm$ 0.04	2.4 $\pm$ 0.15	0.38 $\pm$ 0.04	2.4 $\pm$ 0.15
	48 h starved	1.8 $\pm$ 0.13 (7)*	10.1 $\pm$ 0.82 (7)*	1.11 $\pm$ 0.14*	6.3 $\pm$ 0.74	0.62 $\pm$ 0.06*	3.4 $\pm$ 0.29*	0.62 $\pm$ 0.06*	3.4 $\pm$ 0.29*	0.62 $\pm$ 0.06*	3.4 $\pm$ 0.29*	0.62 $\pm$ 0.06*	3.4 $\pm$ 0.29*	0.62 $\pm$ 0.06*	3.4 $\pm$ 0.29*	0.62 $\pm$ 0.06*	3.4 $\pm$ 0.29*
Citrate synthase	Fed	14.9 $\pm$ 1.03 (8)	102 $\pm$ 3.7 (8)	11.5 $\pm$ 1.06	77.8 $\pm$ 5.29	11.8 $\pm$ 0.90	82.1 $\pm$ 4.35	11.8 $\pm$ 0.90	82.1 $\pm$ 4.35	11.8 $\pm$ 0.90	82.1 $\pm$ 4.35	11.8 $\pm$ 0.90	82.1 $\pm$ 4.35	11.8 $\pm$ 0.90	82.1 $\pm$ 4.35	11.8 $\pm$ 0.90	82.1 $\pm$ 4.35
	48 h starved	13.5 $\pm$ 1.07 (7)	103 $\pm$ 9.4 (7)	10.7 $\pm$ 0.63	72.6 $\pm$ 8.21	9.3 $\pm$ 0.41	62.1 $\pm$ 5.87	9.3 $\pm$ 0.41	62.1 $\pm$ 5.87	9.3 $\pm$ 0.41	62.1 $\pm$ 5.87	9.3 $\pm$ 0.41	62.1 $\pm$ 5.87	9.3 $\pm$ 0.41	62.1 $\pm$ 5.87	9.3 $\pm$ 0.41	62.1 $\pm$ 5.87
Oxoglutarate dehydrogenase	Fed	2.1 $\pm$ 0.20 (7)	19.5 $\pm$ 1.5 (7)	1.6 $\pm$ 0.13	18.4 $\pm$ 1.21	1.3 $\pm$ 0.13	14.3 $\pm$ 0.88	1.3 $\pm$ 0.13	14.3 $\pm$ 0.88	1.3 $\pm$ 0.13	14.3 $\pm$ 0.88	1.3 $\pm$ 0.13	14.3 $\pm$ 0.88	1.3 $\pm$ 0.13	14.3 $\pm$ 0.88	1.3 $\pm$ 0.13	14.3 $\pm$ 0.88
	48 h starved	2.8 $\pm$ 0.22 (7)*	26.1 $\pm$ 1.07 (8)**	1.9 $\pm$ 0.11*	19.2 $\pm$ 1.11	1.2 $\pm$ 0.11	12.6 $\pm$ 1.02	1.2 $\pm$ 0.11	12.6 $\pm$ 1.02	1.2 $\pm$ 0.11	12.6 $\pm$ 1.02	1.2 $\pm$ 0.11	12.6 $\pm$ 1.02	1.2 $\pm$ 0.11	12.6 $\pm$ 1.02	1.2 $\pm$ 0.11	12.6 $\pm$ 1.02
Glutaminase	Fed	7.0 $\pm$ 0.27 (5)	69.1 $\pm$ 8.96 (5)	6.8 $\pm$ 0.38	60.1 $\pm$ 3.32	6.7 $\pm$ 0.33	61.6 $\pm$ 4.14	6.7 $\pm$ 0.33	61.6 $\pm$ 4.14	6.7 $\pm$ 0.33	61.6 $\pm$ 4.14	6.7 $\pm$ 0.33	61.6 $\pm$ 4.14	6.7 $\pm$ 0.33	61.6 $\pm$ 4.14	6.7 $\pm$ 0.33	61.6 $\pm$ 4.14
	48 h starved	6.4 $\pm$ 0.35 (5)	44.9 $\pm$ 3.20 (5)*	6.0 $\pm$ 0.71	41.9 $\pm$ 6.52*	6.0 $\pm$ 0.71	40.6 $\pm$ 3.23*	6.0 $\pm$ 0.71	40.6 $\pm$ 3.23*	6.0 $\pm$ 0.71	40.6 $\pm$ 3.23*	6.0 $\pm$ 0.71	40.6 $\pm$ 3.23*	6.0 $\pm$ 0.71	40.6 $\pm$ 3.23*	6.0 $\pm$ 0.71	40.6 $\pm$ 3.23*

phatase activity and, assuming this to be the case, most of the glucose absorbed from the lumen could be transported directly into the bloodstream. The suggestion that glucose 6-phosphatase activity restricts glycolytic flux requires the operation of a substrate cycle between glucose and glucose 6-phosphate in absorptive cells of the small intestine. Such a cycle has not been demonstrated. In the lower portions of the small intestine, the maximum activities of hexokinase are considerably greater than those of glucose 6-phosphatase, suggesting that some of the glucose absorbed into the cells in this portion of the intestine could be metabolized to lactate. The difference in activities of these two enzymes along the length of the intestine could explain some of the differences in the results concerning the proportion of absorbed glucose metabolized by the absorptive cells (see the introduction).

The importance of glutamine as a fuel for the absorptive cells of the small intestine is well established. This is supported by the high activities of glutaminase reported in Table 1, and especially by the fact that the activities of this enzyme remain almost constant along the length of the intestine and change very little with starvation.

In muscle, the activities of oxoglutarate dehydrogenase provide a quantitative indication of the maximum capacity of the citric acid cycle. If this is the case for absorptive cells of the small intestine, the increased activity of this enzyme, at least in part of the intestine, during starvation suggests an increased maximum capacity of the citric acid cycle and hence of ATP generation by aerobic metabolism. This observation could support the view that glucose conversion into lactate is quantitatively important in the absorption of glucose, since the latter process would generate a considerable quantity of ATP, and to compensate for the loss of this ATP production in starvation a greater capacity of the citric acid cycle would be required.

We thank Professor R. R. Porter, F.R.S., and Professor S. Kowolowski for their interest and encouragement. L. B. gratefully acknowledges support from the

British Council. J. C. is a recipient of an S.E.R.C. Training Scholarship.

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