Biochem. J. (1985) 231, 713–719 (Printed in Great Britain)

Fuel utilization in colonocytes of the rat

M. Salleh M. ARDAWI*† and ERIC A. NEWSHOLME‡

*Department of Biochemistry, College of Medicine and Allied Sciences, P.O. Box 9029, King Abdulaziz University, Jeddah, Saudi Arabia 21413, and ‡Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

1. In incubated colonocytes isolated from rat colons, the rates of utilization O_2, glucose or glutamine were linear with respect to time for over 30 min, and the concentrations of adenine nucleotides plus the ATP/ADP or ATP/AMP concentration ratios remained approximately constant for 30 min. 2. Glutamine, n-butyrate or ketone bodies were the only substrates that caused increases in O_2 consumption by isolated incubated colonocytes. 3. The maximum activity of hexokinase in colonic mucosa is similar to that of 6-phosphofructokinase. Starvation of the donor animal decreased the activities of hexokinase and 6-phosphofructokinase, whereas it increased those of glucose-6-phosphatase and fructose-bisphosphatase. 4. Isolated incubated colonocytes utilized glucose at about 6.8 μmol/min per g dry wt., with lactate accounting for 83% of glucose removed. These rates were not affected by the addition of glutamine, acetoacetate or n-butyrate, and starvation of the donor animal. 5. Isolated incubated colonocytes utilized glutamine at about 5.5 μmol/min per g dry wt., which is about 21% of the maximum activity of glutaminase. The major end-products of glutamine metabolism were glutamate, aspartate, alanine and ammonia. Starvation of the donor animal decreased the rate of glutamine utilization by colonocytes, which is accompanied by a decrease in glutamate formation and in the maximum activity of glutaminase. 6. Isolated incubated coloncytes utilized acetoacetate at about 3.5 μmol/min per g dry wt. This rate was not markedly affected by addition of glucose or by starvation of the donor animal. 7. When colonocytes were incubated with n-butyrate, both acetoacetate and 3-hydroxybutyrate were formed, with the latter accounting for only about 19% of total ketones produced.

INTRODUCTION

Most of the energy required by the epithelial cells of the small intestine is provided by the oxidation of glucose and glutamine in the fed state and of glutamine and ketone bodies in the starved state (for review, see Windmueller, 1980, 1984). However, the oxidation of fatty acids did not contribute significantly to energy formation even in the starved state (Hanson & Parsons, 1977, 1978; Windmueller & Spaeth, 1978, 1980; Watford *et al.*, 1979; Windmueller, 1984). In a similar manner to other rapidly dividing cells (e.g. lymphocytes, thymocytes, tumour cells), these epithelial cells are also characterized by a high rate of glycolysis in either the fed or the starved state (Watford *et al.*, 1979; Hume *et al.*, 1978; Ardawi & Newsholme, 1983*a*). Experiments with incubated lymphocytes suggested that most of the energy requirements in either the fed or the starved state were provided from the utilization of fatty acids, glutamine and glucose, whereas ketone bodies appeared to be much less important (Ardawi & Newsholme, 1982, 1983*a*,*b*, 1984*a*,*b*, 1985). Experiments with incubated colonocytes suggests that most of the energy requirements were provided from the oxidation of fatty acids, ketone bodies and/or glutamine (Roediger, 1982). However, the pathway for the utilization and the metabolic interrelationship between these fuels in colonocytes have not been studied. Hence, to provide further information about metabolism of these fuels in colonocytes, the maximum catalytic activities of key enzymes in the pathways of glucose, glutamine and ketone-body metabolism were measured in colonic mucosa in a similar manner to that done for lymphoid tissues (Ardawi & Newsholme, 1982). The rates of utilization and the metabolic fate of glucose, glutamine, acetoacetate and n-butyrate in incubated colonocytes have been investigated by measurement of the intermediates that accumulate on incubation of these cells with the various fuels. In addition, the effect of starvation of the donor animals on the metabolism of these fuels, together with the effect on the maximal activities of key enzymes, have also been investigated. The main object of the present work was to provide more information on the metabolism of fuels by the epithelial cells of the colon isolated from fed or starved rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (200–230 g) were obtained either from Batin and Kingman, Grimston, Hull HU11 4QE, U.K., or from King Fahd Medical Research Center (KFMRC), College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were maintained on a standard diet and water *ad libitum*.

