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The regulation of glutamine and ketone-body metabolism in the small intestine of the long-term (40-day) streptozotocin-diabetic rat

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The small intestine is the major site of glutamine utilization in the mammalian body. During prolonged (40-day) streptozotocin-diabetes in the rat there is a marked increase in both the size and the phosphate-activated glutaminase activity of the small intestine. Despite this increased capacity, intestinal glutamine utilization ceases in diabetic rats. Mean arterial glutamine concentration fell by more than 50% in diabetic rats, suggesting that substrate availability is responsible for the decrease in intestinal glutamine use. When arterial glutamine concentrations in diabetic rats were elevated by infusion of glutamine solutions, glutamine uptake across the portal-drained viscera was observed. The effect of other respiratory fuels on intestinal glutamine metabolism was examined. Infusions of ketone bodies did not affect glutamine use by the portal-drained viscera of non-diabetic rats. Prolonged diabetes had no effect on the activity of 3-oxoacid CoA-transferase in the small intestine or on the rate of ketone-body utilization in isolated enterocytes. Glutamine (2 mM) utilization was decreased in enterocytes isolated from diabetic rats as compared with those from control animals. However, glutaminase activity in homogenates of enterocytes was unchanged by diabetes. In enterocytes isolated from diabetic rats the addition of ketone bodies or octanoate decreased glutamine use. It is proposed that during prolonged diabetes ketone bodies, and possibly fatty acids, replace glutamine as the major respiratory fuel of the small intestine.

INTRODUCTION

Glutamine functions in the mammalian body to transport carbon and nitrogen between tissues. Under normal physiological conditions the major site of glutamine utilization is probably the small intestine, where it is used as a respiratory fuel for the absorptive columnar epithelial cells (enterocytes) (for reviews, see Kovacevic & McGivan, 1983; Windmueller, 1984). We (Watford *et al.*, 1984) reported that during streptozotocin-diabetes in the rat there was a large increase in the activity of phosphate-activated glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2) in the small intestine, which was more pronounced after prolonged (40-day) diabetes. Despite this increase in the capacity of the small intestine to utilize glutamine, arteriovenous-difference measurements across the portal-drained viscera showed complete cessation of plasma glutamine utilization during diabetes, confirming a previous report by Brosnan *et al.* (1983).

In our previous report, the mean arterial glutamine concentration fell by more than 50%; this lack of substrate availability could explain the decrease in intestinal glutamine use (Watford *et al.*, 1984). The work described in the present paper was designed to determine if ketone bodies could be an alternative fuel for enterocytes in 40-day-diabetic rats and, furthermore, if ketone bodies were metabolized in preference to glutamine. Some of these results have been reported in preliminary form (Erbelding & Watford, 1985; Erbelding *et al.*, 1985).

MATERIALS AND METHODS

Materials

Dithiothreitol was from Calbiochem–Behring (La Jolla, CA, U.S.A.). Streptozotocin, L-glutamine, ethyl acetoacetate, acetoacetyl-CoA, DL-β-hydroxybutyrate, bovine serum albumin (fraction V), glutaminase (grade V) and kits for the determination of glucose (kit no. 510) and of alkaline phosphatase activity (kit no. 245) were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) Sodium acetoacetate was prepared from ethyl acetoacetate as described by Krebs & Eggleston (1945). Bio-Rad reagent for protein determination was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). All other enzymes and coenzymes were products of Boehringer Corp. (Indianapolis, IN, U.S.A.).

Rats

Male Sprague–Dawley rats (150–250 g) obtained from Blue-Spruce (Altamont, NY, U.S.A.) were maintained on a standard diet (Purina) and water *ad libitum*. They were made diabetic by injection of streptozotocin (70 mg/kg body wt., in 8.1% NaCl/50 mM-sodium citrate, pH 4.5) directly into a tail vein and were killed 40 days after administration of streptozotocin.

