Glutamine and ketone-body metabolism in the gut of streptozotocin-diabetic rats

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1. In short- and long-term diabetic rats there is a marked increase in size of both the small intestine and colon, which was accompanied by marked decreases ($P < 0.001$) and increases ($P < 0.001$) in the arterial concentrations of glutamine and ketone bodies respectively. 2. Portal-drained viscera blood flow increased by approx. 14–37% when expressed as ml/100 g body wt., but was approximately unchanged when expressed as ml/g of small intestine of diabetic rats. 3. Arteriovenous-difference measurements for ketone bodies across the gut were markedly increased in diabetic rats, and the gut extracted ketone bodies at approx. 7 and 60 nmol/min per g of small intestine in control and 42-day-diabetic rats respectively. 4. Glutamine was extracted by the gut of control rats at a rate of 49 nmol/min per g of small intestine, which was diminished by 45, 76 and 86% in 7-, 21- and 42-day-diabetic rats respectively. 5. Colonocytes isolated from 7- or 42-day-diabetic rats showed increased and decreased rates of ketone-body and glutamine metabolism respectively, whereas enterocytes of the same animals showed no apparent differences in the rates of acetoacetate utilization as compared with control animals. 6. Prolonged diabetes had no effects on the maximal activities of either glutaminase or ketone-body-utilizing enzymes of colonic tissue preparations. 7. It is concluded that, although the epithelial cells of the small intestine and the colon during streptozotocin-induced diabetes exhibit decreased rates of metabolism of glutamine, such decreases were partially compensated for by enhanced ketone-body utilization by the gut mucosa of diabetic rats.

INTRODUCTION

Most of the energy required by the epithelial cells of the small intestine (enterocytes) is provided by the oxidation of glucose and glutamine in the fed state and of glutamine and ketone-bodies in the starved state (for reviews, see Kovacevic & McGivan, 1983; Windmueller, 1984). Furthermore, both glutamine and ketone bodies are considered to be important respiratory fuels for colonic mucosa (colonocytes) (Roediger, 1982; Ardawi & Newsholme, 1985a).

In the rat, the maximal activity of colonic phosphate-dependent glutaminase (EC 3.5.1.2) [a key enzyme in the metabolism of glutamine (for review, see Kovacevic & McGivan, 1983)] was decreased or unchanged in short- or long-term diabetes respectively (Ardawi, 1987a). That of the small intestine was increased in both short- and long-term streptozotocin-induced diabetes (Watford *et al.*, 1984, 1987; Ardawi, 1987a). However, despite the marked increase in the maximal activity of intestinal phosphate-dependent glutaminase, arteriovenous-difference measurements across the gut showed diminished metabolism (Ardawi, 1987a) or complete cessation of utilization (Brosnan *et al.*, 1983; Watford *et al.*, 1984, 1987) of plasma glutamine. The decrease in glutamine utilization by the gut was found to correlate with the duration of the diabetic state (Ardawi, 1987a) and was accompanied by enhanced release by skeletal muscle and increased uptake by both kidney and liver of the amino acid (Ardawi, 1987a).

In previous work (Watford *et al.*, 1984; Ardawi, 1987a) it was suggested that, in prolonged diabetes,

ketone bodies and possibly fatty acids could replace glutamine as the major respiratory fuel of the small intestine. Indeed, evidence to support this suggestion was provided by the work of Watford *et al.* (1987). However, conclusions based on the use of arteriovenous-difference measurements to estimate substrate utilization by the small intestine may be inaccurate, since blood-flow rates were not determined in those studies (Brosnan *et al.*, 1983; Watford *et al.*, 1984, 1987; Ardawi, 1987a).

This study was designed to determine the effects of blood-flow changes, which are known to be increased across the gut in streptozotocin-diabetic rats (see Korthuis *et al.*, 1986), on the extent of glutamine and ketone-body metabolism by the gut of short- and long-term diabetic rats. In addition, the extent of ketone-body metabolism as an alternative fuel to glutamine for colonocytes as compared with enterocytes of short- and long-term diabetic rats was also investigated. The relevance of these changes to the overall regulation of gut energy metabolism during streptozotocin-induced diabetes is discussed.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (200–240 g) were supplied by King Fahd Medical Research Center, College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were maintained on a standard laboratory diet (see Ardawi, 1986) and water *ad libitum* and were kept in a controlled environment

(constant temperature 24 °C, and a light cycle of 12 h on, 12 h off). Rats were made diabetic by a single intravenous injection of streptozotocin (70 mg/kg body wt. in 50 mM-sodium citrate, pH 4.5) under light ether anaesthesia. Rats showing blood glucose greater than 20 mM were used at 7, 21, and 42 days after induction of diabetes (see Table 1). The daily food intake and body weights of normal and streptozotocin-diabetic rats were recorded.

Chemicals and enzymes

All chemicals and enzymes were obtained from the same sources as described previously (Ardawi & Newsholme, 1985a; Ardawi, 1986, 1987a). *p*-Aminohippuric acid (PAH) was a gift from Mr. Omer Saggaff, King Fahd Medical Research Center.