Chemicals and enzymes

All chemicals and enzymes were obtained from Boehringer Corp. (London), London W5 2TZ, U.K., except for the following: 2-mercaptoethanol, 5,5′-dithobis-(2-nitrobenzoic acid), L-alanine, L-glutamine, L-aspartate, iodoacetamide, D-glucose and glycine were obtained from Sigma Chemical Co., Poole, Dorset, U.K.;

† To whom reprint requests should be addressed.

the scintillant and all inorganic reagents were obtained from Fisons, Loughborough, Leics. LE11 0RG, U.K.; Tes was obtained from Hopkin and Williams, Chadwell Heath, Essex, U.K.; bovine serum albumin was obtained from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. The serum was dialysed against Ca^{2+}-free phosphate-buffered saline as described by Krebs *et al.* (1974). Radiochemical compounds were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of colonocytes

Colonocytes were isolated by using a method similar to that described by Roediger & Truelove (1979). Four different preparation media were used: (1) phosphate-buffered saline from which $CaCl_2$ was omitted (see Culvenor & Weidemann, 1976); (2) the same medium, to which 0.3% (w/v) dialysed bovine serum albumin was added; (3) medium (2), to which 5 mM-EDTA was added; (4) phosphate-buffered saline containing 5 mM-dithiothreitol and 2.5% bovine serum albumin. Animals were killed by cervical dislocation, and colons (from the caecum to the rectal ampulla) were rapidly removed and washed with 100%-O_2-saturated medium (1), and isolated colon was ligated and, by means of a plastic rod, was everted. To distend the colon, it was filled with 100%-O_2-saturated medium (2). The other end of the colon was then ligated, and everted distended colons were placed in a plastic flask containing 100%-O_2-saturated medium (3). Flasks were continuously gassed with 100% O_2 and incubated at 37 °C in a Grant-type shaker (at 50–60 oscillations/min) for 20 min. Colons were then rinsed in fresh medium (1), and stirred vigorously for 2 min to free the cells. The cells were then centrifuged for 3 min at 600 *g* and were washed twice with oxygenated medium (4) and finally resuspended in 5 vol. of 100%-O_2-saturated medium (4). This suspension contained approx. 4–8 mg dry wt. of tissue/2 ml.

Incubation procedures

Incubations were performed at 37 °C in 25 ml plastic flasks. Freshly prepared colonocytes (4–6 mg dry wt.) from fed or 48 h-starved rats were incubated in a total volume of 1 ml of incubation medium, which consisted of phosphate-buffered saline supplemented with 100% O_2, for 20 s and were shaken continuously (55–65 oscillations/min); incubations were initiated by the addition of substrate(s). Incubations were terminated by the addition of 200 μl of $HClO_4$ (25%, w/v) to the incubation flask and cooling the mixture to 0 °C. Precipitated protein was removed by centrifugation at 13500 *g* for 2 min, the supernatant was neutralized with KOH containing 0.5 M-triethanolamine, and the $KClO_4$ removed by centrifugation at 13500 *g* for 3 min.

Freeze-clamping

Animals were killed by cervical dislocation, and the colon was rapidly removed and washed by forcing 100%-O_2-saturated phosphate-buffered saline (pH 7.2) through the lumen, and was then cut longitudinally. Mucosal tissue was separated from the underlying muscle by scraping with a microscope slide and was then immediately frozen between two aluminium plates pre-cooled in liquid N_2 (Wollenberger *et al.*, 1960) and stored under liquid N_2 until required for extraction. The frozen colonic mucosa was powdered in a mortar and pestle under liquid N_2 and was then homogenized in 0.7 M-$HClO_4$ in a Polytron homogenizer [the ratio of tissue to $HClO_4$ was 1:2 (w/v)]. The precipitate was removed by centrifugation at 13500 *g* for 4 min, and the supernatant was neutralized with 3.5 M-K_2CO_3 containing 0.5 M-triethanolamine. Precipitated $KClO_4$ was removed by centrifugation at 13500 *g* for 5 min. The supernatant was used for adenine nucleotide assays.

Assays of metabolites

Metabolites in neutralized extracts of cells plus medium were determined spectrophotometrically (with a Beckman DU-6 recording spectrophotometer) by enzymic methods: glucose by the coupled hexokinase/glucose-6-phosphate dehydrogenase method as described by Bergmeyer *et al.* (1974*a*); glutamine and ammonia by the method of Windmueller & Spaeth (1974); acetoacetate by the method of Mellanby & Williamson (1974); D-3-hydroxybutyrate by the method of Bernt & Bergmeyer (1974); lactate by the method of Gawehn & Bergmeyer (1974); aspartate by the method of Bergmeyer *et al.* (1974*b*); alanine by the method of Williamson (1974); pyruvate by the method of Czok & Lamprecht (1974); ADP and AMP by the method of Jaworek *et al.* (1974); and ATP by the method of Lamprecht & Trautschold (1974).