Infusion studies and arteriovenous-difference measurements

Rats were anaesthetized with an intraperitoneal injection of Nembutal (50 mg/kg body wt.). Solutions were infused, with a compact infusion pump (Harvard Apparatus, Dover, MA, U.S.A.), via a catheter positioned in a jugular vein. Initially, 1 ml of glutamine (300 mM), or ketone bodies (750 mM-DL-β-hydroxybutyrate, 250 mM-acetoacetate, pH 7.0) or saline (0.9% NaCl) was infused during 1 min. This bolus was followed by constant infusion of the solutions at a rate of 0.037 ml/min. After 15 min the abdominal cavity was

opened and blood was drawn simultaneously from the aorta and the portal vein into heparinized syringes fitted with 26-gauge needles. For arteriovenous-difference experiments blood was drawn in a similar manner. Blood samples (0.5–1.0 ml) were immediately added to 1.0 ml of ice-cold $HClO_4$ (10%, v/v). Assays were performed on the neutralized deproteinized supernatants.

Isolated enterocytes and tissue homogenates

Enterocytes were isolated and incubated as previously described (Watford *et al.*, 1979). Approx. 15 mg dry wt. of cells were incubated in a final volume of 4.0 ml for 20 min at 37 °C. Enterocyte viability was assessed by leakage of lactate dehydrogenase activity during the course of incubation [$9.3 \pm 2.8\%$ ($n = 10$) and $3.7 \pm 2.2\%$ ($n = 10$) for cells from diabetic and control rats respectively]. Homogenates for the assay of glutaminase activity were prepared as previously reported (Watford *et al.*, 1984), and those for the assay of 3-oxoacid CoA-transferase and alkaline phosphatase were prepared as described by Hanson & Carrington (1981).

Determination of enzyme activities

Glutaminase activity was determined by the method of Curthoys & Lowry (1973), alkaline phosphatase by the *p*-nitrophenol method (Sigma kit no. 245), lactate dehydrogenase by the method of Wroblewski & LaDue (1955), and 3-oxoacid CoA-transferase (EC 2.8.3.5), in the direction of acetoacetyl-CoA to succinyl-CoA, by that of Williamson *et al.* (1971). All assays were carried out within 24 h of killing the animal and were performed at 37 °C, with at least two different concentrations of enzyme. Rates were linear with time.

Determination of metabolites

Glutamine was determined as glutamate after hydrolysis with glutaminase (Lund, 1974). Glutamate was measured by the method of Bernt & Bergmeyer (1974), ammonia by that of Kun & Kearney (1974), alanine by that of Williamson (1974), acetoacetate by that of Mellanby & Williamson (1974) and D-β-hydroxybutyrate by that of Williamson & Mellanby (1974). Glucose was determined by the glucose oxidase/peroxidase method (Sigma kit 510). Protein was determined by the dye-binding method of Bradford (1976), with a Bio-Rad kit. DNA was measured as described by Burton (1954).

The naturally occurring form of β-hydroxybutyrate is the D-isomer, and the enzymic analysis used throughout this work detects only this form. However, this isomer is not readily available, and therefore DL-β-hydroxybutyrate was used throughout these studies. There have been reports of a cytosolic enzymic activity present in some tissues that is capable of oxidizing L-β-hydroxybutyrate to acetoacetate, and this is believed to be a property of L-gulonate dehydrogenase (see Williamson & Kuenzel, 1971). It is assumed in the present work that the L-form of β-hydroxybutyrate is not metabolized in rat tissues during the course of these experiments (maximum duration 20 min), and that racemization also does not occur at a significant rate.

Statistical analysis

In experiments with isolated enterocytes, all observations for a particular response were used in an overall repeated-measures analysis of variance to estimate the mean squares for error for subsequent *t* tests. Significance was tested by a *t* test comparing diabetes versus control for each of the treatments, by using the mean square for all rats. Comparisons among treatments (substrates) were made by separate *t* tests for diabetes and control incubations by using the error mean square.

Table 1. Characteristics of long-term (40-day)-diabetic rats

Results are expressed as means ± S.E.M. of the numbers of observations given in parentheses: N.D., not determined.

	Control	Diabetic
Body wt. (g)	262 ± 5 (23)	213 ± 9 (19)
Small-intestine wt. (g)	8.94 ± 0.2 (23)	16.6 ± 0.8 (19)
Small-intestine wt. (% of body wt.)	3.4 ± 0.1 (23)	7.8 ± 0.2 (19)
Small-intestine length (cm)	117 ± 1 (16)	163 ± 3 (12)
Blood [glucose] (mM)	6.0 ± 0.4 (12)	24.7 ± 0.8 (12)
Total blood [ketones] (mM)	0.21 ± 0.03 (12)	4.44 ± 0.70 (12)
A–V* total ketones (μmol/ml)	N.D.	0.131 ± 0.078 (11)

* Arteriovenous difference across the portal-drained viscera. Blood was drawn from the aorta and the portal vein.

