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Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man

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SUMMARY Suspensions of isolated epithelial cells (colonocytes) from the human colon were used to assess utilisation of respiratory fuels which are normally available to the colonic mucosa *in vivo*. Cells were prepared from operative specimens of the ascending colon (seven) and descending colon (seven). The fuels that were used were the short chain fatty acid n-butyrate, produced only by anaerobic bacteria in the colonic lumen, together with glucose and glutamine, normally present in the circulation. The percentage oxygen consumption attributable to n-butyrate, when this was the only substrate, was 73% in the ascending colon and 75% in the descending colon. In the presence of 10 mM glucose these proportions changed to 59% and 72%. Aerobic glycolysis was observed in both the ascending and descending colon. Glucose oxidation accounted for 85% of the oxygen consumption in the ascending colon and 30% in the descending colon. In the presence of 10 mM n-butyrate these proportions decreased to 41% in the ascending colon and 16% in the descending colon. Based on the assumption that events in the isolated colonocytes reflect utilization of fuels *in vivo*, the hypothesis is put forward that fatty acids of anaerobic bacteria are a major source of energy for the colonic mucosa, particularly of the distal colon.

The mucosa of the small and of the large bowel depend upon respiratory fuels to maintain cellular turnover and function. Respiratory fuels can either be derived from the bowel lumen or from the circulation.[1] The preferred respiratory fuels of the mucosa in the small bowel are glutamine and ketone bodies rather than glucose which is poorly oxidised and largely converted to lactic acid[2]. This lactic acid may be reconverted to glucose in the liver and is a means of conserving glucose during glucose absorption.[3]

The respiratory fuels used by the colonic epithelial cells (colonocytes) have not been studied. In this regard, anaerobic bacteria produce short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, which are water soluble and readily absorbed.[4] By using a method to prepare isolated surface epithelial cells[5] it was possible to show that over 80% of energy needs for the colonic mucosa in the rat were obtained from the absorbed fatty acid, n-butyrate.[6] A comparison between luminal and circulating respiratory fuels used by colonocytes of the rat, established that luminal fatty acids are the preferred fuels and that the order of preference was SCFA > ketone bodies > amino acid > glucose.

Because the human colon normally contains large quantities of SCFAs[7] it seemed worthwhile to determine whether these fats were used by the human colonic mucosa and whether SCFAs were used differently in the proximal or distal colon. Preliminary observations have already been presented[8] and this report describes the results in detail. They may be of importance in several clinical conditions affecting the colon.

Methods

MATERIALS

Colonocytes were obtained from 14 colectomy specimens, seven from the ascending colon—that is, from the ileocaecal valve to the hepatic flexure—and seven from the descending colon—that is, from the splenic flexure to the peritoneal reflection in the rectosigmoid region. The mucosa had no macro-

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scopic or microscopic evidence of disease. All except two cases were obtained from patients with carcinoma of the colon. Mucosa was taken at least 6 cm from the carcinoma, a distance sufficient to provide mucosa that is cytochemically normal.[9] One specimen of ascending colon and one of the descending were obtained from patients with Crohn's disease, the area in question being unaffected by the disease.

TECHNIQUES

Colonocytes were prepared and assessed for metabolic viability as previously described.[5] The respiratory fuels that were tested, glucose (10 mM), n-butyrate (10 mM), and glutamine (5 mM), as well as a combination of glucose and butyrate with and without NH_4Cl (10 mM), were selected on the results obtained with rat colonocytes.[6] Ammonium chloride, which is usually found in the colon, was used because Visek[10] suggested that it may have an adverse effect on metabolism of mucosal cells. The selected concentration of NH_4Cl is that which is normally found in the colonic lumen.[11] Oxygen consumption was measured manometrically[12] and expressed as μmol per min/g (dry weight) as previously described.[5]