Measurement of O_2 consumption

O_2 consumption was measured polarographically with a Clark-type oxygen electrode attached to a Spectroplus-D spectrophotometer (MSE). Colonocytes (10–15 mg dry wt.) were incubated in 2.8 ml of incubation medium at 37 °C.

Preparation of homogenates

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 were 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 by using a Polytron homogenizer (PCU-2, at position 3) for 10–20 s at 0 °C. The whole homogenate was used for enzyme assays without further treatment, except for the following: for the assay of hexokinase, 6-phosphofructokinase and fructose-bisphosphatase, homogenates were centrifuged at 13500 *g* for 2 min, and the resultant supernatant was used for assay; for the assay of citrate synthase, oxoglutarate dehydrogenase, glutamate dehydrogenase, phosphate-dependent glutaminase (hereafter called 'glutaminase') and ketone-body-utilizing enzymes, mucosal preparations were treated immediately before assay with 0.05 (v/v) Triton X-100.

The extraction media for all enzymes studied were as described previously (see Ardawi & Newsholme, 1982), except for fructose-bisphosphatase, which was extracted in a medium as described by Budohoski *et al.* (1982).

Assay of enzyme activities

Enzyme activities were measured as described in the cited references: hexokinase (Crabtree & Newsholme, 1972); glucose-6-phosphatase (Lackner *et al.*, 1984); 6-phosphofructokinase (Opie & Newsholme, 1967); fructose-bisphosphatase (Crabtree *et al.*, 1972); pyruvate kinase (Zammit *et al.*, 1978); citrate synthase (Alp *et al.*, 1976); oxoglutarate dehydrogenase (Cooney *et al.*, 1981);

Table 1. Rates of utilization of glucose, glutamine and acetoacetate and production of lactate, pyruvate, glutamate, alanine, aspartate, ammonia and 3-hydroxybutyrate by isolated incubated rat colonocytes

Colonocytes isolated from fed rats were incubated as described in the Materials and methods section in the presence of substrate(s) as indicated. Rates are given as means ± S.E.M., with the numbers of separate experiments given in parentheses. A negative sign indicates utilization. Differences in rates between incubations with glucose and glucose plus glutamine or glucose plus acetoacetate or glucose plus n-butyrate for glucose utilization and lactate production, between incubations with glutamine and glucose plus glutamine for glutamine utilization, and for remainder of the rates of production of other metabolites that are statistically significant (Student's t test) are indicated by [a]($P < 0.05$), [b]($P < 0.001$), [c]($P < 0.005$) or [d]($P < 0.001$).

Additions to incubation	Rates of utilization or production (μmol/min per g dry wt.)										
	Oxygen	Glucose	Lactate	Pyruvate	Glutamine	Glutamate	Ammonia	Alanine	Aspartate	Aceto-acetate	3-Hydroxy-butyrate
None	−10.3 ± 2.37 (11)	−0.36 ± 0.21 (5)	0.95 ± 0.17 (5)	—	—	0.37 ± 0.17 (5)	1.74 ± 0.37 (7)	0.27 ± 0.10 (4)	0.24 ± 0.08 (5)	0.25 ± 0.09 (5)	—
Glucose (10 mM)	−13.1 ± 1.66 (12)	−6.88 ± 0.60 (7)	10.90 ± 1.19 (7)	0.48 ± 0.09 (5)	—	0.59 ± 0.16[a] (6)	1.24 ± 0.34[a] (6)	0.61 ± 0.17 (6)	0.45 ± 0.13 (5)	0.17 ± 0.07 (5)	0.09 ± 0.05 (5)
Glutamine (5 mM)	−14.0 ± 0.81 (12)	—	0.39 ± 0.12 (6)	0.06 ± 0.03 (5)	−5.51 ± 0.62 (10)	3.52 ± 0.38 (8)	4.15 ± 0.35 (9)	0.68 ± 0.09 (5)	0.83 ± 0.51 (7)	0.14 ± 0.06 (5)	0.03 ± 0.01 (3)
Acetoacetate (5 mM)	−14.1 ± 1.05 (9)	—	—	—	—	—	—	—	—	−2.48 ± 0.29 (8)	0.23 ± 0.05 (8)
n-Butyrate (10 mM)	−15.3 ± 1.10 (12)	—	—	—	—	—	—	—	—	2.58 ± 0.20 (7)	0.61 ± 0.18[c] (7)
Glucose (10 mM) plus glutamine 5 mM)	−14.4 ± 0.37 (7)	−5.07 ± 0.42[d] (9)	9.40 ± 0.88 (9)	—	−5.66 ± 0.55 (9)	2.74 ± 0.47[c] (9)	4.20 ± 0.47 (8)	0.97 ± 0.16[b] (6)	1.32 ± 0.23[a] (7)	0.14 ± 0.05 (5)	0.03 ± 0.01 (4)
Glucose (10 mM) plus acetoacetate (5 mM)	−15.5 ± 1.71 (8)	−6.20 ± 0.50[a] (6)	11.35 ± 0.57 (6)	—	—	—	—	—	—	−2.73 ± 0.34 (8)	0.50 ± 0.10[b] (6)
Glucose (10 mM) plus n-butyrate (10 mM)	−18.5 ± 1.70 (5)	−6.03 ± 0.31[a] (6)	11.60 ± 0.63 (6)	0.46 ± 0.11 (4)	—	—	1.14 ± 0.31 (6)	0.46 ± 0.15 (4)	0.24 ± 0.09 (4)	2.16 ± 0.29[b] (6)	1.57 ± 0.18[d] (4)