RESULTS

The characteristics of a representative group of control and 40-day-diabetic rats are presented in Table 1. Diabetic rats lost weight during the course of study and by day 40 were severely emaciated. They had exhibited ketosuria (Ketostix; Ames) at least 10 days before being killed, at which time they had elevated concentrations of blood ketone bodies and glucose (Table 1). The size of the small intestine, both in length and in weight, was increased in the 40-day-diabetic rats (Table 1).

Glutamine metabolism across the portal-drained viscera *in vivo*

Arteriovenous-difference measurements across the portal-drained viscera of 40-day-diabetic rats showed a net uptake of ketone bodies (Table 1). The results shown in Table 2 confirm previous findings (Watford *et al.*, 1984) that net glutamine uptake from the circulation by the portal-drained viscera completely ceases in 40-day-diabetic animals. Release into the portal blood of the major products of intestinal glutamine metabolism, alanine and ammonia, is also decreased. The arterial concentration of glutamine in 40-day-diabetic rats is markedly lower than in controls (Table 2), indicating that less plasma glutamine is available. Windmueller & Spaeth (1978) reported that net glutamine uptake cannot be detected across the perfused small intestine at circulating glutamine concentrations of < 0.2 mM. Therefore, the decrease in glutamine uptake in 40-day-diabetic rats (Table 2) may be due to limited substrate availability. Infusion of glutamine into 40-day-diabetic rats (Fig. 1 and Table 3) resulted in elevation of arterial glutamine concentrations to above normal values. At these values there was uptake of glutamine by the portal-drained viscera, with a concomitant increase in

Table 2. Glutamine metabolism in 40-day-diabetic rats *in vivo*

Measurements were made as described in the Materials and methods section. Results are means ± S.E.M. for the numbers of rats shown in parentheses.

Rats	Arterial [glutamine] (μmol/ml)	Arteriovenous difference (μmol/min)			
		Glutamine	Glutamate	Alanine	NH_4^+
Control	0.579 ± 0.086 (7)	+0.119 ± 0.030 (7)	−0.078 ± 0.048 (8)	−0.124 ± 0.027 (8)	−0.422 ± 0.047 (6)
Diabetic	0.282 ± 0.031 (7)	−0.036 ± 0.028 (7)	+0.021 ± 0.024 (7)	−0.020 ± 0.189 (5)	−0.081 ± 0.048 (7)

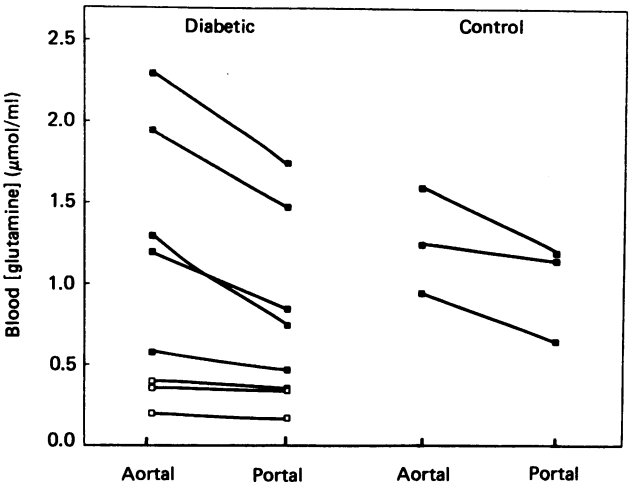

Fig. 1. Arteriovenous differences across the portal-drained viscera after infusion of glutamine

Rats (control and 40-day-diabetic) received infusions of glutamine (300 mM) (■) or saline (0.9% NaCl) (□) as described in the Materials and methods section. Points connected by a line indicate values from the aorta and portal vein of a single rat.

ammonia output (Table 3). Infusion of saline into control or diabetic rats did not significantly change arterial glutamine concentration or glutamine uptake (Tables 3 and 4).

Infusion of ketone bodies in control rats

The above results indicate that the portal-drained viscera of 40-day-diabetic rats will use glutamine when it is available. However, this raises two questions concerning intestinal metabolism in these rats. First, what alternative fuels are being used in place of glutamine? Second, are these fuels used simply because glutamine is not available, or are they used preferentially? When ketone bodies, a possible alternative fuel, were infused into control rats, there were no apparent changes in the uptake of glutamine or production of alanine and ammonia across the portal-drained viscera (Table 4). The arterial concentration of total ketones rose to 7 mM, similar to that seen in long-term diabetes.

