Propionate absorption and metabolism in the rabbit hindgut

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SUMMARY Propionate disappearance from the loops of the hindgut in the rabbit was evaluated by measuring variations in the concentration of propionate 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) propionate into the caecocolonic loops. The rate of disappearance from the loops was always quantitatively significant but was greater in the proximal colon. Hindgut tissue metabolised propionate and the intensity of the metabolism varied with the segment studied; the proximal colon showed by far the highest propionate consumption. Radioactivity was found in a certain number of free amino acids, organic acids, sugars, lipid soluble substances and proteins. Propionate is an efficient respiratory fuel for the colonocyte and a good precursor for gluconeogenesis.

It is well known that volatile fatty acids (VFA) – for example, acetate, propionate and butyrate – are the major end products of microbial fermentation in the digestive tract of polygastric and monogastric animals.^{1,2} In the rabbit a large proportion of its energy requirement (30–40%) is derived from the catabolism of the bacterial metabolites formed in the hindgut;^{3,5} nearly half of the energy supplied is because of the butyrate.⁶ The large intestine tissues metabolise the VFA, the intensity of this metabolism varying with the location in the gut and the excretion pattern.^{5,9} It has been shown that butyrate⁶ is a good respiratory fuel for the colonocyte, and that acetate⁷ is 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. For monogastric animals, much less information is available about the metabolism of propionate by caecal^{10–11} and colonic mucosa. Thus the aim of the present investigation is to determine the extent to which propionate is metabolised during its absorption from the large intestine of the rabbit.

Methods

ANIMALS

Twelve male rabbits (*Oryctolagus cuniculus*) with an average weight of 2.5 kg were provided with oats and lucerne *ad libitum*. All experiments were carried out during the hard faeces period of the animals.^{12,13}

EXPERIMENTAL PROCEDURE

Anaesthesia was achieved by administering a pentobarbital solution, (20 mg/kg), through the marginal vein of the ear. After laparotomy the hindgut was removed and three loops of approximately equivalent parameters (wet weight, serosal area) were made from the caecum (median zone) and the proximal (aboral segment) and distal colon (for anatomical detail, see Snipes *et al.*¹⁴). The sites of the loops were chosen for suitability for cannulation of the venous drainage. Polyethylene catheters (PE 90) were inserted into small caecal and colonic veins draining each loop, and care was taken to ensure that blood circulation was not damaged. A carotid was exposed and cannulated (PE 190). The loops were washed with sodium chloride (9 g/l) at 38°C, then the three loops were filled with 5 ml of the experimental solution. This solution contained (mM): 159 Na, 17 K, 0.7 Ca, 4.3 Mg, 83 Cl, 26 HCO₃, 19 PO₄, 37 acetate, 10 propionate, 15 butyrate, had a pH of 7.1

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at 38°C and an osmolality of 330 mosmol/kg. A non-absorbable marker substance, 2 g polyethylene glycol/l (PEG) was added to the control solution. For each of the four metabolism studies, 30 μ Ci ($1\text{-}^{14}\text{C}$) propionate was added to the test solution. Blood samples (1 ml) were simultaneously collected through the catheters before experimentation and every five minutes for 20 minutes in a heparinised beaker. After each operation the volume of blood withdrawn was replaced with a physiological saline solution and the catheter was filled with a solution of heparin (40 IU/ml physiological saline). At the end of the incubation (20 min) blood samples were taken from the portal and hepatic veins. The loops were then emptied and the liquids 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.