Disappearance of substrates and formation of metabolites—that is, lactate, acetoacetate, β-hydroxybutyrate, alanine, glutamine, and ammonia—were measured enzymatically,[13] and expressed as μmol per min/g (dry weight). Production of $^{14}CO_2$ from [1-^{14}C]-butyrate was estimated as follows: 1 ml of cell suspension, equivalent to approximately 5 mg dry weight, was incubated at 37°C in 1 ml of physiological saline containing 2·5% w/v BSA with radioactively labelled butyrate. Cell suspensions were placed in 25 ml conical flasks sealed with a Suba-seal stopper (Gallenkamp, London, UK) and equipped with a glass centre well. The gas phase was O_2+CO_2 (19:1 v/v). The initial concentration of [1-^{14}C]-butyrate was 10 mM with a specific activity of $2{\cdot}1\times10^4$ cpm/μmol. At the end of incubation 0·5 ml of 10 M NaOH was injected into the centre well and immediately afterwards 0·5 ml 10% $HClO_4$ (v/v) into the cell suspension. The $^{14}CO_2$ was allowed to equilibrate over three hours with NaOH, of which duplicate aliquots of 0·1 ml were taken for counting in a liquid scintillation counter (Beckman model 200s). The scintillation fluid, per litre, was a mixture of the following: 600 ml toluene, 400 ml methoxyethanol, 60 g naphthalene, and 5·5 g Permablend (Packard Instruments Ltd, Caversham, Berks, UK). Counts per minute in each case were corrected for non-specific activity generated by perchloric acid. Production of $^{14}CO_2$ was expressed as μmol per min/g (dry weight).

CALCULATIONS

From the established pathways of butyrate and glucose metabolism[14] and the present results, the oxidations of each respiratory fuel could be calculated. Values were calculated from the mean estimations of metabolites appearing or being utilised and expressed as percentages of the measured oxygen consumption. To calculate the O_2 requirement to account for butyrate metabolism, the following was taken into consideration: conversion of butyrate to two acetyl-coA requires 1 mol O_2, the oxidation of two acetyl-coA requires 4 mol O_2, the conversion of butyrate to acetoacetate requires 1 mol oxygen and the conversion to β-hydroxybutyrate ½ mol O_2. Thus the contribution of butyrate as the fuel of respiration is:

[($^{14}CO_2\times5$) + (acetoacetate$\times1$) + (β-hydroxybutyrate$\times{\cdot}5$)] and is expressed as a percentage value of the oxygen consumption measured manometrically. The rate of glucose oxidation was calculated as follows:

[glucose utilisation—(lactate production$\times{\cdot}5$)] $\times$ 2·5 and expressed as a percentage value of the oxygen consumption measured manometrically.

Results

The cumulative oxygen consumption of colonocytes in the ascending colon (Fig. 1) and descending colon

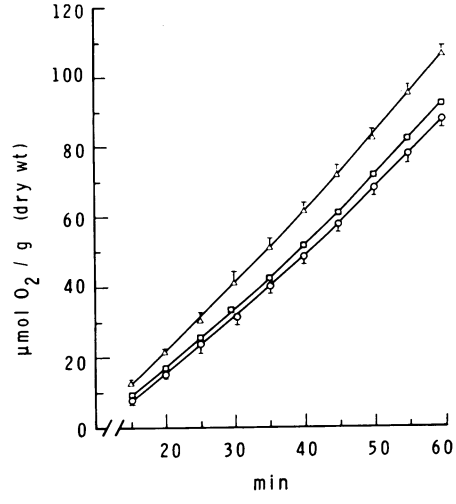

Fig. 1 *Cumulative oxygen consumption of human colonocytes of the ascending colon. Mean values of three experiments with 10 mM butyrate (△———△), 10 mM glucose (□———□) or no substrate (○———○).*

Table 1 *Effect of substrates on rate of oxygen consumption of human colonocytes*

Substrate	*Rate of oxygen uptake (μmol/min/g dry weight)*	
	Ascending colon	*Descending colon*
None	8·29 ± ·57 (7)	7·47 ± ·69 (7)
Glucose (10 mM)	9·29 ± ·60† (7)	7·88 ± ·83* (7)
Glucose (10 mM + butyrate, 10 mM)	10·97 ± ·64‡ (7)	9·31 ± ·58‡ (7)
Butyrate (10 mM)	10·11 ± ·56† (7)	9·30 ± ·83‡ (7)
Glucose (10 mM + butyrate 10 mM + NH_4Cl, 10 mM)	11·32 ± ·83 (6)	10·55 ± 1·01 (5)
Glutamine (5 mM)	9·15 ± ·49 (5)	8·60 ± ·49 (5)