Glutamine and ketone-body metabolism in isolated enterocytes

The results from the studies *in vivo* described above suggest that ketone bodies may be used in place of glutamine by the small intestine of the 40-day-diabetic rat, but that they do not suppress glutamine use in control animals or in 40-day-diabetic animals with artificially increased blood glutamine. The effects of ketone bodies and other alternative fuels, glucose and fatty acids, on glutamine metabolism in enterocytes are shown in Table 5.

Rates of glutamine utilization and ammonia production were lower in enterocytes isolated from 40-day-diabetic rats compared with controls. In enterocytes from diabetic rats, glutamine utilization and ammonia production were decreased by the addition of octanoate, acetoacetate or acetoacetate plus β-hydroxybutyrate. In cells from control animals, glutamine utilization and ammonia production were inhibited only by the addition of acetoacetate. The addition of glucose had no effect on glutamine removal, but did increase alanine synthesis in all cases. Similarly, alanine production from glutamine was decreased in the presence of

Table 3. Arteriovenous differences across the portal-drained viscera after infusion of glutamine or saline

Glutamine (300 mM) or saline (0.9% NaCl) was infused into anaesthetized rats as described in the Materials and methods section. The results are means ± S.E.M., where *n* is the number of animals.

Rat	Infusion	*n*	Arteriovenous difference (μmol/ml)			
			Glutamine	Glutamate	Alanine	NH_4^+
Control	Glutamine	3	+0.115 ± 0.237	−0.036 ± 0.048	−0.035 ± 0.049	−0.273 ± 0.019
Diabetic	Glutamine	5	+0.361 ± 0.162	−0.022 ± 0.008	−0.054 ± 0.026	−0.549 ± 0.144
Diabetic	Saline	5	+0.050 ± 0.021	−0.024 ± 0.089	−0.074 ± 0.038	−0.137 ± 0.307

Table 4. Arteriovenous differences across the portal-drained viscera of non-diabetic rats after infusion of ketone bodies

Ketone bodies (750 mM-DL-β-hydroxybutyrate, 250 mM-acetoacetate) or saline (0.9% NaCl) were infused into anaesthetized control rats as described in the Materials and methods section. The results are means ± S.E.M. for four animals in each group. Arterial ketone-body concentration is the sum of enzymically determined acetoacetate plus D-β-hydroxybutyrate; L-β-hydroxybutyrate was not determined.

Infusion	Arterial [ketone body] (μmol/ml)	Arteriovenous difference (μmol/ml)			
		Glutamine	Glutamate	Alanine	NH_4^+
Ketone bodies	7.0 ± 0.4	+0.150 ± 0.008	−0.009 ± 0.010	−0.069 ± 0.037	−0.395 ± 0.118
Saline	< 0.2	+0.128 ± 0.052	−0.013 ± 0.015	−0.084 ± 0.023	−0.260 ± 0.056

Table 5. Effect of ketone bodies, octanoate and glucose on glutamine metabolism in isolated enterocytes

Enterocytes were isolated from control and 40-day-diabetic rats and incubated as described by Watford *et al.* (1979). The results are expressed as μmol of metabolite removed (−) or produced (+), and are means ± S.E.M. of the numbers of observations given in parentheses.