3-oxoacid CoA-transferase, acetoacetyl-CoA thiolase and D-3-hydroxybutyrate dehydrogenase (Williamson *et al.*, 1971); glutaminase (Curthoys & Lowry, 1973); glutamate dehydrogenase (Williamson *et al.*, 1967); alanine aminotransferase and aspartate aminotransferase (Sugden & Newsholme, 1975). The final volume of assay mixtures in all cases was 1.0 ml. All spectrophotometric measurements were performed in a Gilford recording spectrophotometer (model 240) at 25 °C, except for glucose-6-phosphatase and glutaminase, which were determined at 30 and 37 °C respectively. For all enzymes studied, preliminary experiments established that extraction and assay procedures were such as to provide maximum enzyme activities (see Crabtree *et al.*, 1979).

Expression of results

Changes in concentrations of substrates or metabolites during the incubation were determined from the net change between zero time and 30 min incubation. Rates of substrate utilization or metabolite production are expressed (unless otherwise indicated) as μmol/min per g dry wt. of cells. All maximal enzyme activities are expressed as μmol of substrate utilized/min per g dry wt. of tissue and as nmol of substrate utilized/min per mg of protein. Protein was determined by the procedure of Lowry *et al.* (1951).

RESULTS AND DISCUSSION

O_2 consumption

The rate of respiration by isolated colonocytes in the absence of added substrate was 10.3 μmol/min per g dry wt. (Table 1), which was linear with respect to time for only 30 min (results not shown). This rate is lower than that reported for rat colonocytes (Roediger & Truelove, 1979; Roediger, 1982), but higher than that reported for human colonocytes (Roediger & Truelove, 1979), rat lymphocytes (Ardawi & Newsholme, 1983*a*) or rat thymocytes (Hume *et al.*, 1978), and similar to that observed in rat enterocytes (Watford *et al.*, 1979; Porteous, 1980). Glutamine, n-butyrate or ketone bodies were the only substrates that increased the rate of O_2 consumption (Table 1). The respiratory stimulation by n-butyrate contrasts with that observed in isolated enterocytes (Watford *et al.*, 1979).

In muscle, the maximum activity of oxoglutarate dehydrogenase provides a reasonable quantitative index of the maximal capacity of the tricarboxylic acid cycle (Read *et al.*, 1977; Cooney *et al.*, 1981). The maximum capacity of the cycle in colonocytes, calculated from the rate of O_2 consumption with glucose as substrate, is approx. 4.1 μmol/min per g dry wt., or about 25% of the maximum activity of oxoglutarate dehydrogenase when calculated at 37 °C (Table 3). If the maximum activity of oxoglutarate dehydrogenase indicates the maximum capacity of the cycle in colonocytes, this finding suggests that the rate of the cycle could be markedly increased in other conditions, perhaps during proliferation (as suggested for lymphocytes; see Ardawi & Newsholme, 1982).

Adenine nucleotide concentrations

These were measured in colonic mucosa or isolated colonocytes as soon as possible after scraping or preparation and then at various times after incubation in the presence of normal incubation medium containing 10 mM-glucose. The concentrations of ATP and total nucleotides were similar in both scraped colonic mucosa and incubated colonocytes for 30 min (Table 2). The ATP/AMP concentration ratio decreased considerably after 30 min of incubation. This is in contrast with the situation found in rat enterocytes, in which a more rapid decrease in ATP/AMP concentration ratio was observed (Watford *et al.*, 1979), but similar to that in rat lymphocytes for the same period of incubation (see Ardawi & Newsholme, 1983*a*). These results, together with the fact that rates of O_2, glucose or glutamine utilization, and of lactate or glutamate production, were linear over 30 min (results not shown), indicate that isolated incubated rat colonocytes provide a satisfactory viable system for short-term metabolic studies (i.e. no more than 30 min).