Arteriovenous-difference measurements

Rats were anaesthetized with ether, and blood was withdrawn simultaneously into heparinized syringes from the hepatic portal vein and the abdominal aorta. Samples (1.0–1.5 ml) were quickly added to 1.0 ml of ice-cold HClO₄ (10%, v/v) and used for determination of metabolites after deproteinization and neutralization (see Ardawi, 1987a).

Blood-flow measurements

Portal blood flow was measured by the indicator-dilution technique described by Katz & Bergman (1969). PAH, a non-metabolizable dye, was utilized to measure gut blood flow by the dye-dilution technique. Blood flow was determined from the PAH administered (30 µg/min per 100 g body wt., via a tertiary branch of the mesenteric vein) and arterial (abdominal aorta) and venous (portal-vein) PAH concentration-difference measurements according to the equation:

$$\text{Gut blood flow (ml/min)} = \frac{[\text{PAH administered (mg/min)}]}{[\text{venous PAH (mg/ml)}] - [\text{arterial PAH (mg/ml)}]}$$

PAH was measured in Somogi filtrate [10% (v/v) Ba(OH)₂ and ZnSO₄], treated with *p*-dimethylamino-benzaldehyde alcohol solution as described previously (Brun, 1951). Substrate or metabolite exchange rates were calculated by multiplying gut blood flow by the respective substrate or metabolite arteriovenous concentration difference.

Preparation and incubation of colonocytes and enterocytes

Colonocytes were prepared as previously described by Ardawi & Newsholme (1985a). Enterocytes were prepared as described by Watford *et al.* (1979). Incubations of either isolated colonocytes or enterocytes were as described previously (Ardawi & Newsholme, 1985a).

Preparation of homogenates and assay of enzyme activities

Animals were killed by cervical dislocation, and colons (from the caecum to the rectal ampulla) were rapidly removed, washed by forcing ice-cold 0.9% NaCl through the lumen, and then cut longitudinally. Mucosal tissue of the colon was separated from the underlying muscle by scraping with a microscope slide, weighed, and homogenized in 5 vol. of extraction medium (Ardawi & Newsholme, 1982) by using a Polytron homogenizer (PCU-2, at position 3) for 10–20 s at 0 °C. In some experiments, the whole of the colon (mucosa plus underlying muscle layers) was extracted as described above. Tissue preparations were treated immediately before assay with 0.05% (v/v) Triton X-100.

Phosphate-dependent glutaminase (hereafter referred to as 'glutaminase') was determined by the method of Curthoys & Lowry (1973); the activities of 3-oxoacid CoA-transferase, acetoacetyl-CoA thiolase and D-3-hydroxybutyrate dehydrogenase were determined by the method of Williamson *et al.* (1971). The final volume of assay mixtures in all cases was 1.0 ml. All spectrophoto-

Table 1. Body weight, small-intestine and colon weights, plasma glucose, plasma insulin and plasma ketone bodies in control and streptozotocin-diabetic rats

Values are presented as means ± S.D. for the numbers of rats shown. Diabetic rats are streptozotocin-induced diabetic animals, and controls are age-matched control rats. Significant differences from control rats are indicated by * ($P < 0.001$). Significant differences from 7-day-diabetic rats are indicated by † ($P < 0.05$).

		Duration of diabetic state (days)		
		7	21	42
	Animals No. of rats...	8	8	6
Body wt. (g)	Control	228 ± 12.6	302 ± 6.9	374 ± 13.2
	Diabetic	196 ± 11.6*	248 ± 4.3*	264 ± 12.8*
Food consumption (g/day)	Control	15.92 ± 1.05	14.98 ± 2.68	16.20 ± 0.64
	Diabetic	19.59 ± 1.19	25.27 ± 1.49*†	29.22 ± 1.51*†
Small-intestine wt. (g)	Control	7.78 ± 0.50	10.93 ± 0.30	13.47 ± 0.46
	Diabetic	9.15 ± 0.36*	13.86 ± 0.28*	18.19 ± 1.03*
Colon wt. (g)	Control	1.29 ± 0.07	1.71 ± 0.04	2.15 ± 0.12
	Diabetic	1.63 ± 0.08*	2.07 ± 0.05*	2.34 ± 0.09*
Plasma glucose (mM)	Control	7.19 ± 0.37	6.90 ± 0.48	7.32 ± 0.79
	Diabetic	25.40 ± 0.75*	27.47 ± 0.93*	33.09 ± 0.86*†
Plasma insulin (µunits/ml)	Control	44.41 ± 4.43	48.78 ± 3.09	50.88 ± 4.83
	Diabetic	11.68 ± 1.09*	11.19 ± 0.82*	9.95 ± 2.10*†
Plasma ketone bodies (mM)	Control	0.25 ± 0.04	0.24 ± 0.05	0.23 ± 0.03
	Diabetic	0.33 ± 0.04	1.19 ± 0.19*†	5.62 ± 0.49*

Table 2. Arteriovenous-difference measurements for glutamine, glutamate, alanine, ammonia and total ketone bodies across the portal-drained viscera of control and streptozotocin-diabetic rats

Measurements were made as described in the Materials and method section; results are presented as means $\pm$ S.D., with the numbers of animals used given in parentheses. Values are expressed as μ mol of metabolite/ml of whole blood; negative values indicate uptake. Ketone bodies represent the sum of acetoacetate and 3-hydroxybutyrate concentrations. Significant differences from control rats are indicated by * ($P < 0.01$), ** ($P < 0.005$), *** ($P < 0.001$).

