Acetate absorption and metabolism in the rabbit hindgut

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SUMMARY Acetate disappearance from the loops of the hindgut in the rabbit was evaluated by measuring variations in the concentration of acetate in caecocolonic loops and differences in the arterial and venous plasma. *In vivo* metabolism in gut and liver tissues was studied after introduction of (1-¹⁴C) acetate into caecocolonic loops. The rate of disappearance from the loops was quantitatively significant and showed little variation irrespective of the location in the hindgut. Hindgut tissue metabolised acetate and the intensity of the metabolism varied with the segment studied. The distal position of the gut showed by far the highest acetate uptake. Radioactivity was found in a certain number of free amino acids, organic acids, and sugars. Acetate was mainly converted into aspartate and glutamate. These can be considered as 'stock forms' which can be diverted either towards oxidative metabolism or towards protein synthesis.

A large proportion of the energy requirement of the rabbit is derived from the metabolism of volatile fatty acids (VFA) formed by microbial fermentation of the fodder in the large intestine.^{1,2} Substantial amounts of acetate, propionate, and butyrate appear in caecocolonic contents and are subjected to metabolic activity in the hindgut epithelium during their absorption.³⁻⁷ In an earlier paper⁸ we observed that large intestine tissues metabolise butyrate, the intensity of this metabolism varying with the location in the gut and with the nature of the pellets contained in the colon. We showed that butyrate is the best respiratory fuel for the colonic wall, its oxidation into carbon dioxide in the TCA cycle yielding energy for metabolic activity and for the absorption of electrolytes⁹⁻¹² and of numerous bacterial constituents liberated by the intervention of a lytic protein. This protein isolated and characterised by Bonnafe and Raynaud¹³⁻¹⁵ is responsible for the steady fall in the bacterial population of the proximal and distal colon of the rabbit. Acetate is the major fatty acid present in the hindgut contents (73%),⁶ however, its turnover rate through the caecal pool is rapid and 10-fold higher than that calculated for the rumen of the cow.² Much less information is available about its

metabolism by caecal and colonic mucosa. McMillan *et al*¹⁶ reported that acetate absorption in the caecum occurs with little or no metabolic change; Henning and Hird^{3,4} showed that acetate is only slightly oxidised by hindgut mucosa.

The aim of the present investigation is to determine the extent to which acetate is metabolised during its absorption from the hindgut.

Methods

ANIMALS

Twelve male rabbits (*Oryctolagus cuniculus*) with an average weight of 2.5 kg were provided with oats, lucerne, and *ad libitum* water. Animals were not selected according to the nature of the pellets contained in their colon, as the study of the variations linked to the excretory rhythm were not the purpose of this work.

EXPERIMENTAL PROCEDURE

Anaesthesia of the rabbits was achieved by administering pentobarbital solution (20 mg/kg), through the marginal vein of the ear. After laparotomy the intestine was removed and three loops of approximately equivalent parameters, as indicated in Table 1, were made from the caecum (median zone) and the proximal and distal colon. The sites of the loops were chosen for suitability for cannulation of the venous drainage. Polyethylene catheters (PE90) were inserted into small

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Table 1 Mean weight and serosal area of caecocolonic loops

Parameters	Intestinal loops		
	Caecum	Proximal colon	Distal colon
Dry tissue	1.5±0.08	1.1±0.05	0.9±0.06
Wet tissue	6.3±1.89	6.5±1.24	5.1±1.32
Serosal area	27.0±1.36	26.0±1.37	23.0±1.08

The weight of tissue is expressed in g on a dry or wet basis, the serosal area in cm². Each value represents the mean±SE of 12 samples. There was no statistically significant difference between the weights or the serosal areas of the different loops.

caecal and colonic veins draining each loop and care was taken to ensure that blood circulation was not damaged. A carotid artery was exposed and cannulated (PE190). The content of the loops was carefully washed out with sodium chloride (9 g/l) at 38°C. Each animal so prepared was put into a thermoregulated chamber. The three loops were filled with 5 ml of experimental solution¹⁷ containing 2 g/l polyethylene glycol (PEG). For the six metabolism studies, 30 µCi of (1-¹⁴C) acetate was added to the test solution. Blood samples (1 ml) were simultaneously collected through the catheters before and every five minutes for 20 minutes in a heparinized beaker. After each operation the volume of blood withdrawn was replaced with physiological saline and the catheter was filled with a solution of heparin (40 IU/ml physiological saline). At the end of the incubation (20 min) the loops were emptied and the contents were immediately placed in ice. The intestinal loops together with a portion of each lobe of the liver were resected, blotted, and weighed. The serosal area of the loops was evaluated by planimetry and dry weight determined after drying to constant weight.