Glucose metabolism

Maximal activities of some of the glycolytic enzymes in colonic mucosa are given in Table 3. The activity of hexokinase in colonic mucosa is similar to that reported

Table 2. Concentration of adenine nucleotides in freeze-clamped colonic scrapings and incubated colonocytes of the rat

Freeze-clamping of colonic scrapings, colonocytes incubations and analysis of adenine nucleotides were carried out as described in the Materials and methods section. Values are presented as means ± S.E.M. for eight separate experiments, except where shown in parentheses.

Sample	Incubation time (min)	Concn. (μmol/g dry wt.) ATP	ADP	AMP	Total adenine nucleotides	Ratio [ATP]/[AMP]
Freeze-clamped colonic scrapings	0	9.06 ± 1.42	2.23 ± 0.41	0.84 ± 0.11	12.12 ± 1.65	10.80
Incubated colonocytes	0	6.01 ± 0.81	2.55 ± 0.75	2.91 ± 1.13	11.46 ± 1.63	2.07
Incubated colonocytes plus 10 mM-glucose	20 (n = 12)	8.32 ± 1.39	1.90 ± 0.54	0.98 ± 0.32	11.21 ± 1.45	8.50
	30 (n = 9)	7.83 ± 0.61	1.82 ± 0.23	1.27 ± 0.22	10.92 ± 0.4	6.17
	40	6.94 ± 1.24	1.22 ± 0.40	2.32 ± 0.36	10.52 ± 1.42	3.00
	60	2.47 ± 0.61	2.64 ± 0.43	3.24 ± 0.90	8.36 ± 1.12	0.80

Table 3. Maximal activities of some key enzymes of carbohydrate, ketone-body and amino acid metabolism in mucosal cells of the colon of the rat

Mucosal scrapings of colons isolated from fed and 48 h-starved rats were extracted and enzymes assayed as described in the Materials and methods section. Activities were measured at 25 °C, except for glucose-6-phosphatase and glutaminase, which were measured at 30 °C and 37 °C respectively. Results are presented as means ± S.E.M. for six animals. The statistical significance (Student's *t* test) of the difference in activity between fed and starved animals is indicated by *($P < 0.01$), **($P < 0.005$), ***($P < 0.001$).

Enzyme	Enzyme activities			
	Fed		Starved	
	(μmol/min per g dry wt.)	(nmol/min per mg of protein)	(μmol/min per g dry wt.)	(nmol/min per mg of protein)
Hexokinase	23.62±4.56	29.70±6.15	16.35±3.48*	17.00±3.41***
Glucose-6-phosphatase	0.65±0.24	0.60±0.14	1.09±0.20**	0.78±0.07*
6-Phosphofructokinase	18.25±2.81	21.62±3.40	17.37±6.17	16.39±4.86*
Fructose-bisphosphatase	1.39±0.42	1.61±0.16	2.42±0.33*	2.32±0.33***
Pyruvate kinase	199±35	222±30	158±31	180±30
Citrate synthase	60±12	72±13	67±11	74±10
Oxoglutarate dehydrogenase	6.60±1.70	7.82±2.07	8.20±2.24	8.30±1.60
3-Oxoacid CoA-transferase	62±22	73±9	62±6	66±5
Acetoacetyl-CoA thiolase	40±8	50±9	39±7	43±6
3-Hydroxybutyrate dehydrogenase	11.72±1.26	14.00±1.71	9.90±1.70	10.88±1.18
Phosphate-dependent glutaminase	27.50±6.10	33.65±5.60	20.93±3.60*	23.22±2.53*
Glutamate dehydrogenase	54±5	63±4	57±5	61±4
Aspartate aminotransferase	124±14	144±15	135±20	141±13
Alanine aminotransferase	44±6	62±7	49±9	60±7

for intestinal mucosa (Hanson & Carrington, 1981; Budohoski *et al.*, 1982) and lymphocytes (Ardawi & Newsholme, 1982). The activity of 6-phosphofructokinase is similar to that of hexokinase, supporting the view that glucose is the major carbohydrate that is used by this tissue.