Treatment group	Arterial concentration		Arteriovenous difference				
	Glutamine	Ketone bodies	Glutamine	Glutamate	Alanine	Ammonia	Ketone bodies
Control	0.583 ± 0.03 (6)	0.258 ± 0.03 (6)	-0.120 ± 0.08 (6)	0.056 ± 0.02 (6)	0.133 ± 0.03 (6)	0.348 ± 0.06 (6)	-0.017 ± 0.01 (6)
Diabetic (7 days)	0.643 ± 0.08 (6)	-	$-0.075 \pm 0.03^*$ (6)	0.044 ± 0.03 (6)	$0.082 \pm 0.01^*$ (6)	$0.186 \pm 0.02^{***}$ (6)	-
Diabetic (21 days)	$0.422 \pm 0.08^{***}$ (6)	$1.057 \pm 0.14^{***}$ (4)	$-0.033 \pm 0.01^*$ (6)	$0.029 \pm 0.01^{**}$ (6)	$0.059 \pm 0.01^{***}$ (6)	$0.134 \pm 0.02^{***}$ (6)	$-0.045 \pm 0.02^{***}$ (6)
Diabetic (42 days)	$0.327 \pm 0.07^{***}$ (6)	$5.522 \pm 0.48^{***}$ (5)	$-0.019 \pm 0.01^{***}$ (6)	$0.021 \pm 0.01^{***}$ (6)	$0.029 \pm 0.02^{***}$ (6)	$0.047 \pm 0.02^{***}$ (6)	$-0.170 \pm 0.08^{***}$ (5)

metric measurements were performed in a Gilford recording spectrophotometer (model 260) at 25 °C, except for glutaminase, which was determined at 37 °C. For all enzymes studied, preliminary experiments established that extraction and assay procedures were such as to provide maximum activities (see Crabtree *et al.*, 1979).

Determination of metabolites and plasma insulin

Glutamine and other metabolites in neutralized extracts were determined by the same methods as described previously (Ardawi & Newsholme, 1985a; Ardawi, 1987a). Plasma insulin was measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA, U.S.A.). Protein was determined by the procedure of Lowry *et al.* (1951).

Expression of results

Blood-flow values were expressed as ml/min per g of small intestine, and substrate or metabolite exchange rates by the gut were expressed as nmol/min per g of small intestine. Changes in concentrations of substrates or metabolites during the incubation were determined from the net change between zero time and 30 or 20 min incubation for colonocytes or enterocytes respectively, and such changes were linear with time over the time intervals used. Rates of substrate utilization or metabolite production are expressed as μ mol/min per g dry wt. of cells. All maximal enzyme activities are expressed as nmol/min per mg of protein. The results are expressed as means $\pm$ S.D., and comparisons between sets of data were made by Student's *t* test.

RESULTS

The body-weight gain of diabetic rats was markedly less than that of age-matched controls (Table 1). Food consumption increased by approx. 28–80 % in diabetic rats (Table 1), which correlated well with the duration of the diabetic state. Diabetes resulted in an increase in the weight of both the small intestine and the colon, confirming previous work (Watford *et al.*, 1984, 1987; Ardawi, 1987a); however, the percentage increase in the size of the colon decreased with increasing duration of the diabetic state. This is indicated by the constant colon-weight/body-weight ratio during short or prolonged periods of diabetes (Ardawi, 1987a). The plasma concentrations of glucose in diabetic animals were significantly higher than that found in corresponding controls ($P < 0.001$). Plasma glucose concentrations in diabetic rats of 42 days duration were significantly higher than those in rats with short-term diabetes (i.e. 7 days) ($P < 0.05$). Plasma insulin concentrations in diabetic rats were markedly lower than those of control rats (Table 1). The concentrations of total ketone bodies (acetoacetate plus 3-hydroxybutyrate) increased with increasing duration of the diabetic state (Table 1).