Rates of oxygen consumption were measured manometrically and calculated from 20–45 minute periods of incubation. The data are mean ± SEM with number of observations in parentheses.
Compared against endogenous oxygen consumption; paired *t* test.
*NS ‡P<0·005 †P<0·025

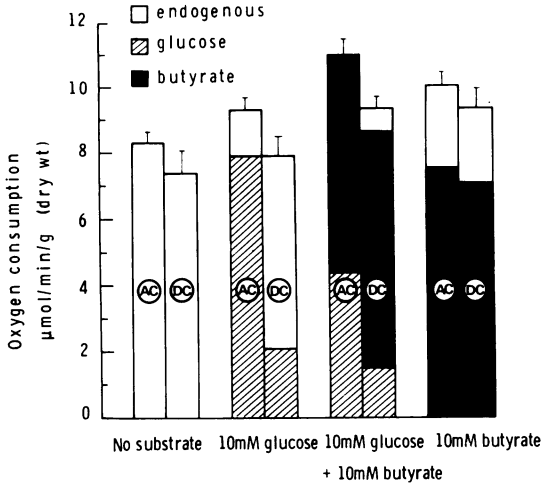

Fig. 2 *Contribution of glucose (10 mM), butyrate (10 mM), and endogenous fuels to the total oxygen consumption of isolated colonocytes from the ascending (AC) and descending (DC) colon. Histograms are mean values ± SEM of oxygen uptake in μmol/min/g dry weight (see Table 1).*

(Fig. 2) was linear indicating that the metabolic performance of cells was good throughout the course of experiments. The addition of glucose, butyrate, and glutamine significantly improved oxygen consumption by isolated cell suspensions (Table 1).

GLUCOSE UTILISATION, GLYCOLYSIS

The total amount of glucose removed by colonocytes for either oxidation or lactogenesis differed in the ascending and descending colon but was not statistically significant (P>0·5, Student's *t* test) (Tables 2, 3). In the presence of 10 mM butyrate, glucose utilisation was not altered at either of the sites in the colon. Of the utilised glucose the proportion appearing as lactate was 45% in the ascending colon and 74% in the descending colon. The percentage oxygen consumption attributable to glucose oxidation is 85·4% in the ascending colon and 30% in the descending colon. Glucose oxidation is suppressed by butyrate from 85·4% to 40·3% in the ascending colon and from 30% to 15·8% in the descending colon (Table 3).

BUTYRATE OXIDATION AND KETOGENESIS

Oxygen consumption with butyrate, measured by the amount of CO_2 generated, was linear over

Table 2 *Metabolic changes on addition of substrates to human colonocyte suspension—ascending colon*

	Substrate added					
	None	*Glucose*	*Glucose + butyrate*	*Butyrate*	*Glucose − butyrate + NH_4Cl*	*Glutamine*
Glucose		−5·74 ± ·97 (7)	−4·13 ± ·51 (7)		−4·39 ± ·91 (6)	
Lactate	·32 ± ·04 (7)	5·14 ± ·55 (7)	4·73 ± ·50 (7)		5·26 ± ·72 (6)	·33 ± ·06 (5)
Acetoacetate	·37 ± ·09 (6)	·15 ± ·03 (7)	·89 ± ·10 (7)	1·89 ± ·15 (7)	·99 ± ·14 (6)	·20 ± ·05 (5)
Hydroxybutyrate			·54 ± ·07 (7)	·42 ± ·07 (7)	·56 ± ·09 (6)	
Ammonia	2·82 ± ·21 (6)	2·64 ± ·25 (6)	2·29 ± ·28 (6)	2·63 ± ·28 (6)	·77 ± ·44 (4)	4·41 ± ·41 (4)
Glutamate			·39 ± ·04 (7)	·44 ± ·05 (7)	·38 ± ·03 (6)	·98 ± ·04 (5)
Alanine			·43 ± ·02 (7)	·14 ± ·03 (7)	·49 ± ·02 (7)	·45 ± ·05 (5)

Cells were incubated for 60 minutes. The initial concentration of glucose, butyrate, and NH_4Cl was 10 mM and of glutamine 5 mM. The data are mean ± SEM of glucose removed (−) or metabolite production expressed as μmol/min/g dry weight.

