Activity of 3-oxo acid CoA-transferase, D-3-hydroxybutyrate dehydrogenase, hexokinase and carnitine palmitoyltransferase in the stomach and small and large intestine of the rat

Peter J. HANSON and Janet M. CARRINGTON*

Department of Biological Sciences, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET, U.K.

(Received 1 June 1981/Accepted 23 July 1981)

1. Activities of 3-oxo acid CoA-transferase, D-3-hydroxybutyrate dehydrogenase, hexokinase and carnitine palmitoyltransferase have been measured in the gastrointestinal tract. 2. Activity of 3-oxo acid CoA-transferase in the glandular mucosa of the stomach was as high as that in heart and kidney, and was 2–4 times greater than that in other regions of the gastrointestinal tract. It is suggested that metabolism of acetoacetate might support acid secretion on re-feeding after a period without food. 3. All regions of the gastrointestinal tract have the capacity to use ketone bodies, and it is likely that both muscle and mucosa will contribute to their utilization. 4. Activity of hexokinase was twice the rate of glucose utilization by the jejunum under anaerobic conditions. The maximal rate of glucose metabolism in the jejunum may not be substantially different from that in other regions of the gastrointestinal tract. 5. Starvation decreased the capacity for metabolism of glucose in several regions of the intestine. 6. Activities of carnitine palmitoyltransferase in the stomach, jejunum and colon were similar, and about one-third of that in the liver. Activity in the jejunum was much higher than the apparent rate of oxidation of exogenous fatty acid. 7. The results do not suggest any large variation between tissues of the gastrointestinal tract in metabolism of glucose or fatty acids, whereas metabolism of ketone bodies may be more prominent in the stomach.

The relative importance of the metabolic fuels that supply energy in the rat jejunum has recently been established (Hanson & Parsons, 1977, 1978; Windmueller & Spaeth, 1978, 1980; Watford *et al.*, 1979). Glutamine is an important metabolic fuel for the small intestine (Windmueller & Spaeth, 1974; Hanson & Parsons, 1977), and measurement of the distribution of glutaminase (EC 3.5.1.2) activity (Pinkus & Windmueller, 1977) suggests, in agreement with other work (Windmueller & Spaeth, 1974), that the mucosa of the small intestine is the region of the gastrointestinal tract that is predominant in glutamine metabolism.

One aim of this work was to obtain an estimate of the pattern of utilization of some other potential fuels in regions of the gastrointestinal tract of fed and starved rats by measuring the activities of key enzymes of some metabolic pathways involved in

fuel metabolism. Activities of hexokinase (EC 2.7.1.1), carnitine palmitoyltransferase (EC 2.3.1.21), 3-oxo acid CoA-transferase (EC 2.8.3.5) and D-3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) were therefore measured in regions of the stomach and small and large intestine. Hexokinase and carnitine palmitoyltransferase may provide quantitative information about the maximum activities of the pathways of glycolysis and fatty acid oxidation respectively (Crabtree & Newsholme, 1972*a,b*), whereas activities of D-3-hydroxybutyrate dehydrogenase and 3-oxo acid CoA-transferase probably only provide qualitative information about the extent of ketone-body utilization (Sugden & Newsholme, 1973).

Additional aims were to establish whether both muscle and mucosa could contribute to the metabolism of ketone bodies in rat jejunum (Hanson & Parsons, 1978; Windmueller & Spaeth, 1978), whether a low activity of carnitine palmitoyltransferase was responsible for fatty acids being poorly metabolized by this tissue (Windmueller & Spaeth,

* Permanent address: Department of Biochemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand.

1978) and whether hexokinase activity determined the maximum rate of the glycolytic pathway.

Materials and methods

Animals

Male Wistar rats, of about 200 g body weight, were obtained from Banting & Kingman, Hull, U.K. Rats were fed on Heygates breeding diet (modified 41 b diet) supplied by Pilsbury, Edgbaston, Birmingham, U.K. Starved rats were deprived of food for 2 days before they were required for use.