Arteriovenous-difference measurements and fluxes for ketone-body and glutamine metabolism

The arterial concentration of total ketone bodies was increased by 3- and 20-fold in 21- and 42-day-diabetic rats respectively (Table 2). Arteriovenous-difference measurements of total ketone bodies across the gut of control, short- and long-term diabetic rats are presented in Table 2. Control rats exhibited low net total ketone-body uptake from the circulation, confirming previous

Table 3. Blood-flow measurements and gut exchange rates of ketone bodies, glutamine, glutamate, alanine and ammonia in control and streptozotocin-diabetic rats

Measurements and calculations were done as described in the Materials and methods section; results are presented as means $\pm$ S.D., with the numbers of animals used given in parentheses. Blood-flow values are expressed as ml/min per g of small intestine, and exchange rates of substrates and metabolites are expressed as nmol/min per g of small intestine. Negative values indicate uptake. Ketone bodies represent the sum of acetoacetate and 3-hydroxybutyrate concentrations. Significant differences from control rats are indicated by * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Treatment group	Gut blood flow (ml/min per g of small intestine)	Gut exchange rates (nmol/min per g of small intestine)				
		Ketone bodies	Glutamine	Glutamate	Alanine	Ammonia
Control	0.387 $\pm$ 0.058 (6)	-7 $\pm$ 3 (6)	-49 $\pm$ 10 (6)	22 $\pm$ 9 (6)	52 $\pm$ 15 (6)	137 $\pm$ 38 (6)
Diabetic (7 days)	0.373 $\pm$ 0.029 (6)	-	-27 $\pm$ 11 (6)*	15 $\pm$ 11 (6)	30 $\pm$ 5 (6)**	69 $\pm$ 7 (6)***
Diabetic (21 days)	0.357 $\pm$ 0.021 (6)	-55 $\pm$ 18 (4)***	-12 $\pm$ 4 (6)***	10 $\pm$ 4 (6)*	21 $\pm$ 5 (6)**	47 $\pm$ 7 (6)***
Diabetic (42 days)	0.357 $\pm$ 0.011 (6)	-60 $\pm$ 29 (5)***	-7 $\pm$ 4 (6)***	7 $\pm$ 4 (6)**	10 $\pm$ 5 (6)***	16 $\pm$ 9 (6)***

work (for review, see Windmueller, 1984), whereas in 21- and 42-day-diabetic rats total ketone-body uptake was increased by 7.9- and 8.6-fold respectively (Table 2).

Arterial glutamine concentration was decreased by 28% and 44% in 21- and 42-day-diabetic rats ($P < 0.001$) respectively, with no apparent change in 7-day-diabetic animals (Table 2). These results confirm previous findings (see Watford *et al.*, 1984; Ardawi, 1987a). Control rats, however, exhibited a net glutamine removal from the circulation, which was accompanied by the production of glutamate, alanine and ammonia. Net glutamine removal from the circulation was diminished by 45% ($P < 0.01$), 76% ($P < 0.01$) and 86% ($P < 0.001$) in 7-, 21- and 42-day-diabetic rats respectively, which was associated with decreased rates of release of glutamate, alanine and ammonia (Table 2).

Portal-drained viscera blood flow was approximately unchanged in diabetic rats when expressed per g of small intestine (Table 3). However, it increased by approx. 14–37% when expressed per 100 g body wt. [control values being 2.98 ± 0.34 ml/min per 100 g body wt.], consistent with other work (Korthuis *et al.*, 1986). Calculation of substrate-exchange rates from measured arteriovenous concentration differences and blood flow across the gut demonstrated several significant alterations in net gut glutamine and ketone-body fluxes in diabetic animals (Table 3). These findings are consistent with increased and decreased ketone-body and glutamine metabolism by gut mucosa of diabetic rats respectively.

Ketone-body and glutamine metabolism by isolated colonocytes and enterocytes

Acetoacetate and 3-hydroxybutyrate were utilized at similar rates by colonocytes isolated from control or diabetic rats (Table 4). Some acetoacetate was converted into 3-hydroxybutyrate, or vice versa. The rates of ketone-body utilization by colonocytes of diabetic rats were increased by about 34% ($P < 0.005$) or 30% ($P < 0.001$) when acetoacetate or 3-hydroxybutyrate was the substrate respectively (Table 4). The addition of glutamine increased the utilization of acetoacetate and production of 3-hydroxybutyrate in colonocytes isolated from control or diabetic rats (Table 4). This is an unexpected finding, and no explanation can be provided at present, but it is possible that glutamine and/or one of its metabolites may activate one or several steps in the

pathway of ketone-body utilization. Similarly, enterocytes metabolized ketone bodies, but at higher rates than that of colonocytes of the same animals (Table 5), and there were no apparent differences in the rates of acetoacetate utilization or 3-hydroxybutyrate formation in cells isolated of 7- or 42-day-diabetic rats (Table 5). These findings are consistent with those in other work (Watford *et al.*, 1987).

The rate of glutamine utilization by incubated colonocytes was $5.65 \mu\text{mol/min per g dry wt.}$, when measured in cells isolated from control rats. Glutamate, alanine and ammonia were the major nitrogenous end products (Table 4), confirming previous work (Ardawi & News-holme, 1985a; Ardawi, 1987a,b). However, other amino acids (e.g. citrulline, proline, serine and aspartate) are known to be formed from glutamine metabolized by the gut (see Windmueller, 1984), and were not measured in the present work. Diabetes, however, caused a decrease in the rates of glutamine metabolism (Table 4). In colonocytes from control or diabetic rats, the rates of glutamine utilization and production of its metabolites were markedly decreased by the addition of acetoacetate (Table 4). Similar changes were obtained in isolated enterocytes (Table 5).