METABOLITE DETERMINATION

For the studies on ¹⁴C incorporation we adapted the method of Schurmann¹⁸ and Lavergne *et al*¹⁹ which separates free amino acids, organic acids, and sugars on the same autoradiographs. The tissues were homogenised in ice cold water using an Ultra-Turrax electric blender. The homogenates were extracted in boiling water and the insoluble fraction was removed by centrifugation (30 min at 10000 g). In order to obtain an adequate aqueous phase the lipid-soluble substances were eliminated with chloroform (three extractions v/v) in a separation funnel. For plasma samples protein precipitation with sulphosalicylic acid (15 g/l)

preceded the chloroform extraction procedure. The aqueous phase was recovered and concentrated in a Rotavapor (Büchi). An aliquot part of this liquor was first submitted to electrophoresis (2100 V, 40 mA, 1 h) in an acid medium: formic acid/acetic acid/water (17:57:926, v/v) at pH 2. After drying, double development ascendant chromatography was used in butan-2-ol/formic acid/water (6:1:2, v/v). The autoradiographs (Fig. 1) were obtained by leaving the electrophoresis paper in prolonged contact with photographic plates (Kodak Kodirex). The radioactive spots were eluted and ¹⁴C activity was determined in a liquid scintillation counter (Packard 460). The remains of the extract was fractionated on cationic (Dowex 50×4) and anionic (Dowex 1×4) exchange resin columns in preparation for quantitative analysis. Free amino acid determination was made on a technicon autoanalyser.²⁰ Organic acids were evaluated by potentiometry as described by Carles.²¹ Acetate was determined by a gas liquid chromatography (Intersmat IGC 120 DSL), after preparation using the technique described by Rémésy and Demigné.²² Polyethylene glycol concentration was determined according to the procedure described by Hyden.²³

STATISTICS

The formula used in calculating net acetate transport in the intestinal loops was:

$$\text{Net acetate transport } (\mu\text{mol}) = C_0V_0 - CtV_0\text{PEG}_0/\text{PEGt}$$

where V_0 is the volume of fluid in loops at the beginning of the experiment (ml); PEG_0 and PEGt are the PEG concentrations at the beginning and at the end of the experiment and C_0 and Ct are the acetate concentrations (µmol/ml) at the beginning and at the end of the experiment.

The formula used in calculating specific radioactivity (SR) for a radioactive metabolite A was:

$$\text{SR } (\mu\text{Ci}/\mu\text{mol}) = \text{Rt} \times \text{QI}/\text{At}$$

where Rt represents the percentage of ¹⁴C incorporated into labelled metabolite A, it is expressed as a percentage of the total ¹⁴C detected on the autoradiograph; QI is the quantity of (1-¹⁴C) acetate that has disappeared from the loop (µCi/20 min); At is the concentration of the unlabelled metabolite A in the tissue concerned (µmol/100 g wet wt) or in the plasma (µmol/100 ml).

The data were expressed as means±SE; the statistical significance of the differences between means was assessed with Student's *t* test or with the paired *t*-test when appropriate.

Fig. 1 Autoradiograph of metabolites incorporated into gut or liver tissues after introduction of (1-¹⁴C) acetate into caecocolonic loops. Free amino acids: alanine, aspartate, asparagine, cysteine, glutamate, glutamine, leucine and valine. Organic acids: TCA cycle acids (citrate, fumarate, malate, succinate), lactate and glycolate. Sugars: fructose, glucose and phosphorylating sugars (P-oses)

Results

Disappearance of acetate in intestinal loops

The concentration of acetate used in the present work was: 37.0±0.53 mM which is regarded as physiological.¹⁷ Twenty minutes after introduction of the test solution, the rate of acetate disappearance from the caecal loops did not differ from either that of the proximal or distal colonic loops. At the end of incubation 30–35% of the acetate inserted into the loops had disappeared (Table 2; Fig. 2).

Arterial and venous plasma concentrations of acetate

There was an aborally decreasing gradient of acetate in the venous hindgut plasma (Fig. 2). Differences in the concentrations of acetate in the arterial and venous plasma in the distal colon was

not significant. The mean value of acetate in arterial plasma was 177±19.9 μmol/100 ml, which represents 88% of the total VFA.

Table 2 Mean disappearance of acetate in the caecocolonic loops

Disappearance expressions	Intestinal loops		
	Caecum	Proximal colon	Distal colon
Per g dry tissue	57.6±4.84	66.1±5.59	54.4±2.97
Per g wet tissue	14.4±1.21	11.6±1.88	15.1±1.67
Per cm ²	2.5±0.19	3.0±0.63	2.7±0.16

The net flux of acetate through the caecocolonic loops is expressed in μmol/g wet or dry wt/20 min. Each value represents the mean±SE of 12 samples. There was no statistically significant difference between the net flux of acetate in the different loops.