Table 3 *Metabolic changes on addition of substrates to human colonocyte suspension—descending colon*

	Substrate added					
	None	*Glucose*	*Glucose + butyrate*	*Butyrate*	*Glucose + butyrate + NH_4Cl*	*Glutamine*
Glucose		−3·61 ± ·90 (5)	−3·38 ± ·79 (7)		−3·59 ± ·96 (4)	
Lactate	·25 ± ·05 (7)	5·36 ± ·55 (7)	5·58 ± ·56 (7)		5·73 ± ·60 (6)	·55 ± ·10 (5)
Acetoacetate	·27 ± ·03 (7)	·14 ± ·03 (7)	·55 ± ·06 (6)	1·39 ± ·25 (6)	·78 ± ·15 (6)	·16 ± ·05 (5)
Hydroxybutyrate	·06 ± ·03 (4)		·44 ± ·06 (7)	·31 ± ·05 (7)	·43 ± ·06 (6)	
Ammonia	2·63 ± ·60 (6)	2·34 ± ·82 (6)	2·07 ± ·56 (6)	1·98 ± ·57 (5)	1·77 ± ·31 (6)	3·49 ± ·28 (4)
Glutamate			·30 ± ·03 (7)	·39 ± ·04 (7)	·27 ± ·02 (6)	·92 ± ·19 (5)
Alanine			·50 ± ·03 (7)	·19 ± ·04 (7)	·51 ± ·04 (7)	·43 ± ·06 (5)

Conditions as given in Table 2.

60 minutes (Fig. 1). Because of lack of cell suspensions evaluation of $^{14}CO_2$ was measured over only 40 minutes (Table 4) but was linear up to 60 minutes in three cases where this could be tested. Production of $^{14}CO_2$ was similar in the ascending and descending colon and addition of glucose or NH_4Cl did not alter the oxidation of butyrate to CO_2.

Human colonocytes produced ketone bodies, mainly acetoacetate, from n-butyrate. Addition of glucose decreased the total ketone body production but increased the appearance of β-hydroxybutyrate. Ketogenesis diminished in the distal colon, which was significant with regard to acetoacetate but not β-hydroxybutyrate: for acetoacetate ascending versus descending colon $P = < 0{\cdot}05$ (Student's *t* test).

Based on the mean values of $^{14}CO_2$ and metabolite formation, n-butyrate contributed 73% of the oxygen consumption in the ascending colon and 75% in the descending colon. In the presence of 10 mM glucose these proportions changed to 59% and 72%.

GLUTAMINE METABOLISM

The proportion of glutamine not accounted for as glutamate, ammonia, and alanine can be calculated from the evolution of ammonia, as there is a one to one stoichiometry between glutamine breakdown and ammonia formation (reaction 1, Fig. 3). Glutamine metabolised beyond glutamate was 0·37 μmol per min/g (dry weight) in the ascending colon and 0·04 in the descending colon. Of these values the flux across glutamate dehydrogenase to oxoglutarate in the tricarboxylic acid cycle was 18% in the ascending colon and 0% in the descending colon.

Table 4 *Production of $^{14}CO_2$ from sodium n[1-^{14}C] butyrate*

	10 mM [1-^{14}C] butyrate	*10 mM [1-^{14}C] butyrate + 10 mM D-glucose*	*10 mM [1-^{14}C] butyrate + 10 mM NH_4Cl*
Ascending colon	1·05 ± ·10 (5)	1·06 ± ·05 (4)	0·95 ± ·04 (4)
Descending colon	1·09 ± ·19 (5)	1·18 ± ·22 (5)	1·20 ± ·10 (3)

Data are means ± SEM (μmol/min/g dry weight) of the number of observations in parentheses.