Activities of glutaminase and ketone-body-utilizing enzymes

The maximal activities of glutaminase and ketone-body-utilizing enzymes in rat colonic mucosa (measured in scrapings or whole colon) are presented in Table 6. A marked decrease (33–48%) in the activity of glutaminase was observed in 7-day-diabetic rats when measured in colonic scrapings or whole colon. These findings are consistent with previous work (Ardawi, 1987a), but contrast with those found for glutaminase activity in the small intestine (see Watford *et al.*, 1984, 1987; Ardawi, 1987a).

There was no apparent change in the maximal activities of 3-oxoacid CoA-transferase, acetoacetyl-CoA thiolase and 3-hydroxybutyrate dehydrogenase in colonic tissue preparations of diabetic rats (Table 6). These findings are consistent with the response of ketone-body-utilizing enzymes to diet, starvation or diabetes (Robinson & Williamson, 1980; Hanson & Carrington, 1981; Watford *et al.*, 1987).

Table 4. Glutamine and ketone-body metabolism by isolated colonocytes of control and streptozotocin-diabetic rats

Colonocytes isolated from control, 7- or 42-day diabetic rats were incubated as described in the Materials and methods section in the presence of various substrates as indicated. Rates are given as means $\pm$ S.D., with (n) the number of separate experiments. A negative sign indicates utilization. Significant differences from control rats are indicated by ^a($P < 0.05$), ^b($P < 0.01$), ^c($P < 0.005$), ^d($P < 0.001$). Values significantly different from those in the presence of glutamine only are indicated by ^e($P < 0.01$). Values significantly different from those in the presence of acetoacetate only are indicated by ^f($P < 0.01$).

Additions to incubations	Animals	n	Rates of utilization or production (μ mol/min per g dry wt.)						
			Glutamine	Glutamate	Alanine	Ammonia	Acetoacetate	3-Hydroxybutyrate	Total ketone bodies
Glutamine (5 mM)	Control	7	-5.65 ± 0.79	3.59 ± 0.55	0.67 ± 0.11	3.90 ± 0.33	-	-	-
	Diabetic (7 days)	7	-4.85 ± 0.18^b	2.79 ± 0.15^c	0.53 ± 0.04^d	3.18 ± 0.09^d	-	-	-
	Diabetic (42 days)	7	-3.43 ± 0.52^d	2.19 ± 0.35^d	0.41 ± 0.06^d	2.40 ± 0.38^d	-	-	-
Acetoacetate (5 mM)	Control	6	-	-	-	-	-2.23 ± 0.24	0.27 ± 0.05	-1.96 ± 0.26
	Diabetic (7 days)	5	-	-	-	-	-2.99 ± 0.44^e	0.37 ± 0.064^b	-2.63 ± 0.42^b
	Diabetic (42 days)	6	-	-	-	-	-3.04 ± 0.50^e	0.35 ± 0.06^e	-2.69 ± 0.48^b
3-Hydroxybutyrate (5 mM)	Control	5	-	-	-	-	0.42 ± 0.09	-2.27 ± 0.25	-1.85 ± 0.25
	Diabetic (7 days)	4	-	-	-	-	0.75 ± 0.28^e	-3.14 ± 0.17^d	-2.39 ± 0.42^e
	Diabetic (42 days)	6	-	-	-	-	0.81 ± 0.19^e	-3.23 ± 0.30^d	-2.42 ± 0.32^b
Glutamine (5 mM) plus acetoacetate (5 mM)	Control	5	-3.79 ± 0.50^f	2.55 ± 0.48^e	0.46 ± 0.09^e	2.89 ± 0.52^e	-2.94 ± 0.27^f	0.37 ± 0.08^f	-2.57 ± 0.28^f
	Diabetic (7 days)	5	-2.96 ± 0.82^e	2.04 ± 0.57^e	0.37 ± 0.10^e	2.25 ± 0.58^e	-3.96 ± 0.69^{df}	0.60 ± 0.16^{df}	-3.36 ± 0.62^{df}
	Diabetic (42 days)	5	-2.39 ± 0.41^{de}	1.53 ± 0.26^{de}	0.29 ± 0.06^e	1.64 ± 0.25^{de}	-4.83 ± 0.91^{df}	0.56 ± 0.13^{df}	-4.27 ± 0.85^{df}

Table 5. Glutamine and ketone-body metabolism by isolated enterocytes of control and streptozotocin-diabetic rats

Enterocytes isolated from control, 7- or 42-day diabetic rats were incubated as described in the Materials and methods section in the presence of various substrates as indicated. Rates are given as means $\pm$ s.d., with (*n*) the number of separate experiments. A negative sign indicates utilization. Significant differences from control rats are indicated by ^a(*P* < 0.05) or ^b(*P* < 0.001). Values significantly different from those in the presence of glutamine only are indicated by ^c(*P* < 0.001). Values significantly different from those in the presence of acetoacetate only are indicated by ^a(*P* < 0.01).