Fig. 2 Acetate disappearance from 12 caecocolonic loops is expressed as a % of the initial acetate concentration in the test solution. Acetate concentration in venous plasma is expressed as a % of the acetate concentration in the arterial plasma. Each column with its bar represent mean $\pm$ SE; ** significantly different at $p < 0.01$; *** significantly different at $p < 0.001$ from controls.

^{14}C incorporation into metabolites

All tissues were able to convert acetate. On the autoradiographs radiocarbon was always detected in a number of metabolites: free amino acids, organic acids and sugars (Table 3; Fig. 1). In gut walls practically all the ^{14}C was recovered in free amino acids: alanine; aspartate, asparagine, cysteine; glutamate, glutamine, leucine and valine; but the radioactivity was mainly accounted for by glutamate (75–80%). Radiocarbon was also detected in organic acids, in this case most of the labelling concerns the TCA cycle acids (75–85%); some label was recovered in glycerate and lactate. Radioactivity was regularly found in fructose, glucose, and phosphorylating sugars. The total ^{14}C incorporation increased in an aboral direction, the values were respectively seven and 13 times higher in the proximal and distal colon than in the caecum. The high values obtained in distal colon were not only due to an increased labelling of

organic acids but also to that of free amino acids which were 14 times more labelled in the distal colon. Among the amino acids aspartate, glutamate and glutamine were respectively 50, 14, and nine times more labelled in the distal hindgut. Irrespective of the total ^{14}C content in the large intestine tissues the labelling balance of the free amino acids, organic acids and sugars was remarkably constant (Fig. 3).

Most of the labelled metabolites present in hindgut walls were detectable in venous plasma, this is not the case in arterial plasma where radiocarbon was only found in citrate, lactate and glucose.

For 1 g of wet tissue the ^{14}C content of the liver was identical to the ^{14}C content of the caecal wall, but the accumulation of glutamate was not as great: 60% instead of 75%.

Specific radioactivities (SR)

The specific radioactivity for aspartate and glutamate were relatively large and similar in the three gut tissues (Table 4). The specific radioactivity for cysteine decreased in an aboral

Fig. 3 ^{14}C incorporation into free amino acids, organic acids and sugars in caecal (CC), proximal and distal colon (PC, DC) and liver (L) tissues after introduction of ($1\text{-}^{14}\text{C}$) acetate into caecocolonic loops. Values are expressed as a % of the total ^{14}C incorporation.

Table 3 Incorporation of ^{14}C into free amino acids, organic acids and sugars in gut and liver tissues and plasmas 20 minutes after introduction of ($1\text{-}^{14}\text{C}$) acetate into caecocolonic loops

		Tissues				Plasmas	
		Caecum	Proximal colon	Distal colon	Liver	Venous	Arterial
Free amino acids							
	Alanine	0.3±0.14	1.0±0.16	1.1±0.31	0.7±0.01	0.3±0.13	—
	Aspartate	0.3±0.07	8.8±3.21	14.7±3.08	0.9±0.15	0.2±0.13	—
	Asparagine	0.4±0.09	1.1±0.29	1.9±0.27	0.3±0.04	—	—
	Cysteine	0.3±0.03	0.6±0.24	1.6±0.42	0.1±0.09	0.2±0.11	—
	Glutamate	5.5±1.58	48.3±11.18	78.8±15.67	4.2±0.35	0.6±0.19	—
	Glutamine	0.6±0.07	0.5±0.11	5.1±0.32	0.4±0.18	—	—
	Leucine	—	—	0.3±0.24	0.1±0.09	—	—
	Valine	—	1.1±0.38	1.5±0.48	0.2±0.15	—	—
	Total	7.4±0.21	61.4±12.53	105.0±18.57	6.9±0.41	1.3±0.99	—
Organic acids							
TCA cycle	Citrate	0.2±0.09	4.6±1.80	7.3±2.32	0.5±0.09	1.4±0.28	0.3±0.09
	Fumarate	0.1±0.09	0.7±0.28	1.6±0.42	0.3±0.04	—	—
	Malate	0.4±0.18	1.7±0.47	6.5±3.06	0.7±0.12	0.2±0.08	—
	Succinate	0.5±0.09	1.7±0.43	4.1±1.73	0.6±0.02	0.4±0.24	—
	Glycerate	0.1±0.01	0.3±0.10	1.4±0.56	0.2±0.15	—	—
	Glycolate	0.1±0.09	0.1±0.04	0.1±0.08	—	—	—
	Lactate	0.3±0.09	1.0±0.22	2.3±1.04	0.7±0.35	0.3±0.09	0.3±0.07
	Total	1.7±0.26	10.1±3.20	23.3±8.97	3.0±0.54	2.3±0.73	0.6±0.16
Sugars							
	Fructose	0.4±0.03	0.5±0.22	2.0±0.86	—	—	—
	Glucose	0.3±0.08	1.0±0.25	0.7±0.27	1.2±0.15	1.5±0.12	0.7±0.07
	P. oses	0.3±0.06	1.4±0.41	1.2±0.36	0.1±0.10	0.1±0.05	—
	Sucrose	—	0.4±0.21	0.3±0.13	—	—	—
	Total	1.0±0.27	3.3±0.76	4.2±1.27	1.3±0.17	1.6±0.18	0.7±0.07
Total ¹⁴ C incorporation		10.0±0.10	74.8±15.24	132.5±11.15	11.2±0.88	5.2±0.47	1.3±0.87