Substrate added	Rats	Metabolite removal (−) or production (+) (μmol/min per g dry wt.)			
		Glutamine	Glutamate	Alanine	NH_4^+
None (5)	Control	+0.17 ± 0.13	+0.23 ± 0.07	+0.62 ± 0.19	+1.49 ± 0.51
	Diabetic	+0.13 ± 0.13	+0.33 ± 0.11	+0.56 ± 0.24	+1.46 ± 0.72
2 mM-glutamine (10)	Control	−8.80 ± 0.35	+3.50 ± 0.27	+3.19 ± 0.21§	+10.11 ± 1.0
	Diabetic	−6.99 ± 0.55†	+2.91 ± 0.27	+3.27 ± 0.37§	+7.31 ± 1.03†
2 mM-glutamine + 5 mM-DL-β-hydroxybutyrate‡ (3)	Control	−8.80 ± 1.06	+3.49 ± 0.24	—	+7.69 ± 0.22*
	Diabetic	−5.94 ± 0.66†	+2.82 ± 0.26	—	+4.32 ± 0.36*†
2 mM-glutamine + 5 mM-acetoacetate (5)	Control	−6.85 ± 1.31*	+3.11 ± 0.39	—	+7.08 ± 0.92*
	Diabetic	−4.85 ± 0.51*†	+2.34 ± 0.18†	—	+4.09 ± 0.29*†
2 mM-glutamine + 3 mM-DL-β-hydroxybutyrate‡ + 1 mM-acetoacetate (10)	Control	−8.38 ± 0.45	+3.69 ± 0.37	+2.39 ± 0.35§*	+8.08 ± 0.79*
	Diabetic	−5.35 ± 0.47*†	+3.11 ± 0.30	+2.50 ± 0.43§*	+5.68 ± 0.59*
2 mM-glutamine + 3 mM-DL-β-hydroxybutyrate‡ + 1 mM-acetoacetate + 10 mM-glucose (5)	Control	−10.06 ± 0.80	+3.70 ± 0.61	+3.99 ± 0.67*	+9.32 ± 0.74
	Diabetic	−5.67 ± 0.62†	+3.18 ± 0.29	+4.65 ± 0.60*	+5.31 ± 0.57*†
2 mM-glutamine + 10 mM-glucose (5)	Control	−10.17 ± 0.70	+3.65 ± 0.32	+5.42 ± 0.47*	+10.61 ± 0.48
	Diabetic	−6.15 ± 0.40†	+2.93 ± 0.29	+5.37 ± 0.37*	+6.58 ± 0.86†
2 mM-glutamine + 1 mM-octanoate (5)	Control	−8.57 ± 0.19	+4.30 ± 0.51*	+2.59 ± 0.44	+10.78 ± 0.81
	Diabetic	−5.21 ± 0.95*†	+4.35 ± 0.35*	+2.24 ± 0.33*	+7.54 ± 0.64†
10 mM-glucose (5)	Control	+0.33 ± 0.23	+0.37 ± 0.07	+1.08 ± 0.19	—
	Diabetic	+0.59 ± 0.42	+0.30 ± 0.08	+0.93 ± 0.27	—
1 mM-octanoate (5)	Control	+0.19 ± 0.15	+0.44 ± 0.15	+0.65 ± 0.10	—
	Diabetic	−0.12 ± 0.13	+0.37 ± 0.14	+0.56 ± 0.14	—

* Significantly different ($P < 0.05$) from incubations with glutamine alone.
† Significantly different ($P < 0.05$) control versus diabetic.
‡ DL-β-Hydroxybutyrate was added at the concentrations indicated.
§ $n = 5$.

ketone bodies, but this was prevented by the addition of glucose. Octanoate caused an accumulation of glutamate regardless of the source of enterocytes, but only inhibited glutamine use in cells from diabetic rats.

The utilization of ketone bodies by enterocytes was investigated in a separate series of experiments (Table 6). Acetoacetate and β-hydroxybutyrate, alone or in combination, were utilized at similar rates by cells from both control and 40-day-diabetic rats. Utilization of ketone bodies was concentration-dependent. The addition of glutamine increased the utilization of acetoacetate. We are unable to account for this observation, but have ruled

Table 6. Ketone-body metabolism in rat enterocytes

Enterocytes were isolated and incubated as described by Watford *et al.* (1979). The results are expressed as μmol of metabolite removed (−) or produced (+) and are the means ± S.E.M. of four separate experiments for control and diabetic (40-day) rats. Abbreviations: AcAc, acetoacetate; D-βOH, D-β-hydroxybutyrate.