The preparation of the colonic tissues used in this study has been described previously^{5,7} and is briefly summarised below. The tissues were homogenised in ice cold water using an Ultra-Turrax electric blender and with the homogenates, an extraction in boiling water allowed the volatile fraction to be recovered; the insoluble fraction (proteins) being removed by centrifugation (30 min at 10 000 g). After elimination of lipid soluble substances with chloroform (three extractions v/v) in a separating funnel, the aqueous fraction was recovered and concentrated in a Rotavapor (Büchi). From the aqueous fractions free amino acids, organic acids and sugars were isolated on a cationic (Dowex 50 $\times$ 4) and an anionic (Dowex 1 $\times$ 4) exchange resin column. The ^{14}C incorporation into volatile, insoluble, lipid soluble and aqueous fractions was measured in a liquid scintillation counter (Packard 460 C). The propionate of the blood was determined after ethanolic extraction as described by Rémésy and Demigné,¹⁵ while propionate of the test colonic solutions was measured after acidification of 1 ml of the sample with 100 μ l formic acid. The fatty acid was subsequently analysed by gas liquid chromatography (Intersmat IGC 120 DSL). Polyethylene glycol concentration was determined according to the procedure described by Hyden.¹⁶

CALCULATIONS

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

$$\text{Net propionate transport (\%)} = \frac{100 - \text{CoVo} - \text{CtVoPEG} / \text{PEGt} : \text{CoVo}}{100}$$

where Vo is the volume of fluid in the loops at the beginning of the experiment and Co and Ct are the propionate concentrations (μ mol/ml) at the begin-

ning and at the end of the experiment. ^{14}C incorporation in metabolites was expressed per g wet tissue; the mean weight (g) of caecal, proximal and distal colonic loops were respectively: 6.3 ± 1.89 , 6.5 ± 1.24 , 5.1 ± 1.32 and the corresponding mean serosal areas (cm^2) were: 27.0 ± 1.36 , 26.0 ± 1.37 and 23.0 ± 1.08 .

From values for hepatic blood supply, it was assumed that for the rabbit¹⁷ the portal vein and the hepatic artery represent 90 and 10% of the afferent blood flow respectively. Thus afferent plasma concentrations are: $0.9 \times (\text{portal vein}) + 0.1 \times (\text{aorta})$; the percentage of propionate hepatic uptake is calculated with the ratio:

$$\frac{(\text{hepatic plasma}) - (\text{afferent plasma})}{(\text{afferent plasma})} \times 100$$

All the results are presented as means with their standard errors. Differences were evaluated statistically using paired or unpaired Student's *t* test as appropriate.

Results

DISAPPEARANCE OF PROPIONATE IN INTESTINAL LOOPS

The concentration of propionate used in the present work was: 10.8 ± 0.15 mM which is regarded as physiological.^{12,13,18} Twenty minutes after introduction of the test solution the rate of propionate disappearance from the intestinal loops was always high; moreover it was invariably greater in the proximal colon compared with the caecum and distal colon. At the end of incubation 40–50% of the fatty acid inserted into the loop had disappeared (Fig. 1). If the disappearance is expressed in μ mol/unit serosal area/20 min the corresponding net propionate flux is: 1.2 ± 10 for the caecum, 1.5 ± 0.16 for the proximal colon and 1.1 ± 0.04 for the distal colon.

ARTERIAL AND VENOUS PLASMA CONCENTRATIONS OF PROPIONATE

The mean value of propionate in arterial plasma was 11 ± 0.8 μ mol/100 ml. Differences in the concentrations of propionate in the arterial and venous plasma were greater in the caecum than in the colon (Fig. 1). The concentration of the propionate in portal and hepatic plasma was 55 ± 3.1 and 11 ± 1.5 (μ mol/100 ml) respectively; therefore $80 \pm 2.4\%$ of the absorbed propionate was removed by the liver.

^{14}C INCORPORATION INTO BIOCHEMICAL FRACTIONS

Twenty minutes after introduction of ($1\text{-}^{14}\text{C}$) propionate in the intestinal loops radioactivity was found to be present in caecal, colonic, and hepatic tissues (Table 1). On a per gram wet tissue basis the ^{14}C

Fig. 1 Propionate disappearance from 10 caecocolonic loops is expressed as % of the initial propionate concentration in the test solution after PEG correction. Propionate concentration in venous plasma is expressed as % of the propionate concentration in the arterial plasma. Each column with its bar represent mean $\pm$ SE; propionate disappearance was significantly different at *** $p < 0.001$ between proximal colon and caecum or distal colon; propionate concentration in the venous plasma was significantly different at: *** $p < 0.001$, or ** $p < 0.01$ from propionate concentration in the arterial plasma.

content was higher in the colon than in the caecum and the liver: approximately 4–8 times more ($p < 0.001$). It is to be noted that the amount of radioactivity recovered in the caecocolonic tissue is small compared with the quantities of ^{14}C -propionate absorbed: 0.5–0.8% in the caecum, 1.8–2.9% in the proximal colon and 3.4–6.2% in the distal colon.