Colonocytes utilized glucose at a high rate (6.88 μmol/min per g dry wt.) (Table 1), which is higher than that reported by Roediger (1982) and is about 12% of the maximal activity of hexokinase (calculated at 37 °C) (Table 3). Thus hexokinase activity may not be indicative of the maximum capacity of glycolysis in colonocytes. This rate of glucose utilization is considerably higher than that reported for thymoctyes (Culvenor & Weidemann, 1976; Yassmeen *et al.*, 1977; Hume *et al.*, 1978; Brand *et al.*, 1984), lymphocytes (Ardawi & Newsholme, 1983*a*, 1984*a*), but about 50% lower than that reported for enterocytes (Watford *et al.*, 1979; Porteous, 1980). About 83% of the glucose removed was accounted for as lactate and small amounts of pyruvate. There were also small increments of glutamate, alanine and aspartate over the endogenous amounts (Table 1). The proportion of glucose converted into lactate was 93% in the presence of glutamine, 92% in the presence of acetoacetate and 96% in the presence of n-butyrate (Table 1). Thus it is likely that glucose oxidation in colonocytes accounts for a very small percentage of glucose utilization and does not replace the oxidation of endogenous fuels, which is similar to the findings with a variety of intestinal and colonic preparations (Windmueller & Spaeth, 1978; Hanson & Parsons, 1978; Watford *et al.*, 1979; Porteous, 1980). The addition of glucose plus glutamine to incubated coloncytes caused an increase in the yield of both aspartate and alanine, but a decrease in glutamate production. The rate of glucose removal was decreased by about 26%, whereas glutamine utilization was not affected. This is in contrast with the effect of glutamine on glucose utilization in either enterocytes or lymphocytes (Watford *et al.*, 1979; Ardawi & Newsholme, 1983*a*). However, the amount of glutamine utilized beyond the stage of glutamate was increased to 57% of the total glutamine removed in the presence of glucose. The production of ammonia from glutamine was not significantly affected by the addition of glucose (Table 1).

Starvation of the donor animal decreased the activities of hexokinase and 6-phosphofructokinase in colonic mucosa, whereas it increased those of glucose-6-phosphatase and fructose-bisphosphatase (Table 3), which is similar to the effect of starvation on intestinal mucosa (Anderson, 1974; Budohoski *et al.*, 1982) but in contrast with the effect on lymphocytes (Ardawi & Newsholme, 1982). Starvation of the rat is known to decrease by almost 50% the rate of glucose utilization by the small intestine (Hanson & Parsons, 1977); incubated coloncytes isolated from 48 h-starved rats exhibited a decrease in the rates of glucose utilization and lactate production (results not shown), which may be explained in part by decreases in activities of hexokinase and 6-phosphofructokinase and increases in those of glucose-6-phosphatase and fructose bisphosphatase (Table 3).

Glutamine metabolism

The rate of glutamine utilization by incubated rat colonocytes was 5.51 μmol/min per g dry wt. (Table 1), which is about 21% of the maximal activity of glutaminase (Table 3). Thus glutaminase activity may not be indicative of the maximum capacity for glutamine utilization in colonocytes. This rate of glutamine utilization is higher than that reported for kidney slices (Vinay *et al.*, 1980), lymphocytes (Ardawi & Newsholme,

1983*a*, 1984*a*) and thymocytes (Brand *et al.*, 1984), but lower than that for kidney tubules (Vinay *et al.*, 1980) and enterocytes (Watford *et al.*, 1984). The major end-products of glutamine metabolism in colonocytes are glutamate, aspartate, alanine and ammonia (Table 1). This is in contrast with the cells of the small intestine, which produce alanine rather than aspartate (Hanson & Parsons, 1977; Watford *et al.*, 1979, 1984; Porteous, 1980), and lymphocytes or thymocytes, in which aspartate production predominates (Ardawi & Newsholme, 1983*a*, 1984*a*; Brand *et al.*, 1984). Of the glutamine utilized by colonocytes, ammonia production accounted for 38% of the glutamine nitrogen, which, since the glutaminase reaction produces ammonia, suggests that glutamate is metabolized via transaminase reactions rather than by glutamate dehydrogenase. This is expected from the low activity of the latter enzyme in colonic mucosa (Table 3). Glutamate accounted for about 64% of the glutamine removed. For glutamine metabolized beyond the stage of glutamate, about 33% was accounted for as aspartate and 21% as alanine. This finding is expected from the maximum activities of aspartate aminotransferase and alanine aminotransferase in colonic mucosa (Table 3).

On the basis of end-products of metabolism, and maximal activities of some enzymes (Tables 1 and 3), it is considered that glutamine is utilized by colonocytes via a pathway which is similar to that proposed for the small intestine (Hanson & Parsons, 1980) or lymphocytes (Ardawi & Newsholme, 1984*a*, 1985). Assuming that glutamine carbon contributed only to the production of glutamate, aspartate and alanine, it was calculated that glutamine contributed about 32% to respiration (assuming that endogenous rates of glutamate, aspartate and alanine production were not affected by metabolism of glutamine).