	Metabolite removal (−) or production (+) (μmol/min per g dry wt.)					
Rat . . .	Control			Diabetic		
Substrate added	AcAc	D-βOH	Total	AcAc	D-βOH	Total
Acetoacetate (1 mM)	−1.85±0.70	+0.19±0.07	−1.67±0.41	−1.54±0.25	+0.25±0.08	−1.29±0.31
DL-β-Hydroxybutyrate (2 mM)†	+0.55±0.23	−1.77±0.15	−1.23±0.15	−0.82±0.13	−2.00±0.11	−1.19±0.14
Acetoacetate (1 mM) + DL-β-hydroxybutyrate (6 mM)†	−1.25±0.61	−4.96±0.72	−6.21±0.46	+1.81±0.23	−4.15±0.26	−5.96±0.44
Acetoacetate (1 mM) + glutamine (5 mM)	−2.62*±0.50	+0.24±0.11	−2.39*±0.36	−3.49*±0.41	+0.35±0.09	−3.13*±0.46
DL-β-Hydroxybutyrate (2 mM)† + glutamine (5 mM)	+0.49±0.56	−2.48±0.35	−1.99±0.35	+0.45±0.11	−1.82±0.25	−1.38±0.33
Acetoacetate (1 mM) + DL-β-hydroxybutyrate (6 mM)† + glutamine (5 mM)	−2.39*±0.45	−3.55*±0.35	−5.92*±0.28	−2.23±0.19	−2.73*±0.31	−4.96*±0.28

* Values significantly different ($P < 0.05$) in the presence of glutamine. No significant differences were seen between enterocytes from control or diabetic rats.

† DL-β-Hydroxybutyrate was added to the flasks at the concentrations indicated. The enzymic analysis of β-hydroxybutyrate only detects the D isomer. Therefore changes in [βOH] represent changes in the D isomer only. DL-β-Hydroxybutyrate is approx. 41% D.

out interference in the acetoacetate assay by glutamine, or by the products of its metabolism, ammonia, glutamate and alanine.

Activity of 3-oxoacid CoA-transferase and glutaminase

The activity of 3-oxoacid-CoA transferase of the small intestine was not affected by 40-day diabetes when activity was expressed relative to unit tissue weight or protein (control, 6.39±0.33 units/g wet wt., 164±10 munits/mg of protein, versus diabetic, 5.54±0.58 units/g wet wt., 159±17 munits/mg of protein; means ± S.E.M. of four observations in each group). But there was a 2-fold increase when activity was expressed relative to body weight (control, 22.1±0.8 units/100 g body wt., versus diabetic, 46.8±4.3 units/100 g body wt.). This is similar to the response of alkaline phosphatase activity (results not shown). In contrast, a 4-fold increase in intestinal glutaminase activity was observed in diabetic animals (Table 7).

Lower rates of glutamine utilization by enterocytes from 40-day-diabetic rats (Table 5) could indicate lower glutaminase activity in these cells, despite an overall increase in the whole organ (Watford *et al.*, 1984). The results presented in Table 7 show the large increase in glutaminase activity of the whole organ when expressed as units/g wet wt., units/100 g body wt. or munits/mg of protein. The increase is less pronounced when expressed as munits/mg of DNA, indicating that cell number had increased. However, when specific activity was determined in isolated enterocytes, there was no difference between control and 40-day-diabetic animals.

It should be noted that the values of glutaminase activity for whole intestine samples, but not those for enterocytes (reported in Table 7), are considerably higher

Table 7. Activity of glutaminase in the small intestine of 40-day-diabetic rats

Activities were determined as described by Watford *et al.* (1984) and are expressed as μmol of glutamate formed/min at 37 °C. Results are means ± S.E.M. of the numbers of observations given in parentheses.

	Glutaminase activity				
	Whole small intestine				Isolated enterocytes (munits/mg of protein)
Rat	(units/g wet wt.)	(units/100 g body wt.)	(munits/mg of protein)	(munits/μg of DNA)	
Control	4.20±1.18 (7)	13.9±4.3 (7)	46±11 (7)	0.98±0.18 (4)	124±17 (6)
Diabetic	8.43±0.85 (7)	65.4±7.2 (7)	103±11 (7)	1.57±0.15 (4)	139±17 (5)

(2–4-fold) than values we reported previously (Watford *et al.*, 1984). We are unable to offer any explanation for these differences. The rats and tissues were treated similarly in both cases. The higher values in no way affect the conclusions of our earlier work, that diabetes increases glutaminase activity in the small intestine and that this is due, at least in part, to the increased size of the organ.