The labelling balance of the biochemical fractions varied (Fig. 2). In the hindgut walls about 67% of the total radioactivity was recovered in the aqueous fraction and 16% in the insoluble fraction (proteins); radioactivity was also found in the volatile (15%) and the lipid soluble (2%) fractions. For the liver tissue 80% of the ^{14}C detected was incorporated in the aqueous fraction, 17% in the insoluble fraction, the remainder (3%) was distributed on the lipid soluble and volatile fractions.

^{14}C INCORPORATION INTO METABOLITES ISOLATED FROM THE AQUEOUS FRACTION

On the autoradiographs, radiocarbon was always detected in a number of metabolites – that is, free amino acids, organic acids and sugars (Table 2; Fig. 3). In the gut walls the radioactivity of the aqueous fraction was mainly accounted for by organic acids (56%), but also by amino acids (24%) and sugars (20%). In the case of the liver a high percentage of the radioactivity was detected in the free amino acids (86%) and some label was recovered in sugars (11%) and organic acids (3%).

The details of the ^{14}C distribution into metabolites present in the aqueous fraction are reported in Table 2. It is to be noted that in the hindgut walls the radioactivity in the free amino acids was mainly associated with glucogenic substrates – that is,

Table 1 Incorporation of ^{14}C into volatile, lipid-soluble, aqueous and insoluble fractions in hindgut and liver tissues 20 minutes after introduction of ($1\text{-}^{14}\text{C}$) propionate into caecocolonic loops

	Fractions				
	Volatile	Lipid soluble	Aqueous	Insoluble	Total
Caecum	7.8±1.24	0.8±0.27	9.6±1.69	9.3±2.12	27.5±6.32
Proximal colon	19.1±4.78	0.1±0.09	93.8±23.45	10.6±3.82	124.0±31.07
Distal colon	7.5±2.58	0.4±0.15	225.5±56.38	15.1±5.06	248.5±74.53
Liver	1.0±0.32	0.1±0.07	23.3±9.45	5.0±2.31	29.4±12.18

^{14}C incorporation into the different fractions is expressed in 10^3 dpm/g wet wt/20 min. Each value represents the mean±SE of four samples of tissue. Differences in the variations of the ^{14}C incorporation into the four groups were significantly different at $p<0.001$ between caecal or liver and proximal or distal colon, and between proximal and distal colon.

glutamate+glutamine (28%), methionine (25%), alanine (20%), aspartate+asparagine (18%) and glycine (8%). The labelled organic acids were mainly adipic and sebacic acids (41%); nevertheless ^{14}C was also found in TCA cycle acids (36%) – that is, succinate, fumarate, malate and oxaloacetate. Finally, 16% of the ^{14}C was incorporated into lactate.

In the case of the liver tissue the highest labelling rate found in the free amino acids was mainly due to glutamate+glutamine (44%) and to aspartate+asparagine (38%).

Certain of the labelled metabolites present in the gut tissues were detectable in the venous plasma. The total radioactivity measured in the aqueous fraction of the portal plasma was 3.8×10^3 dpm/ml, and 30% of the total ^{14}C was recovered in the free amino acids (alanine, aspartate and glutamate), 39% in the organic acids (succinate, malate and lactate), the remainder (31%) in the glucose. The radioactivity found in arterial plasma ranged between 1.1 to 1.3×10^3 dpm/ml, and the ^{14}C was only found in the lactate and glucose.

Fig. 2 ^{14}C incorporation into volatile, lipid soluble, aqueous and insoluble fractions in caecal (CC), proximal and distal colon (PC, DC) and liver (L) tissues after introduction of ($1\text{-}^{14}\text{C}$) propionate into caecocolonic loops. Values are expressed as % of the total ^{14}C incorporation.