Starvation of the donor rats for 48 h caused a decreased rate of glutamine utilization by isolated colonocytes, which is accompanied by a decrease in the rate of glutamate formation (results not shown). These effects are predicted by the effect of starvation on the maximal activity of glutaminase in colonic mucosa (Table 3), and are consistent with the effect of starvation on glutamine metabolism by intestinal preparations (Hanson & Parsons, 1980), but contrast with the situation reported in lymphocytes (Ardawi & Newsholme, 1982, 1983*a*).

Ketone bodies and n-butyrate metabolism

The rate of acetoacetate utilisation by incubated colonocytes was about 3.5 μmol/min per g dry wt. (Table 1); this rate was linear over the period of incubation. Some acetoacetate was converted into 3-hydroxybutyrate. This rate is similar to that reported by Roediger (1982), but considerably higher than that reported for thymocytes (Hume *et al.*, 1978), lymphocytes (Ardawi & Newsholme, 1984*b*) or enterocytes (Hanson & Parsons, 1978). If the observed rate of acetoacetate utilization reflects complete oxidation, then it would account for about 60% of oxygen consumption by the colonocytes. However, this value may be too high, since some of the acetoacetate could be converted into fatty acids; the rate of lipogenesis was not measured in the present work.

Isolated colonocytes readily oxidize n-butyrate (Roediger, 1982). In the present work, n-butyrate stimulated respiration by about 48%, which was not significantly altered by the addition of glucose, acetoacetate or glutamine (Table 1). When isolated colonocytes were incubated with n-butyrate, both acetoacetate and 3-hydroxybutyrate were formed, but 3-hydroxybutyrate accounted for only about 19% of the total ketones produced. However, a marked increase in the proportion of 3-hydroxybutyrate occurred when colonocytes were incubated with n-butyrate plus glucose (Table 1). In addition, more lactate was formed, even though less glucose was utilized, which is indicative of an inhibition of pyruvate oxidation. This may have been caused by an increase in the [acetyl-CoA]/[CoA] ratio in the mitochondria and inhibition of pyruvate dehydrogenase activity (Denton & Halestrap, 1979).

Pattern of fuel utilization

In a similar manner to other dividing cells [(e.g. enterocytes, tumour cells or cells with the potential for rapid cell division (lymphocytes; thymocytes)], colonocytes are characterized by a high rate of glycolysis (i.e. glucose conversion into lactate) and a high rate of glutamine utilization and partial oxidation to glutamate, aspartate and alanine. The present work also suggests that the energy requirement of colonocytes can be supported by oxidation of acetoacetate and/or n-butyrate. Although acetoacetate or n-butyrate decreased the rate of glucose utilization, the effect was small (about 10%) and unlikely to be of physiological significance. Furthermore, although starvation decreased glucose utilization by colonocytes, the effect was also small. Although the metabolism of glucose by the colon *in vivo* may be quantitatively significant, most if not all of the glucose is converted into lactate, pyruvate or alanine, all of which can give rise to glucose via gluconeogenesis during starvation, so that carbohydrate is not lost from this tissue, and consequently the operation of the 'glucose/fatty acid cycle' is unimportant. The ability of colonocytes to oxidize acetoacetate and n-butyrate may reflect the availability of these short-chain fatty acids for these particular cells, owing to bacterial fermentation in the large intestine (Henning & Hird, 1972).

We are grateful to the late Professor R. R. Porter, F.R.S., for his interest and encouragement. This work was supported by grants to M.S.M.A. from King Fahd Medical Research Center (KFMRC), College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

REFERENCES

Alp, P. R., Newsholme, E. A. & Zammit, V. A. (1976) Biochem. J. **154**, 689–700

Anderson, J. W. (1974) Am. J. Physiol. **226**, 226–229

Ardawi, M. S. M. & Newsholme, E. A. (1982) Biochem. J. **208**, 743–748

Ardawi, M. S. M. & Newsholme, E. A. (1983*a*) Biochem. J. **212**, 835–842

Ardawi, M. S. M. & Newsholme, E. A. (1983*b*) Hoppe-Seyler's Z. Physiol. Chem. **364**, 1251–1252

Ardawi, M. S. M. & Newsholme, E. A. (1984*a*) in Glutamine Metabolism in Mammalian Tissues (Haussinger, E. & Sies, H., eds.), pp. 235–246, Springer-Verlag, Berlin, Heidelberg and New York