DISCUSSION

Glutamine metabolism in the small intestine during diabetes

Decreased glutamine utilization by the small intestine during diabetes (Brosnan *et al.*, 1983; Watford *et al.*, 1984) was unexpected, because rapidly replicating/short-lived cells, such as enterocytes, were thought to have an absolute requirement for high rates of glutamine catabolism (Krebs, 1980; McKeehan, 1982; Zielke *et al.*, 1984; Newsholme *et al.*, 1985*a*,*b*). In contrast, no decrease in glutamine use by the portal-drained viscera of streptozotocin-diabetic rats was observed in a similar study reported by Schrock & Goldstein (1981). The use of arteriovenous-difference measurements to estimate glutamine utilization by the small intestine in this and previous studies (Table 2; Brosnan *et al.*, 1983; Watford *et al.*, 1984) may be inaccurate, since blood-flow rates were not determined. A preliminary communication (Korthuis *et al.*, 1986) suggests that, in 24 h-starved long-term (4 weeks)-diabetic rats, blood flow to the small intestine increases by only 64–107%. Therefore it is unlikely that our results were simply due to changes in blood flow. In addition, intraluminal substrates cannot be ruled out in the present study, as all rats were used in the fed state. However, decreased release of alanine and ammonia into the portal circulation in diabetic rats (Brosnan *et al.*, 1983; Table 2) suggests that luminal glutamine was not replacing that normally obtained from the circulation.

A possible explanation for decreased glutamine utilization by the small intestine during long-term diabetes is that glutamine is not available. The mean arterial glutamine concentration during long-term diabetes (0.282 mM; Table 2) approached that at which Windmueller & Spaeth (1978) saw no net glutamine uptake by this organ. The finding that glutamine is removed from the circulation by the portal-drained viscera of the 40-day-diabetic rat when arterial glutamine concentrations are artificially elevated (Fig. 1 and Table 3) further supports this hypothesis.

Effects of alternative fuels on glutamine metabolism

The findings reported in Fig. 1 and Table 3 suggest that glutamine is the preferred respiratory fuel for the small intestine in 40-day-diabetic rats and that alternative fuels cannot suppress glutamine utilization when the latter is available. Possible alternative fuels are glucose, fatty acids and ketone bodies, all of which are more available in uncontrolled streptozotocin-diabetes and are known to be oxidized by the small intestine (Neptune, 1967; Hulsmann, 1971; Hulsmann *et al.*, 1970, 1981; Iemhoff & Hulsmann, 1971; Hanson & Parsons, 1976, 1977, 1978; Windmueller & Spaeth, 1978). The results presented in Table 4 suggest that, if ketone bodies are the alternative fuel, they are not able to suppress glutamine use in control rats with normal arterial concentrations of glutamine. The possibility that ketone bodies do not affect glutamine metabolism and are only used when glutamine concentrations are low seems unlikely, as Brosnan *et al.* (1983) and Windmueller & Spaeth (1978) suggest ketone-body use when arterial glutamine concentrations are maintained. This would mean that ketone bodies not only replace glutamine as the major respiratory fuel but actually suppress glutamine use, although a period of adaptation may be required.

Ketone-body utilization has been shown to affect the use of other respiratory fuels in the small intestine, although the exact response depends on the physiological state of the animal. Hanson & Parsons (1978) have shown that rates of ketone-body utilization by the perfused jejunum from fed and 48 h-starved rats are similar, but that glucose oxidation was partially inhibited by ketone bodies in preparations from fed rats and completely inhibited in those from starved rats. They also reported that fatty acids suppressed glucose oxidation in the intestine of starved and diabetic rats. Nagy & Kretchmer (1986*a*) imply that glutamine oxidation in enterocytes isolated from the intestinal crypts of starved/re-fed rats may be suppressed by the addition of β-hydroxybutyrate. In isolated enterocytes (Table 5) there was an apparent sparing of glutamine by ketone bodies and octanoate, and this was more pronounced in cells isolated from diabetic animals, again suggesting some form of adaptation.

Another possible alternative fuel, glucose, had no significant effect on glutamine utilization in enterocytes from 40-day-diabetic rats. This is in agreement with previous work in enterocytes from fed, starved or short-term-diabetic rats (Watford *et al.*, 1979, 1984) and the perfused small intestine of fed or starved rats (Hanson & Parsons, 1977).