Additions to incubations	Animals	<i>n</i>	Rates of utilization or production (μ mol/min per g dry wt.)					
			Glutamine	Glutamate	Alanine	Ammonia	Acetoacetate	3-Hydroxy-butyrate
Glutamine (5 mM)	Control	5	-12.18 $\pm$ 1.04	3.53 $\pm$ 0.42	2.43 $\pm$ 0.25	9.21 $\pm$ 1.52	-	-
	Diabetic (7 days)	4	-11.41 $\pm$ 1.46	3.22 $\pm$ 0.13	2.57 $\pm$ 0.34	9.56 $\pm$ 1.50	-	-
	Diabetic (42 days)	5	-9.17 $\pm$ 0.72 ^a	2.77 $\pm$ 0.10 ^a	2.31 $\pm$ 0.20	6.76 $\pm$ 0.41 ^b	-	-
Acetoacetate (5 mM)	Control	5	-	-	-	-	-3.67 $\pm$ 0.24	0.47 $\pm$ 0.08
	Diabetic (7 days)	5	-	-	-	-	-3.80 $\pm$ 0.25	0.57 $\pm$ 0.14
	Diabetic (42 days)	4	-	-	-	-	-3.65 $\pm$ 0.45	0.54 $\pm$ 0.17
Glutamine (5 mM) plus acetoacetate (5 mM)	Control	4	-8.16 $\pm$ 0.75 ^c	2.17 $\pm$ 0.23 ^c	1.59 $\pm$ 0.14 ^c	5.53 $\pm$ 0.36 ^c	-4.67 $\pm$ 0.31 ^d	0.60 $\pm$ 0.03
	Diabetic (7 days)	5	-5.58 $\pm$ 0.53 ^{bc}	1.47 $\pm$ 0.11 ^{bc}	1.06 $\pm$ 0.11 ^{bc}	4.23 $\pm$ 0.27 ^{bc}	-5.15 $\pm$ 0.14 ^{bd}	0.60 $\pm$ 0.20
	Diabetic (42 days)	5	-5.48 $\pm$ 0.67 ^{bc}	1.36 $\pm$ 0.17 ^{bc}	1.04 $\pm$ 0.28 ^{bc}	4.25 $\pm$ 0.23 ^{bc}	-5.17 $\pm$ 0.33 ^{bd}	0.48 $\pm$ 0.11
								Total ketone bodies
								-3.21 $\pm$ 0.23
								-3.01 $\pm$ 0.30
								-3.10 $\pm$ 0.32
								-4.07 $\pm$ 0.33
								-4.56 $\pm$ 0.27 ^{ad}
								-4.68 $\pm$ 0.28 ^{ad}

Table 6. Maximal activities of glutaminase and ketone-body-utilizing enzymes in colonic mucosal scrapings and whole colon of control and streptozotocin-diabetic rats

Rat treatments and tissue extractions were carried out as described in the Materials and methods section. Activities were measured at 25 °C, except for glutaminase, which was measured at 37 °C. Results are presented as means $\pm$ S.D., with the numbers of rats given in parentheses. Significant differences from control rats are indicated by * ($P < 0.01$), ** ($P < 0.001$).

Tissue preparation	Animals	Enzyme activities (nmol/min per mg of protein)			
		Glutaminase	3-Oxoacid CoA-transferase	Acetoacetyl-CoA thiolase	3-Hydroxybutyrate dehydrogenase
Colonic mucosal scrapings	Control	35.4 $\pm$ 6.46 (7)	72.01 $\pm$ 6.16 (5)	52.20 $\pm$ 5.63 (5)	14.01 $\pm$ 3.16 (5)
	Diabetic (7 days)	18.25 $\pm$ 4.62*	69.50 $\pm$ 8.06 (4)	55.80 $\pm$ 5.12 (4)	15.30 $\pm$ 3.30 (4)
	Diabetic (42 days)	35.65 $\pm$ 2.73 (7)	73.00 $\pm$ 8.81 (6)	56.70 $\pm$ 8.04 (6)	19.20 $\pm$ 3.49 (6)
Whole colon	Control	17.99 $\pm$ 2.60 (7)	33.80 $\pm$ 6.98 (5)	22.04 $\pm$ 2.96 (5)	4.90 $\pm$ 0.48 (5)
	Diabetic (7 days)	12.01 $\pm$ 1.79** (7)	32.51 $\pm$ 3.27 (4)	23.41 $\pm$ 1.54 (4)	4.50 $\pm$ 0.84 (4)
	Diabetic (42 days)	19.92 $\pm$ 0.82 (7)	34.05 $\pm$ 4.61 (4)	—	6.77 $\pm$ 1.48 (6)

DISCUSSION

The results of the present work have demonstrated that the gut of short- and long-term diabetic rats exhibited marked increases and decreases in the rates of ketone-body and glutamine metabolism respectively, as compared with control animals.