Discussion

This study shows that the absorption capacity of the large intestine for the propionate did not diminish with the increasing distance from the caecocolonic junction, as the variations in plasma propionate concentration might suggest. In the rabbit^{7,19} as in ruminants, the metabolic activity of the gut epithelium explains the variations in VFA concentrations in the venous plasma. The low concentrations of this nutrient in the colonic bloodstream imply that its catabolism by the gut wall is extremely efficient, particularly in the proximal colon. In gut walls, the radioactivity found in various metabolites confirms that propionate is implicated in the metabolism of the colonocyte, but the amount of label recovered in the tissues is small compared with the quantities of ^{14}C -propionate absorbed. It appears, therefore, that a large proportion is metabolised into carbon dioxide, and to a lesser extent into free amino acids, organic acids, and sugars. Some of the molecules can be stored or transported away from the gut, as most of the labelled metabolites present in gut tissues are recovered in the blood.

For polygastric animals an unknown and probably variable (30–60%) of the absorbed propionate is oxidised or converted to other products during the absorption process through the rumen wall.^{20,22} Evidence of extensive metabolism of propionate to lactate by the rumen epithelium has now been challenged by the results of a number of studies^{20,21,23} carried out with a variety of experimental techniques. But, in experiments with calves, Weigand *et al.*^{24,25} estimated that the true conversion of this bacterial metabolite into lactate averaged less than 5%, and when estimates were corrected to lactate derived directly from propionate *via* glucose they averaged 2.3%. Similar low estimates were found *in vitro* for the rumen epithelium of ewes.^{22,26} The nature and the distribution of the ^{14}C in the rabbit hindgut tissues show that the propionate is used by the colonocyte as a source of energy and as substrates for glucone-

Table 2 Incorporation of ^{14}C into free amino acids, organic acids and sugars of the aqueous fraction in hindgut and liver tissues 20 minutes after introduction of ($1\text{-}^{14}\text{C}$) propionate into caecocolonic loops

	Tissues	
	Hindgut	Liver
Free amino acids		
Alanine	20.2 ± 5.26	6.0 ± 1.59
Aspartate + asparagine	18.3 ± 11.64	37.9 ± 10.36
Glutamate + glutamine	28.0 ± 6.75	44.1 ± 9.62
Glycine + serine	8.2 ± 5.51	8.0 ± 1.59
Methionine	25.3 ± 1.51	4.0 ± 1.47
Total	100	100
Total ^{14}C incorporation (10^3 dpm/g wet wt/20 min)	19.6 ± 6.56	20.0 ± 6.02
Organic acids		
TCA cycle		
Succinic	4.0 ± 1.36	(-)
Fumaric	21.8 ± 6.23	25.0 ± 10.50
Malic	7.1 ± 2.31	(-)
Oxaloacetic	3.3 ± 1.66	(-)
Adipic	28.0 ± 13.26	51.3 ± 12.52
Glyceric	3.6 ± 1.86	(-)
Glycolic	2.4 ± 8.37	(-)
Lactic	16.4 ± 3.73	23.7 ± 3.17
Sebacic	13.4 ± 4.26	(-)
Total	100	100
Total ^{14}C incorporation (10^3 dpm/g wet wt/20 min)	45.7 ± 13.43	0.7 ± 0.87
Sugars		
Fructose	17.1 ± 16.97	(-)
Glucose	73.5 ± 26.30	77.5 ± 27.67
Poses	9.4 ± 9.30	22.5 ± 10.28
Total	100	100
Total ^{14}C incorporation (10^3 dpm/g wet wt/20 min)	16.6 ± 7.83	2.6 ± 1.44

^{14}C incorporation into the different metabolites of each biochemical group is expressed as % of the total ^{14}C incorporation in the aqueous fraction. Each value represents the mean ± SE of 12 samples of gut tissues and four samples of liver tissues; (-) represents nil results.