Reagents

D-(+)-carnitine chloride was a gift from Otsuka Pharmaceutical Factory, Naruto, Tokushima, Japan. NAD⁺, ATP and enzymes were obtained from Boehringer Corporation (London), Lewes, East Sussex, U.K. All other biochemicals were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. Inorganic chemicals were purchased from BDH Chemicals, Poole, Dorset, U.K.

Preparation of tissue

Rats were killed by cervical fracture and the regions of the gastrointestinal tract were excised and washed with copious amounts of ice-cold saline (0.9% NaCl). Mucosa from the glandular portion of the stomach, duodenum, jejunum, ileum, caecum and colon was separated from the underlying muscle by scraping with a microscope slide. The duodenum was taken to be the region of small intestine from the pylorus to the ligament of Trietz, and the jejunum, the upper, and the ileum, the lower half of the remaining small intestine. A muscle extract was prepared with the pooled smooth muscle derived from jejunum and ileum.

Preparation of homogenates and assay of enzyme activity

3-oxo acid CoA-transferase. Tissue was homogenized for 20 s with 15–30 vol. of a medium containing 25 mM-Tris/HCl, 1 mM-EDTA and 1 mM-mercaptoethanol at pH 7.4 with an Ultra-Turrax homogenizer (Janke & Kunkel, Belmont, Surrey, U.K.). After sonication of the homogenate at 0°C for 30 s (Rapidis A 350G sonic disintegrator working at 100 W), activity was measured in the direction of acetoacetate formation (Williamson *et al.*, 1971) by following the succinate-dependent decrease in A_{303} caused by the breakdown of the acetoacetyl-CoA-Mg²⁺ complex (ϵ_{303} 21 100 litre · mol⁻¹ · cm⁻¹).

D-3-Hydroxybutyrate dehydrogenase. Tissue was extracted by homogenization (Teflon/glass homogenizer, except for muscle, when the Ultra-Turrax instrument was used) in 5–10 vol. of a medium

containing 50 mM-triethanolamine, 1 mM-EDTA, 2 mM-MgCl₂ and 2 mM-dithiothreitol at pH 7.5. Activity was assayed by following the D-3-hydroxybutyrate-dependent formation of NADH as described by Beis (1978) after sonication of the homogenate at 0°C for six periods of 30 s.

Carnitine palmitoyltransferase. Tissue was homogenized in 10–15 vol. of a medium containing 100 mM-Tris/HCl, 1 mM-EDTA at pH 7.4 using the Ultra-Turrax homogenizer for 20 s. Just before assay extracts were sonicated for two periods of 15 s. Activity was assayed by following the L-(–)-carnitine-dependent formation of reduced CoA from palmitoyl-CoA as described by Zammit & Newsholme (1979), except that Triton X-100 (0.45 ml/litre) was included in the sample and reference cuvettes.

Hexokinase. Tissue was homogenized for 20 s in 10–20 vol. of a medium containing 50 mM-Tris/HCl, 100 mM-KCl, 5 mM-MgCl₂, 5 mM-mercaptoethanol and 1 mM-EDTA at pH 7.6 (Tyrrell & Anderson, 1971) by using the Ultra-Turrax homogenizer. Enzyme activity was assayed as described by Hülsmann (1977), except that the concentrations of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) in the cuvette were those used by Srivastava *et al.* (1968), and 4 mM-mercaptoethanol was also present.

All assays were performed at 25°C using a Pye-Unicam SP30 double-beam spectrophotometer with reaction-rate accessory. Enzyme activity in homogenates stored, unsonicated, at 0°C was stable for at least 1 h. Activity was in all cases proportional to the concentration of protein in the cuvette.

Protein was measured by the method of Lowry *et al.* (1951).

Expression of results

Enzyme activities are expressed both with respect to g wet weight of tissue (Table 1) and with respect to tissue protein (Table 2). This procedure largely avoids differences in tissue water, fat or protein content causing erroneous conclusions to be made about differences in enzyme activity, as can occur if only one mode of expression is used.

Comparisons between activity in fed and starved animals were made by using Student's *t* test.