Ardawi, M. S. M. & Newsholme, E. A. (1984*b*) Biochem. J. **221**, 255–260

Ardawi, M. S. M. & Newsholme, E. A. (1985) Essays Biochem., in the press

Bergmeyer, H. J., Bernt, E., Schmidt, F. & Stork, H. (1974*a*) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed), pp. 1196–1202, Academic Press, London and New York
Bergmeyer, H. U., Bernt, E., Mollering, H. & Pfleiderer, G. (1974*b*) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1696–1700, Academic Press, London and New York
Bernt, E. & Bergmeyer, H. U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1704–1708, Academic Press, London and New York
Budohoski, L., Challis, R. A. J. & Newsholme, E. A. (1982) Biochem. J. **206**, 169–172
Brand, K., Williams, J. F. & Weidemann, M. J. (1984) Biochem. J. **221**, 471–475
Cooney, G. J., Taegtmeyer, H. & Newsholme, E. A. (1981) Biochem. J. **200**, 701–703
Crabtree, B. & Newsholme, E. A. (1972) Biochem. J. **126**, 49–58
Crabtree, B., Higgins, S. J. & Newsholme, E. A. (1972) Biochem. J. **130**, 391–396
Crabtree, B., Leech, A. R. & Newsholme, E. A. (1979) in Techniques in Life Science, B 211 (Pogson, C. I., ed.), pp. 1–37, Elsevier/North-Holland, Amsterdam
Culvenor, J. & Weidemann, M. J. (1976) Biochim. Biophys. Acta **437**, 354–363
Curthoys, N. P. & Lowry, O. H. (1973) J. Biol. Chem. **248**, 162–168
Czok, R. & Lamprecht, W. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1446–1451, Academic Press, London and New York
Denton, R. M. & Halestrap, A. P. (1979) Essays Biochem. **15**, 37–77
Gawehn, K. & Bergmeyer, H. U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1492–1495, Academic Press, London and New York
Hanson, P. J. & Carrington, J. M. (1981) Biochem. J. **200**, 349–355
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
Hanson, P. J. & Parsons, D. S. (1980) Biochem. Soc. Trans. **8**, 506–509
Henning, S. J. & Hird, F. J. R. (1972) Biochem. J. **130**, 785–790
Hume, D. A., Radik, J. L., Ferber, E. & Weidemann, M. J. (1978) Biochem. J. **174**, 703–709
Jaworek, D., Gruber, W. & Bergmeyer, H. U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 2127–2131, Academic Press, London and New York
Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) in Regulation of Hepatic Metabolism (Lundquist, F. & Tygstrup, N., eds.), pp. 726–750, Munksgaard, Copenhagen
Lackner, R., Challiss, R. A. J., West, D. & Newsholme, E. A. (1984) Biochem. J. **218**, 649–651
Lamprecht, W. & Trautschold, I. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 2101–2110, Academic Press, London and New York
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Mellanby, J. & Williamson, D. H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1840–1843, Academic Press, London and New York
Opie, L. H. & Newsholme, E. A. (1967) Biochem. J. **104**, 353–360
Porteous, J. W. (1980) Biochem. J. **188**, 619–632
Read, G., Crabtree, B. & Smith, G. H. (1977) Biochem. J. **164**, 349–355
Roediger, W. E. W. (1982) Gastroenterology **83**, 424–429
Roediger, W. E. W. & Truelove, S. C. (1979) Gut **20**, 484–488
Sugden, P. H. & Newsholme, E. A. (1975) Biochem. J. **150**, 105–111
Vinay, P., Lemieux, G., Gougoux, A. & Lemieux, C. (1980) Int. J. Biochem. **12**, 89–98
Watford, M., Lund, P. & Krebs, H. A. (1979) Biochem. J. **178**, 589–596
Watford, M., Smith, E. M. & Erbelding, E. J. (1984) Biochem. J. **224**, 207–214
Williamson, D. H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), pp. 1679–1682, Academic Press, London and New York
Williamson, D. H., Lund, P. & Krebs, H. A. (1967) Biochem. J. **103**, 514–527
Williamson, D. H., Bates, M. W., Page, M. A. & Krebs, H. A. (1971) Biochem. J. **121**, 41–47
Windmueller, H. G. (1980) in Glutamine: Metabolism, Enzymology and Regulation (Mora, J. & Palacios, R., eds.), pp. 235–257, Academic Press, New York
Windmueller, H. G. (1984) in Glutamine Metabolism in Mammalian Tissues (Haussinger, E. & Sies, H., eds), pp. 61–77, Springer-Verlag, Heidelberg and New York
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
Wollenberger, A., Ristau, O. & Schoffa, G. (1960) Pflugers Arch. **270**, 399–405
Yassmeen, D., Laird, A. J., Hume, D. A. & Weidemann, M. J. (1977) Biochim. Biophys. Acta **500**, 89–102
Zammit, V. A., Beis, I. & Newsholme, E. A. (1978) Biochem. J. **174**, 989–998

Received 25 February 1985/17 June 1985; accepted 8 July 1985