genesis, lipogenesis and protein synthesis. In the rabbit, as in the rat¹¹⁻²⁷ and the guinea-pig,²⁸ as well as in ruminants,²⁹ propionate enters the metabolic pool via the methylmalonyl pathway. Indeed, the results obtained are consistent with the conversion of this fatty acid into succinate and the oxidation of succinate into oxaloacetate which is the precursor of labelled amino acids – that is, aspartate, methionine, glycine, serine and glutamate. Subsequently, the latter metabolites can be diverted towards either gluconeogenesis and/or protein synthesis. Acetate is considered to be the primary substrate for fatty acid synthesis in the ruminant³⁰⁻³² and the rabbit.³³⁻³⁴ Interestingly the present investigation suggests that propionate could also be a lipogenic precursor because a substantial labelling of free fatty acids (adipic, sebacic) and lipid soluble substances is found. This is in agreement with Prior *et al.*³⁵⁻³⁶ and with Whitehurst *et al.*³⁷ showing that lactate and

Fig. 3 ^{14}C incorporation into free amino acids, organic acids and sugars of the aqueous fraction in hindgut and liver tissues after introduction of ($1\text{-}^{14}\text{C}$) propionate into caecocolonic loops. Values are expressed as % of the total ^{14}C incorporation in the aqueous fraction.

propionate are important precursors for fatty acids *in vivo* in the sheep and the steer fed *ad libitum*. Thus the existence of this metabolic pathway may explain the low portion of ^{14}C detected on glucose and lactate in the hindgut walls of the rabbit. It is to be noted that both the absorption and metabolism of propionate and butyrate,⁶ as for electrolyte handling^{12,13} are relatively intense in the proximal colon. Indeed in an earlier work¹¹ on the rabbit, it was shown in the large intestine, that the absorption of the VFA and cations (Na, K) is linked, likewise in the colon of the rat,³⁸ goat,³⁹ pig,⁴⁰ sheep,⁴¹ and man;⁴² the link has been postulated to be the recycling of hydrogen ions.^{13,40-43} But the close relationship between VFA and cation absorption, as well as VFA metabolism in the rabbit, the goat,³⁹ the equine⁴³ and man⁴⁴ also supports the hypothesis that the energy gained from colonic VFA metabolism may be used for active transfer of cations across the hindgut epithelia.

In the present experimental conditions, propionate was largely removed (80%) from the portal blood during its passage through the liver; similar uptake values have been obtained for ruminants⁴⁵⁻⁴⁶ and the rat.⁴⁷ The biochemical pathway through which propionate is normally launched into the TCA cycle in the liver is the same as that for the gut tissues. But the labelling of glucogenic amino acids in the former organ is greater compared with the latter, which agrees with the report of Gaitonde *et al.*²⁷ for the rat, these authors have shown that ^{14}C propionate is a better source of free amino acids in the liver than in other tissues. Thus, as demonstrated by previous studies of the liver in rabbits,^{5,48} rats,⁴⁹ and ruminants^{50,51} propionate is an efficient precursor of substrates for hepatic gluconeogenesis.

In arterial plasma, there is no detectable labelling

of free amino acids suggesting that most of the molecules entering the portal circulation are taken by the hepatocyte.^{51,52} Nevertheless very slight radioactivity was found in lactate and glucose which are directly assimilable by peripheric tissues. Surprisingly, in the rabbit the propionate not subject to liver metabolism is not metabolised by extra-hepatic tissues^{19,53} as is the case in ruminants.⁵⁴

In conclusion, like butyrate⁶ and acetate,⁷ the metabolic fate of propionate depends on the tissue which metabolises the compound. Propionate is clearly an important nutrient for the large intestine mucosa, particularly for the colonic mucosa, it is an efficient respiratory fuel for the colonocyte. In addition, it is also a good precursor for gluconeogenesis, lipogenesis and protein synthesis.

Scintillation counting and analysis of amino acids, organic acids and sugars were performed at the Centre de Physiologie Végétale LA CNRS 241-UPS. The author wishes to thank Ms Marty and Mr Abravanel for their invaluable help and advice in the different techniques used.

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