Discussion

More than 70% of the oxygen consumed by colonocytes from the ascending and descending colon was due to butyrate oxidation, a value which is not greatly different from the 80% observed in colono-

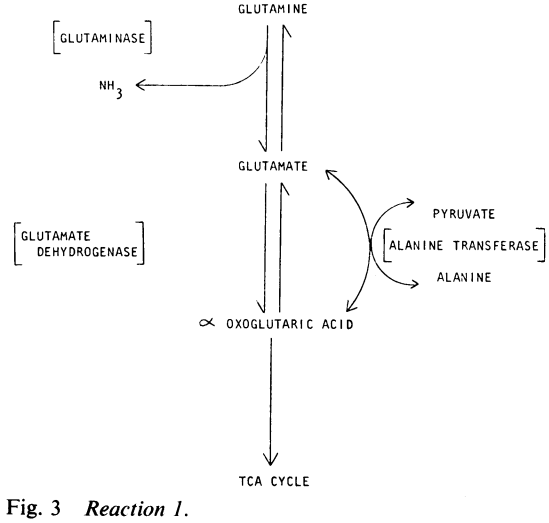

Fig. 3 *Reaction 1.*

cytes of the rat.[6] Part of the butyrate that is oxidised is converted to ketone bodies in a similar way to the mucosa of the rumen and the colon of rabbits.[15] Ketogenesis thus does not occur only in the mucosa of herbivores but also in the colonic mucosa of omnivores such as man. Ketogenesis generally is an indicator that fats are being oxidised,[16] and this was confirmed presently by the rate of radioactively labelled CO_2 produced from butyrate. For many years ketogenesis in herbivores was taken to indicate energy conservation for the whole organism, especially ruminants which absorb large quantities of SCFAs.[17] In the light of the present findings it would be preferable to regard SCFAs in man not just as providers of energy to the whole organism but as the predominant energy source for the epithelium of the colonic mucosa.

Colonocytes of the distal colon produce less ketone bodies than those of the proximal colon, an observation also made in the rabbit colon.[15] In human colonocytes CO_2 production from butyrate is the same at both sites. This implies that, for each mole of butyrate that is absorbed and utilised, more enters into the tricarboxylic acid cycle in the distal than proximal colon and suggests that this fatty acid is more important as a respiratory fuel in the distal than proximal colon. Rates of absorption for butyrate are equal per unit area of mucosa in the ascending and descending colon[18] and changes in ketone body production could therefore not be due to differences in rate of fatty acid absorption. In the presence of glucose butyrate remains a premier fuel of colonocytes and is most pronouncedly oxidised in the distal colon.

Ketogenesis does not occur in the mucosa of small bowel of the rabbit and rat[15 19] and is unlikely to occur in the mucosa of the small bowel of man. Lack of ketogenesis is not unexpected because SCFAs are scarce in the mammalian small bowel,[20] and there is no measurable butyrate in ileostomy effluent of man (Kennedy, unpublished observation). The ileum is capable of absorbing large quantities of SCFAs[21] but the epithelial cells of the small bowel (enterocytes) do not increase their oxygen consumption or form ketone bodies when exposed to n-butyrate.[15 19] The ability to produce ketone bodies and oxidise butyrate is one cardinal metabolic difference between the mucosa of the small and large bowel. Evaluation of ketogenesis and oxidation of butyrate would be worthwhile in disease of the colonic mucosa—as, for example, ulcerative colitis, which is confined entirely to the large bowel.

Another difference between the proximal and distal colon is the extent to which glutamine is metabolised beyond glutamate. As far as glutamine metabolism is concerned, the proximal colon appears to resemble the small bowel, in which (at any rate in the rat) glutamine is extensively oxidised and converted to alanine.[19] The present finding in the human colon is in line with the suggestion of Pinkus and Windmueller[22] that there is an aborally decreasing gradient of glutamine utilisation from jejunum to colon. This suggestion was based on finding a decreasing activity of glutaminase (reaction 1) in the mucosa of the small and the large bowel.

All the SCFAs with the exception of acetate are produced by anaerobic bacteria from oligosaccharides,[23] degradeable fibre, and oligopeptides under conditions of anaerobiosis. The present results indicate that n-butyrate is a major respiratory fuel of the colonic mucosa, though it remains to be shown what precise contribution these fatty acids make to the well-being of the colonic mucosa *in vivo*.

In conclusion, short chain fatty acids in the colon appear to play an important nutritive role in colonocytes, mediating a symbiotic existence between bacteria and the colonic mucosa of man. These acids warrant further investigation in mucosal diseases of the colon.

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