Results and discussion

3-Oxo-acid CoA-transferase and D-3-hydroxybutyrate dehydrogenase

Stomach. Activity of 3-oxo acid CoA-transferase in the stomach glandular mucosa was high, being similar to that found in heart and kidney (Williamson *et al.*, 1971) and was greater than that in all

other regions of the gastrointestinal tract (Tables 1 and 2; $P < 0.01$). Parietal cells, which in mammals can constitute up to 20% of the mass of the gastric glandular mucosa (Sachs *et al.*, 1977), have a very high content of mitochondria (Berglindh & Öbrink, 1976), but this cannot be the cause of the elevated 3-oxo acid CoA-transferase activity, for although this enzyme is over 2.5 times more active in the stomach than in the jejunum, other mitochondrial enzymes such as carnitine palmitoyltransferase (Table 4) and glutamate dehydrogenase (Volman-Mitchell & Parsons, 1974) are not elevated in the stomach in comparison with the jejunum.

The high activity of 3-oxo acid CoA-transferase thus suggests that acetoacetate could be an important fuel for the stomach glandular mucosa, but any quantitative assessment of the maximal capacity of the tissue to metabolize acetoacetate is precluded by the probable equilibrium nature of the transferase enzyme (Sugden & Newsholme, 1973). A physiological circumstance in which acetoacetate utilization by the stomach might be important is that of re-feeding after a period without food, when energy has to be provided to support digestion and absorption of nutrients before the metabolic state of the animal can be altered. It thus might be preferable for utilization of acetoacetate rather than glucose to meet the high energy demands of gastric acid secretion under such circumstances. Acetoacetate can support acid secretion in stomachs of fed mice *in vitro* (Davenport & Chavré, 1951), but this is unlikely to be physiologically important because of the low concentration of acetoacetate in the blood of fed animals (Hawkins *et al.*, 1971).

Activity of branched-chain-amino-acid amino-transferase (EC 2.6.1.42) is much higher in the gastric glandular mucosa and the pancreas than in other rat tissues, including the remainder of the gastrointestinal tract (Ichihara *et al.*, 1975). The gastric mucosa can also oxidize leucine (Ichihara *et al.*, 1975). 3-Oxo acid CoA-transferase could therefore also be present in gastric mucosa at high activity to metabolize acetoacetate generated endogenously from leucine oxidation.

A substance was present in extracts of gastric mucosa that rapidly inactivated D-3-hydroxybutyrate dehydrogenase in extracts from other tissues, and because this inhibitor could explain the absence of measurable activity in the stomach, it is not possible to conclude whether the stomach might use D-3-hydroxybutyrate as well as acetoacetate. By contrast addition of stomach homogenate did not affect activity of 3-oxo acid CoA-transferase in extracts from other tissues.

Small and large intestine. Activities of 3-oxo acid CoA-transferase and D-3-hydroxybutyrate dehydrogenase did not differ substantially between the muscle and mucosa of the small intestine (Tables 1

Table 1. *Distribution of activity of 3-oxo acid CoA-transferase, D-3-hydroxybutyrate dehydrogenase and hexokinase in the rat gastrointestinal tract*

Results of activity, expressed as $\mu\text{mol}/\text{min}$ per g wet wt. of tissue, are presented as means $\pm$ s.e.m. with the numbers of animals in parentheses. The muscle was from the jejunum and ileum; other results are for mucosal enzyme activity. Starvation was for 48 h. Significant differences between results from fed and starved animals are denoted as follows: * $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$.