^{14}C incorporation is expressed in 10^3 dpm/g wet wt/20 min or 10^3 dpm/ml/20 min. Each value represents the mean±SE of six samples of tissue; the venous plasma is mean of the three plasma (caecal and colonic) withdrawn during the six experiments; (—) represents nil results. Differences in the variations of the ^{14}C incorporation into the three groups of metabolites were not statistically significant between proximal and distal colon; in contrast they were significant at $p<0.01$ between caecal and proximal colon; at $p<0.001$ between caecal and distal colon and between arterial and venous plasma.

direction, in contrast the specific radioactivity for alanine was low and constant. The specific radioactivity of organic acids is often missing (Table 4), this is because if labelling does occur, organic acid concentrations are often so low that they could not be quantitatively estimated.

Discussion

This study shows that the rabbit is able to absorb substantial amounts of acetate. Indeed we found an absorption rate of about $8\text{ }\mu\text{mol}/\text{cm}^2/\text{h}$ which is greater than that observed in the rumen epithelium²⁴ and the large intestine of man,^{25, 26} pig²⁷ and pony.²⁸ As the rate of disappearance in caecocolonic loops is comparable when a solution of identical concentration is introduced into the loops, it appears that the absorptive capacity in the hindgut does not diminish in an aboral direction. *In vitro* studies of acetate and transport across the hindgut epithelium of pony²⁸ and pig²⁷ showed that

acetate is absorbed at an approximately equivalent rate in caecal and colonic tissues. Its release onto the serosal side diminished in an aboral direction for the pig, however, but not for the pony. The authors concluded that, for the pig, a large portion of acetate was metabolised by the epithelium itself.

In the rabbit the high concentration of acetate normally present in the large intestine contents,^{6, 9, 29} its rapid absorption,^{2, 5, 30} its strikingly low concentration in colonic plasma and the labelling of the TCA cycle acids suggest that acetate is well utilised by the gut wall. The pattern of the distribution of ^{14}C into free amino acids, organic acids and sugars shows that irrespective of the tissue acetate follows the same pathway. In addition, the specific radioactivities show that acetate is mainly converted into aspartate and glutamate. The intense labelling of these two amino acids in the large intestine tissue raises some speculation as to the metabolism of acetate. We never found such an accumulation in earlier works

Table 4 Specific radioactivity (SR) of free amino acids and organic acids in gut and liver tissues and plasmas 20 minutes after introduction of (1-¹⁴C) acetate into caecocolonic loops

	Tissues				Plasmas	
	Caecum	Proximal colon	Distal colon	Liver	Venous	Arterial
<i>Free amino acids</i>						
Alanine	0.3±0.12	0.6±0.28	0.2±0.06	1.3±0.25	2.3±0.89	(1)
Aspartate+asparagine	19.5±4.81	20.1±1.08	17.4±3.35	13.6±1.64	12.0±1.06	(1)
Cysteine	6.6±0.90	2.4±0.86	3.2±0.88	0.5±0.45	17.7±3.58	(1)
Glutamate+glutamine	6.4±0.23	7.1±0.39	5.5±0.71	3.5±0.25	11.4±5.14	(1)
Leucine	(1)	(1)	0.4±0.29	0.4±0.38	(1)	(1)
Valine	(1)	2.5±0.84	1.0±0.35	1.4±1.38	(1)	(1)
<i>Organic acids</i>						
TCA cycle	Citrate	1.0±0.48	0.4±0.16	(2)	3.2±0.25	3.7±0.82
	Fumarate	1.4±0.67	(2)	(2)	4.4±2.18	(1)
	Malate	0.5±0.46	0.2±0.13	(2)	1.7±0.95	(1)
	Succinate	4.2±0.65	25.3±6.12	(2)	1.1±0.08	19.0±10.51
	Glycerate	0.2±0.06	(2)	(1)	0.1±0.06	(1)
	Glycolate	0.2±0.15	(2)	(2)	(1)	(1)
	Lactate	0.6±0.18	0.2±0.13	0.6±0.32	1.3±0.56	1.9±0.76