Enzyme activities

The activities of enzymes of ketone-body metabolism, including 3-oxoacid CoA-transferase, in the small intestine and most other tissues are not known to change in response to diet, starvation or diabetes (Robinson & Williamson, 1980; Hanson & Carrington, 1981). During diabetes the activity of 3-oxoacid CoA-transferase in the small intestine increased only when expressed relative to body weight, suggesting that regulation is related to changes in the amount of tissue. We previously suggested that the increase in glutaminase activity in the small intestine of diabetic rats is also due to increased enterocyte number. However, comparing the effects of diabetes on the activity of glutaminase and 3-oxoacid CoA-transferase, it is apparent that glutaminase is regulated differently. This suggests that glutaminase activity per enterocyte increases significantly. However, we found no difference in glutaminase activity per enterocyte (Table 7), and Nagy & Kretchmer (1986*b*) report that short-term diabetes decreases glutaminase activity in enterocytes. Pinkus & Windmueller (1977) determined glutaminase to be evenly distributed along the villus–crypt axis; however, Nagy & Kretchmer (1986*b*) report higher activity in enterocytes at the villus–crypt junction. If this differential distribution is more pronounced in long-term diabetes, it could explain differences observed with whole intestine compared with isolated enterocytes: the preparation of enterocytes reported in the present paper represents predominantly villus cells (Watford *et al.*, 1979).

Conservation of glutamine carbon

Brosnan *et al.* (1983) have proposed that, during diabetes, ketone bodies replace glutamine as respiratory fuel for the small intestine in a manner similar to the glucose-sparing effect of fat-derived fuels (Cahill, 1970). The carbon skeleton of glutamine thus spared oxidation could then be recovered via hepatic or renal gluconeogenesis.

However, it has been suggested that glutamine catabolism in the small intestine would not mean loss of glucose units (Caldecourt *et al.*, 1985; Wagenmakers *et al.*, 1985), as alanine, a good substrate for hepatic gluconeogenesis, would be formed. Alanine formation in various preparations of small intestine accounts for about 30% of the nitrogen (Watford *et al.*, 1979; Windmueller & Spaeth, 1974, 1978; Hanson & Parsons, 1976), but < 5% of the carbon (Windmueller & Spaeth, 1974, 1975, 1980; Windmueller, 1984; Watford, 1977), derived from glutamine metabolism. Therefore the amount of glutamine carbon incorporated into alanine in the small intestine is small and, in all likelihood, glucose provides the carbon for alanine synthesis in this tissue, as it does in muscle (Caldecourt *et al.*, 1985; Wagenmakers *et al.*, 1985). Mallet *et al.* (1986), from studies with [^{14}C]succinate as substrate, have proposed that enterocytes convert considerable amounts of glutamine carbon into alanine. Several methodological problems, including the use of succinate as respiratory fuel (see Watford, 1977; Towler *et al.*, 1978; Watford *et al.*, 1979; Porteous, 1980), loss of cell viability during prolonged incubations (see Watford *et al.*, 1979) and absence of glucose from the medium, raise questions as to the significance of their (Mallet *et al.*, 1986) findings. The evidence suggests, then, that decreased utilization of glutamine by the small intestine during diabetes really does represent a sparing of glucose precursors.

Glutamine metabolism in 'active' cells

Newsholme *et al.* (1985*a*,*b*) have proposed that high rates of glutamine oxidation in 'active' cells may be related to the need for glutamine in very active biosynthetic pathways (e.g. nucleic acid synthesis). Furthermore, Nagy & Kretchmer (1986*a*) report that high rates of glutamine oxidation are required to maintain normal rates of DNA synthesis in enterocytes isolated from intestinal crypts. The results presented here (Tables 1 and 2, Fig. 1), indicate that rat enterocytes can decrease glutamine utilization at a time of extensive intestinal growth. This, and other reports that extensive glutamine oxidation does not occur in enterocytes of some species (Windmueller & Spaeth, 1974; Pinkus & Windmueller, 1977; Porteous, 1980), mean that the use of glutamine as the primary respiratory fuel is not an absolute requirement for some types of 'active' cells, and other fuels may be used when glutamine is limiting. However, the glutamine-sparing effect of ketones might not be seen in all such cell types; for example, certain tumour cells are reported to have very low capacity for ketone-body oxidation (Rofe *et al.*, 1986).

Conclusions

Glutamine has been proposed to play a role in the regulation of lipolysis, and possibly ketogenesis (Cersosimo *et al.*, 1986*a*,*b*), illustrating important interactions between availability and use of respiratory fuels. The results in the present paper, and reports by others (Brosnan *et al.*, 1983: Nagy & Kretchmer, 1986*a*,*b*), suggest that during diabetic ketosis in the rat ketone bodies, and possibly fatty acids, replace glutamine as the major respiratory fuel in the small intestine.

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