Enzyme	Dietary status	Enzyme activity ($\mu\text{mol}/\text{min}$ per g wet wt.)						
		Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Muscle
3-Oxo acid CoA-transferase	Fed	23 $\pm$ 2.7 (4)	9.8 $\pm$ 1.0 (5)*	9.0 $\pm$ 0.8 (5)	8.0 $\pm$ 0.9 (5)	5.3 $\pm$ 0.3 (4)	6.8 $\pm$ 1.0 (4)	7.8 $\pm$ 0.9 (5)
	Starved	25 $\pm$ 2.8 (5)	6.7 $\pm$ 0.8 (5)	7.6 $\pm$ 1.0 (5)	6.6 $\pm$ 0.7 (5)	3.7 $\pm$ 0.6 (5)	5.1 $\pm$ 0.7 (4)	6.7 $\pm$ 1.1 (5)
D-3-Hydroxybutyrate dehydrogenase	Fed	†	0.90 $\pm$ 0.05 (5)	0.54 $\pm$ 0.02 (6)	0.32 $\pm$ 0.04 (4)*	1.4 $\pm$ 0.15 (6)	1.2 $\pm$ 0.10 (5)	0.31 $\pm$ 0.02 (4)
	Starved	†	1.1 $\pm$ 0.10 (5)	0.58 $\pm$ 0.06 (4)	0.55 $\pm$ 0.06 (5)	1.3 $\pm$ 0.04 (5)	0.99 $\pm$ 0.09 (5)	0.37 $\pm$ 0.03 (5)
Hexokinase	Fed	2.3 $\pm$ 0.2 (5)*	2.8 $\pm$ 0.2 (5)	2.5 $\pm$ 0.1 (6)**	2.0 $\pm$ 0.1 (6)*	3.4 $\pm$ 0.2 (5)*	2.5 $\pm$ 0.1 (5)***	1.6 $\pm$ 0.1 (5)
	Starved	1.7 $\pm$ 0.1 (4)	2.3 $\pm$ 0.2 (4)	2.0 $\pm$ 0.1 (4)	1.6 $\pm$ 0.03 (4)	2.6 $\pm$ 0.2 (4)	1.7 $\pm$ 0.2 (4)	1.3 $\pm$ 0.1 (4)

† Inhibitor present.

Table 2. Variation in specific activity of 3-oxo acid CoA-transferase, D-3-hydroxybutyrate dehydrogenase and hexokinase in the rat gastrointestinal tract. Results of specific activity are presented as means $\pm$ S.E.M. for the number of animals indicated in parentheses. The muscle was from the jejunum and ileum; other results are for mucosal enzyme activity. Starvation was for 48 h. Significant differences between results from fed and starved animals are denoted as follows: * $P < 0.05$; ** $P < 0.025$; *** $P < 0.01$; **** $P < 0.001$.

Enzyme	Dietary status	Specific activity (nmol/min per mg of protein)						
		Stomach	Duodenum	Jejunum	Ileum	Caecum	Colon	Muscle
3-Oxo acid CoA-transferase	Fed	185 $\pm$ 21 (5)	74 $\pm$ 7 (5)	69 $\pm$ 4 (5)	64 $\pm$ 5 (5)	38 $\pm$ 2 (5)	55 $\pm$ 6 (5)	84 $\pm$ 8 (5)
	Starved	204 $\pm$ 23 (5)	55 $\pm$ 7 (5)	57 $\pm$ 7 (5)	54 $\pm$ 6 (5)	28 $\pm$ 5 (5)	45 $\pm$ 5 (5)	68 $\pm$ 13 (5)
D-3-Hydroxybutyrate dehydrogenase	Fed	†	8.1 $\pm$ 0.3 (5)***	5.3 $\pm$ 0.2 (6)	3.1 $\pm$ 0.2 (4)***	11.4 $\pm$ 1.1 (6)	10.7 $\pm$ 0.8 (5)	3.4 $\pm$ 0.5 (4)
	Starved	†	10.4 $\pm$ 0.6 (5)	6.1 $\pm$ 0.4 (4)	6.2 $\pm$ 0.6 (5)	11.8 $\pm$ 0.7 (5)	8.8 $\pm$ 0.7 (5)	4.1 $\pm$ 0.4 (5)
Hexokinase	Fed	20 $\pm$ 1 (5)***	29 $\pm$ 2 (5)***	28 $\pm$ 1 (6)****	21 $\pm$ 1 (6)***	31 $\pm$ 2 (5)***	25 $\pm$ 3 (5)	19 $\pm$ 1 (5)**
	Starved	14 $\pm$ 1 (4)	19 $\pm$ 1 (4)	17 $\pm$ 1 (4)	15 $\pm$ 0.3 (4)	19 $\pm$ 1 (4)	16 $\pm$ 2 (4)	15 $\pm$ 1 (4)

† Inhibitor present.