These observations are consistent with the findings of Watford *et al.* (1987), which showed enhanced ketone-body metabolism by the small intestine *in vivo* but without any change in incubated enterocytes of 40-day diabetic rats. Moreover, the findings reported in other work (Brosnan *et al.*, 1983; Watford *et al.*, 1984; Ardawi, 1987a), that the intestinal capacity to metabolize glutamine is suppressed in diabetic rats, has been confirmed in the present work, and was associated with a diminished capacity to metabolize glutamine by both colonic and intestinal mucosa (Tables 4 and 5). However, the present results contrast with other reports of little change in (Felig *et al.*, 1973; Schrock & Goldstein, 1981) or complete cessation of (Brosnan *et al.*, 1983; Watford *et al.*, 1987) glutamine metabolism *in vivo* by the small intestine of diabetic animals (see Table 3).

Uncontrolled diabetes is associated with acidosis (see Newsholme & Leech, 1983). This raises the question of whether the adaptive changes in glutamine metabolism that occur in response to diabetes are partly due to the acidosis associated with the diabetic state. In 7-day-acidotic rats glutaminase activity was decreased, whereas that of 7-day-diabetic rats exhibited an increase (Watford *et al.*, 1984; Ardawi, 1987a). Moreover, in chronic NH_4Cl - and HCl -induced acidosis, no significant changes in glutamine metabolism could be detected in the non-hepatic splanchnic bed (Schrock & Goldstein, 1981; Welbourne *et al.*, 1986), which contrasts with that found in chronic diabetic rats (Tables 2 and 3). Therefore it is suggested that the adaptive changes in glutamine metabolism by the gut of diabetic rats could not be due to the acidosis associated with diabetes; further work is needed to confirm this suggestion.

The major differences between the results of the present work and those of Watford *et al.* (1987) mainly concern the quantitative assessment of glutamine and ketone-body extraction and metabolism by the gut of diabetic rats at various durations of diabetes (7, 21 and 42 days). Moreover, blood-flow measurements were included, and the extent of glutamine and ketone-body metabolism by the colonic mucosa were also studied.

The use of arteriovenous-difference measurements to estimate glutamine and ketone-body metabolism by the small intestine in previous work (Watford *et al.*, 1984, 1987; Ardawi, 1987a) may be inaccurate, since blood-flow rates were not determined. In the present work the increase in blood flow in diabetic rats appears to be a consequence of increased organ weight (possibly owing to increased food intake), and blood flow is approximately unchanged if expressed as ml/min per g of small intestine. Therefore, it is misleading to express blood flow as ml/min per 100 g body wt., when there is substantial hypertrophy of the organ concerned, and therefore the results of the present work were not simply due to changes in blood flow. The latter confirms previous work (Watford *et al.*, 1987; Ardawi, 1987a). Furthermore, intraluminal substrates cannot be ruled out as contributing to the changes in arteriovenous-difference measurements obtained in the present work, as all animals were used in the fed state. However, diminished release of alanine and ammonia into the portal circulation in diabetic rats (Table 2) suggests that luminal glutamine was not replacing that normally obtained from the circulation.

The shift in oxidative fuel utilization by the gut in diabetic animals could be attributed to three factors. (1) The low arterial glutamine concentration resulting in substrate unavailability, owing to either increased utilization by other tissues and/or decreased release from skeletal muscle. Indeed, it has been shown that, in 7- or 42-day-diabetic rats, hepatic and renal glutamine removal from the circulation was markedly increased (Brosnan *et al.*, 1983; Ardawi, 1987a), which was accompanied by

an enhanced release from skeletal muscle (see Ardawi, 1987a). Moreover, infusions of glutamine in 40-day-diabetic rats resulted in an enhanced uptake of glutamine across the portal-drained viscera (see Watford *et al.*, 1987). (2) Increased availability of other fuels (e.g. glucose, fatty acids, other amino acids and ketone bodies) to the gut, thus sparing glutamine for other tissues (e.g. liver and kidney). This was indicated by increased ketone-body uptake and metabolism by gut mucosa of diabetic rats both *in vivo* and *in vitro* (Tables 4 and 5), which could partially replace glutamine as an important fuel for these cells (see Watford *et al.*, 1987). These observations are consistent with the known absolute requirement of rapidly dividing cells for glutamine (for review see McKeehan, 1982; Ardawi & Newsholme, 1985b). (3) An inactivation of glutaminase protein (a key enzyme in the metabolism of glutamine) by ketone bodies (Nagy & Kretchmer, 1986) or fatty acids (Kvamme, 1982), which are known to be increased in uncontrolled streptozotocin-induced diabetes. In addition, differential inactivation of the putative glutamine/alanine exchanger in enterocytes (Bradford & McGivan, 1982) and colonocytes (Ardawi, 1986) may play a role to decrease glutamine uptake by these cells in diabetic rats.