Specific radioactivity is expressed in $\mu\text{Ci}/\mu\text{mol}/100$ g wet wt or in $\mu\text{Ci}/\mu\text{mol}/100$ ml plasma. (1): metabolites isolated but not containing any significant counts; (2): quantitative estimations of these metabolites were not obtained. Each value represents the mean $\pm$ SE of six samples of tissues; the venous plasma is the mean of the three plasmas (caecal and colonic) withdrawn during the six experiments. There was no statistically significant difference between the different specific radioactivity in the gut tissues except for the cysteine specific radioactivity ($p < 0.001$). In the liver the specific radioactivity of alanine and glutamine was statistically different from those of the other tissues ($p < 0.001$).

with labelled butyrate⁸ or propionate (unpublished results). It is well known that the preferred respiratory fuels for the small intestine of rat and other species³¹⁻³³ are glutamine and glutamate. When glutamate is extensively oxidized more than half of its carbon skeleton may be converted into alanine.^{31, 32} In the hindgut of rat³⁴ and man³⁵ there is an aborally decreasing gradient of glutamate utilisation, linked with decreasing glutaminase (EC 3.5.1.2) and glutamate dehydrogenase (EC 2.3.2.1) activities in caecocolonic mucosa. The present finding is in line with that of preceding investigators.^{34, 35} In effect a high glutamate concentration does not raise alanine production since the ratio Glu/Ala increases from the caecum to the rectum, while alanine variations are not significant. The rapid degradation of glutamate, however, must be initiated by the amino-transferases.³⁶ Our results may explain the observations of Henning and Hird⁴ in the rabbit colon: In experiments with (1-¹⁴C) acetate applied to the epithelial surface of everted colonic sacs, they found that almost all the ¹⁴C lost from the incubation fluids was taken up by the wall and that little carbon dioxide was released. In the present *in vivo* experiments, it appears that (1-¹⁴C) acetate was rapidly converted into other metabolites: aspartate and glutamate which are more slowly oxidised into carbon dioxide and hardly exported at all. Substantial labelling of glutamate was also

reported by Mayfield *et al.*³⁷ in *in vitro* incubations of various sheep tissues with (1-¹⁴C) acetate.

The low levels of acetate in the colonic bloodstream imply that acetate catabolism by the gut wall is extremely efficient, particularly in the distal colon. The nature and the distribution of the radioactive carbon shows that acetate is used by the colonocytes as a source of energy but especially for protein³⁸ and fat³⁹ synthesis. Thus it appears that the tissues of the rabbit large intestine use acetate as efficiently as those of the rumen.⁴⁰⁻⁴²

For the rabbit, the liver is an important site of acetate uptake, as it removes 43% of acetate reaching it⁴³ and practically all the labelled metabolites produced by the gut walls. The liver does not appear to accumulate glutamate to a great extent, it is possible that this amino acid is not stored, but rapidly utilised throughout the TCA cycle with production of alanine and glucose.⁴⁴⁻⁴⁶ The presence of an acetyl CoA synthetase (EC 6.2.1.1) mainly located in the cytosol fraction⁴⁷ indicates that in the rabbit liver as in sheep⁴⁸ and rat,⁴⁹ acetate is largely converted into fatty acids and carbon dioxide, because the enzymes involved in fatty acid synthesis are cytosolic. Leung and Bauman considered that in the rabbit the liver accounted for approximately two-thirds of the fatty acid synthesis.

In arterial plasma, acetate is the only VFA present in significant concentrations.^{5, 7, 16} There is

no detectable labelling of free amino acids, meanwhile very slight radioactivity was found in citrate, lactate, and glucose, metabolites which, like acetate, are directly assimilable by extra-hepatic tissues.⁵⁰⁻⁵⁴

In conclusion, acetate is clearly an important nutrient for the colonic mucosa particularly for the distal colon, mediating a symbiotic existence between bacteria and the hindgut mucosa. In the light of our present and previous⁸ findings it would be reasonable to regard VFA in rabbit and certain other species not just as providers of energy to the whole organism, but as a predominant energy source for the colonic mucosa.

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