and 2), which suggests that both of these regions will make a contribution to the utilization of ketone bodies by the intact intestine (Hanson & Parsons, 1978; Windmueller & Spaeth, 1978). The presence of a moderate activity [see Williamson *et al.* (1971) for values for other tissues] of 3-oxo acid CoA-transferase in the colonic mucosa is consistent with the ability of acetoacetate to support fluid transport by the colonic epithelium (Parsons & Paterson, 1965). It also suggests that the epithelial cells may produce ketone bodies from what are physiological concentrations of butyrate (Roediger, 1979) by a reversal of the utilization pathway (extrahepatic ketogenesis; Krebs, 1969), although proof of this suggestion requires the demonstration that mitochondrial hydroxymethylglutaryl-CoA lyase (EC 4.1.3.5), a key enzyme of the liver ketone-body biosynthetic pathway (Clinkenbeard *et al.*, 1975), is absent from the mitochondria of the colonic mucosa.

All of the tissues of the gastrointestinal tract have the capacity to use ketone bodies, but the distribution of 3-oxo acid CoA-transferase does not parallel that of D-3-hydroxybutyrate dehydrogenase (e.g. caecal activity of D-3-hydroxybutyrate dehydrogenase is high but that of 3-oxo acid CoA-transferase is low; Tables 1 and 2). Whether this has any physiological significance depends on the extent to which enzyme activity can influence utilization of ketone bodies. This seems somewhat unlikely in the jejunum of rats starved for 48 h, for with ketone bodies in the vascular perfusate at physiological concentrations (Hawkins *et al.*, 1971), the maximal activity of D-3-hydroxybutyrate dehydrogenase is 8 times, and that of 3-oxo acid CoA-transferase 12 times the flux through the enzyme (Table 3).

Starvation. Starvation had a significant effect both on activity expressed per g wet weight and per mg of protein only for D-3-hydroxybutyrate dehydrogenase in the ileum (Tables 1 and 2). The rise in D-3-hydroxybutyrate dehydrogenase activity in this tissue on starvation is not readily explained, but with this exception, it seems that as in other tissues (Williamson *et al.*, 1971) changes in the activity of 3-oxo acid CoA transferase and D-3-hydroxybutyrate dehydrogenase are not associated with the increased utilization that accompanies the rise in the concentration of ketone bodies in the plasma on starvation.

Hexokinase

Small intestine. Srivastava & Hübscher (1966) obtained rather tenuous evidence from work with homogenates that hexokinase activity might limit the maximal rate of glucose metabolism in rat jejunum. The maximal activity of hexokinase in the intestinal wall, measured at a saturating glucose concentration of 10 mM, was about twice the rate of utilization of glucose by jejunal segments *in vitro*

Table 3. Comparison of maximal enzyme activity with measurements of net metabolic flux through the enzyme in rat jejunum

Where necessary data have been recalculated assuming wet wt./dry wt. = 4.5, and dry wt. mucosa/dry wt. whole wall = 0.74. Enzyme activity at 37°C was assumed to be twice the enzyme activity at 25°C (Scrutton & Utter, 1968). Conditions are aerobic unless stated otherwise. 48h-starved rats were used for experiments concerned with ketone-body utilization, and animals that were starved overnight were used for measurement of oleate oxidation (calculated from conversion of [1-¹⁴C]oleate into CO₂ and acid-soluble products); all other animals were fed. 3-Oxo acid CoA-transferase activity has been divided by 5 to correct for assay in the non-physiological direction (Williamson *et al.*, 1971), and carnitine palmitoyltransferase by 2 because of the dual involvement of the enzyme in fatty acid oxidation (McGarry *et al.*, 1978).