It is concluded therefore that, although the epithelial cells of the small intestine and colon during streptozotocin-induced diabetes exhibit decreased rates of metabolism of glutamine, such decreases were partially compensated for by an increase in ketone-body utilization by the gut of diabetic animals (Table 3). These findings emphasize the importance of glutamine for the epithelial cells of the gut, which utilize the amino acid at a very high rate under normal conditions, not only as a major source of energy, but also for the use in the very 'active' biosynthetic pathways (e.g. nucleic acid synthesis) (see Ardawi & Newsholme, 1985b). However, when the role of glutamine as a major source of energy is diminished, as seen in chronic diabetes, the epithelial cells of the small intestine still utilize glutamine, but at a much lower rate, presumably not as a major source of energy, but to be used in the very 'active' biosynthetic pathways. The latter would represent only a small percentage of the rates of glutamine utilization by these cells (approx. 5–10% of normal rates of utilization) (see Ardawi & Newsholme, 1985b; Newsholme *et al.*, 1985a,b) which was made available by switching over to other fuels for the supply of energy (e.g. ketone bodies and fatty acids).

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REFERENCES

Ardawi, M. S. M. (1986) *Biochem. J.* **238**, 131–135

- Ardawi, M. S. M. (1987a) *Diabetologia* **30**, 109–114
 Ardawi, M. S. M. (1987b) *Biochem. J.* **242**, 75–80
 Ardawi, M. S. M. & Newsholme, E. A. (1982) *Biochem. J.* **208**, 743–748
 Ardawi, M. S. M. & Newsholme, E. A. (1985a) *Biochem. J.* **231**, 713–719
 Ardawi, M. S. M. & Newsholme, E. A. (1985b) *Essays Biochem.* **21**, 1–44
 Bradford, N. M. & McGivan, J. D. (1982) *Biochim. Biophys. Acta* **689**, 55–62
 Brosnan, J. T., Man, K. C., Hall, D. E., Colbourne, S. A. & Brosnan, M. E. (1983) *Am. J. Physiol.* **244**, E151–E158
 Brun, C. A. (1951) *J. Lab. Clin. Med.* **37**, 955–958
 Crabtree, B., Leech, A. R. & Newsholme, E. A. (1979) in *Techniques in Life Sciences*, vol. B211 (Pogson, C., ed.), pp. 1–37, Elsevier/North-Holland, Amsterdam
 Curthoys, N. P. & Lowry, O. H. (1973) *J. Biol. Chem.* **248**, 162–168
 Felig, P., Wahren, J., Karl, I., Cerasi, E., Luft, R. & Kipnis, D. M. (1973) *Diabetes* **22**, 573–576
 Hanson, P. J. & Carrington, J. M. (1981) *Biochem. J.* **200**, 349–355
 Katz, M. L. & Bergman, E. N. (1969) *Am. J. Physiol.* **216**, 946–952
 Korthuis, R. J., Benoit, J. N., Kviety, P. R., Taylor, A. R. & Granger, D. N. (1986) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **45**, 416 (abstr. 1522)
 Kovacevic, A. & McGivan, J. D. (1983) *Physiol. Rev.* **63**, 547–604
 Kvamme, E. (1982) in *Renal Ammonia Metabolism* (Tannen, R. L., Goldstein, L., Lemieux, G., Simpson, D. & Vinay, R., eds.), pp. 60–70, Karger, Basel
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
 McKeehan, W. L. (1982) *Cell Biol. Int. Rep.* **6**, 635–647
 Nagy, L. E. & Kretchmer, N. (1986) *Arch. Biochem. Biophys.* **248**, 80–88
 Newsholme, E. A. & Leech, A. R. (1983) *Biochemistry for the Medical Sciences*, pp. 531–533, John Wiley and Sons, Chichester
 Newsholme, E. A., Crabtree, B. & Ardawi, M. S. M. (1985a) *Biosci. Rep.* **5**, 393–400
 Newsholme, E. A., Crabtree, B. & Ardawi, M. S. M. (1985b) *Q. J. Exp. Physiol.* **70**, 473–489
 Robinson, A. M. & Williamson, D. H. (1980) *Physiol. Rev.* **60**, 143–187
 Roediger, W. E. W. (1982) *Gastroenterology* **83**, 424–429
 Schrock, H. & Goldstein, L. (1981) *Am. J. Physiol.* **240**, 5500–5506
 Watford, M., Lund, P. & Krebs, H. A. (1979) *Biochem. J.* **178**, 589–596
 Watford, M., Smith, E. M. & Erbeling, E. J. (1984) *Biochem. J.* **224**, 207–214
 Watford, M., Erbeling, E. J. & Smith, E. M. (1987) *Biochem. J.* **242**, 61–68
 Welbourne, T. C., Childress, D. & Givens, G. (1986) *Am. J. Physiol.* **251**, R858–R866
 Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) *Biochem. J.* **121**, 41–47
 Windmueller, H. G. (1984) in *Glutamine Metabolism in Mammalian Tissues* (Haussinger, E. & Sies, H., eds.), pp. 61–77, Springer-Verlag, Berlin, Heidelberg and New York

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