Enzyme	Preparation	Conditions	Rate (μmol/h per g dry wt.)		Source of flux measurement
			Maximal enzyme activity at 37°C	Net flux of substrate(s)	
D-3-Hydroxybutyrate dehydrogenase	Vascularly perfused	D-3-Hydroxybutyrate (2 mM) in vascular bed	284	36	Hanson & Parsons (1978)
3-Oxo acid CoA-transferase	Vascularly perfused	D-3-Hydroxybutyrate (2 mM) and acetoacetate (1 mM) in vascular bed	796*	64	Hanson & Parsons (1978)
Carnitine palmitoyltransferase	<i>In vivo</i>	Oleate (1 mM) infused into artery	79	1.6	Windmueller & Spaeth (1978)
Hexokinase	Isolated segment	Glucose (10 mM), anaerobic conditions	1224	600	Hanson & Parsons (1978)
	Vascularly perfused	Glucose (10 mM) in vascular bed and lumen	1224	401	Hanson & Parsons (1978)
	Isolated mucosal cells	Glucose (10 mM)	1350	720	Watford <i>et al.</i> (1979)

* This may be an underestimate because enzyme was not saturated with substrate (see Beis, 1978).

under anaerobic conditions (10 mM-glucose; Table 3). There are three potential explanations for this difference. First utilization of glucose under anaerobic conditions may not be maximal because entry of glucose into the tissue is limiting metabolism (Leese & Bronk, 1975). This is unlikely because increasing the concentration of glucose from 10 mM to 28 mM, which should increase entry of glucose, did not increase lactate production (Hanson & Parsons, 1976). Secondly, it is possible that hexokinase is not the enzyme limiting glucose utilization, but this is not consistent with the low maximal catalytic activity of hexokinase in comparison with other glycolytic enzymes (Shakespeare *et al.*, 1972). Thirdly it is possible that not all of the hexokinase is fully active under anaerobic conditions *in vitro*. It is conceivable that the distribution of hexokinase between mitochondria and cytosol in the epithelial cells could be involved in restriction of activity (Jones & Mayer, 1973).

If it is assumed that, although hexokinase activity does not exactly equal the maximal rate of glucose utilization by intestinal tissues, it nevertheless provides a comparative indication of maximal rates of glycolysis from glucose, then it appears that the maximal capacity of the jejunum to metabolize

glucose is not particularly different from several other regions, for example stomach and colon (Table 1). The jejunal mucosa, when exposed to glucose from both the basolateral and luminal aspects of the epithelial cell, appears to metabolize it at a rate that is quite a substantial fraction of the maximum under aerobic conditions *in vitro* (Table 3). However, this phenomenon is not associated with an unusually high maximal glycolytic capacity in comparison with other regions of the gastrointestinal tract. There is thus no association of high maximal glycolytic capacity with regions of the intestine involved in active absorption of glucose from the lumen, such as might be expected if large amounts of glucose were metabolized during the process of absorption (Shapiro & Shapiro, 1979).

A significant gradient of hexokinase activity decreasing between jejunum and ileum was found (determined by paired *t* test: $P < 0.025$ and < 0.01 for fed animals; results expressed as μmol/min per g wet wt. and μmol/min per mg of protein respectively), which is consistent with the gradient of metabolism in the small intestine that was observed when glucose was available from the lumen (Hanson & Parsons, 1976), and that supports the use of hexokinase as an indicator of glycolytic activity.

Starvation. Starvation for 48 h caused a fall in hexokinase activity not just in the small intestine (Tables 1 and 2; Srivastava *et al.*, 1968; Anderson & Tyrrell, 1973) but also in regions such as the stomach and caecum, which are not normally exposed to luminal glucose in the rat. It is unlikely therefore that the fall in hexokinase activity in whole homogenates of the small intestine is specifically related to the absence of the substrate glucose from the lumen.

Carnitine palmitoyltransferase

Windmueller & Spaeth (1978) found that the oxidation of exogenous [^{14}C]oleate *in vivo* by the jejunum from rats starved overnight was low in comparison with other substrates. Large-scale oxidation of endogenous fatty acids, with a consequent decrease in the oxidation of plasma fatty acids, was probably not occurring since CO_2 output could be accounted for solely by the oxidation of exogenous substrates (Windmueller & Spaeth, 1978). Oleate could be an unimportant fuel for the jejunum because access to the site of metabolism from the blood was restricted, because there was insufficient enzyme activity to effect a high rate of metabolism or because the pathway involved in metabolism was inhibited. In the liver carnitine palmitoyltransferase is thought to be the enzyme controlling the β -oxidation of fatty acids (McGarry & Foster, 1980), and its activity was therefore measured in the jejunal mucosa. The maximal activity of the enzyme was found to be substantially greater than the rate of oxidation of exogenous oleate (Tables 3 and 4), and the extent to which carnitine palmitoyltransferase can be used as a quantitative guide to fatty acid oxidation in the intestine thus becomes questionable

(cf. muscle; Crabtree & Newsholme, 1972*b*). It is possible that, in some cases, long-chain acyl-CoA synthetase rather than carnitine palmitoyltransferase is the rate-limiting enzyme for fatty acid utilization. However, from our evidence, lack of enzyme capacity does not seem to be the factor that restricts oxidation of exogenous fatty acids in the jejunum. A possibility is that carnitine palmitoyltransferase is inhibited; indeed some regulation of carnitine palmitoyltransferase does seem likely as its activity is 80% of that of glycerophosphate acyltransferase (EC 2.3.1.15) (Hülsmann & Kurpershoek-Davidov, 1976; activity corrected to 25°C) and both enzymes will presumably compete for the same pool of fatty-acyl-CoA. Monoacylglycerol acyltransferase (EC 2.3.1.22), by contrast, may have access to a separate pool of fatty-acyl-CoA (Hülsmann & Kurpershoek-Davidov, 1976) and may not compete with carnitine palmitoyltransferase. Although the contents of villous epithelial cells probably formed the major component of the homogenate, its derivation from a heterogeneous population of cells means that any conclusions about enzyme interaction must be treated with caution.

Activity of the enzyme was similar in the stomach, jejunum and colon (Table 4), whereas activity in the liver (1.2 $\mu\text{mol/min per g wet wt.}$; J. M. Carrington & P. J. Hanson, unpublished work; Mannaerts *et al.*, 1979) was about three times that in the intestinal tract. Starvation for 48 h did not alter activity in the stomach or jejunum, so a change in enzymic capacity cannot explain why oleate stimulated lactate production in vascularly perfused jejunum from starved but not fed rats (Hanson & Parsons, 1978).

Pattern of fuel utilization

All regions of the gastrointestinal tract have the capacity to metabolize ketone bodies, and the results suggest that acetoacetate might be an important fuel for the glandular mucosa of the stomach. There is no evidence that the maximal activity of the pathway of glycolysis from glucose differs substantially between regions of the gastrointestinal tract. The results, therefore, do not suggest any large variation between regions of the gastrointestinal tract in metabolism of glucose or fatty acids, although metabolism of ketone bodies may be more prominent in the stomach. Starvation may decrease the capacity for utilization of glucose in several regions of the intestinal tract.

References

- Anderson, J. W. & Tyrrell, J. B. (1973) *Gastroenterology* **65**, 69–76
- Beis, A. (1978) Ph.D. Thesis, University of Oxford
- Berglindh, T. & Öbrink, K. J. (1976) *Acta Physiol. Scand.* **96**, 150–159

Table 4. *Activity of carnitine palmitoyltransferase in the rat gastrointestinal tract*

Results of activity are means $\pm$ S.E.M. for the numbers of animals shown in parentheses, except where only one or two animals were used, when the individual values are given. Starvation was for 48 h.

Tissue	Dietary status	Enzyme activity	
		($\mu\text{mol/min per g wet wt.}$)	($\text{nmol/min per mg of protein}$)
Stomach mucosa	Fed	0.36 ± 0.01 (4)	2.7 ± 0.2 (4)
	Starved	0.36 ± 0.04 (5)	2.5 ± 0.2 (5)
Jejunal mucosa	Fed	0.32 ± 0.03 (5)	2.6 ± 0.2 (5)
	Starved	0.35 ± 0.04 (5)	2.6 ± 0.4 (5)
Colonic mucosa	Fed	0.45, 0.40	—
Muscle from jejunum and ileum	Fed	0.21	—

- Clinkenbeard, K. D., Reed, W. D., Mooney, R. A. & Lane, M. D. (1975) *J. Biol. Chem.* **250**, 3108–3116
- Crabtree, B. & Newsholme, E. A. (1972a) *Biochem. J.* **126**, 49–58
- Crabtree, B. & Newsholme, E. A. (1972b) *Biochem. J.* **130**, 697–705
- Davenport, H. W. & Chavré, V. J. (1951) *Am. J. Physiol.* **166**, 456–461
- Hanson, P. J. & Parsons, D. S. (1976) *J. Physiol. (London)* **255**, 775–795
- Hanson, P. J. & Parsons, D. S. (1977) *Biochem. J.* **166**, 509–519
- Hanson, P. J. & Parsons, D. S. (1978) *J. Physiol. (London)* **278**, 55–67
- Hawkins, R. A., Alberti, K. G. M. M., Houghton, C. R. S., Williamson, D. H. & Krebs, H. A. (1971) *Biochem. J.* **125**, 541–544
- Hülsmann, W. C. (1977) in *Intestinal Permeation* (Kramer, M. & Lauterbach, F., eds.), p. 232, Excerpta Medica, Amsterdam and Oxford
- Hülsmann, W. C. & Kurpershoek-Davidov, R. (1976) *Biochim. Biophys. Acta* **450**, 288–300
- Ichihara, A., Noda, C. & Goto, M. (1975) *Biochem. Biophys. Res. Commun.* **67**, 1313–1318
- Jones, G. M. & Mayer, R. J. (1973) *Biochem. J.* **132**, 125–128
- Krebs, H. A. (1969) *Curr. Top. Cell. Regul.* **1**, 45–55
- Leese, H. J. & Bronk, J. R. (1975) *Biochim. Biophys. Acta* **401**, 40–48
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mannaerts, G. P., Debeer, L. J., Thomas, J. & De Schepper, P. J. (1979) *J. Biol. Chem.* **254**, 4585–4595
- McGarry, J. D. & Foster, D. W. (1980) *Annu. Rev. Biochem.* **49**, 395–420
- McGarry, J. D., Leatherman, G. F. & Foster, D. W. (1978) *J. Biol. Chem.* **253**, 4128–4136
- Parsons, D. S. & Paterson, C. R. (1965) *Q. J. Exp. Physiol.* **50**, 220–231
- Pinkus, L. M. & Windmueller, H. G. (1977) *Arch. Biochem. Biophys.* **182**, 506–517
- Roediger, W. E. W. (1979) in *Topics in Gastroenterology* (Truelove, S. C. & Willoughby, C. P., eds.), vol. 7, pp. 281–287, Blackwell, Oxford
- Sachs, G., Spenney, J. G. & Rehm, W. S. (1977) *Int. Rev. Physiol.* **12**, 141
- Scrutton, M. C. & Utter, M. F. (1968) *Annu. Rev. Biochem.* **37**, 250–302
- Shakespeare, P., Ellis, R. B., Mayer, R. J. & Hübscher, G. (1972) *Int. J. Biochem.* **3**, 671–676
- Shapiro, A. & Shapiro, B. (1979) *Biochim. Biophys. Acta* **586**, 123–127
- Srivastava, L. M. & Hübscher, G. (1966) *Biochem. J.* **100**, 458–466
- Srivastava, L. M., Shakespeare, P. & Hübscher, G. (1968) *Biochem. J.* **109**, 35–42
- Sugden, P. H. & Newsholme, E. A. (1973) *Biochem. J.* **134**, 97–101
- Tyrrell, J. B. & Anderson, J. W. (1971) *Endocrinology* **89**, 1178–1185
- Volman-Mitchell, H. & Parsons, D. S. (1974) *Biochim. Biophys. Acta* **334**, 316–327
- Watford, M., Lund, P. & Krebs, H. A. (1979) *Biochem. J.* **178**, 589–596
- Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) *Biochem. J.* **121**, 41–47
- Windmueller, H. G. & Spaeth, A. E. (1974) *J. Biol. Chem.* **249**, 5070–5079
- Windmueller, H. G. & Spaeth, A. E. (1978) *J. Biol. Chem.* **253**, 69–76
- Windmueller, H. G. & Spaeth, A. E. (1980) *J. Biol. Chem.* **255**, 107–112
- Zammit, V. A. & Newsholme, E. A. (1979) *Biochem. J.* **184**, 313–322