

Absorption and metabolism of the volatile fatty acids in the hind-gut of the rabbit

BY JOSETTE MARTY* AND MICHELE VERNAY†

Institut de Physiologie, 2 rue F. Magendie, 31400–Toulouse, France

(Received 12 April 1983 – Accepted 5 October 1983)

1. Volatile fatty acids (VFA) absorption in the large intestine of the anaesthetized rabbit was evaluated by measuring variations in the concentration of VFA in intestinal loops and plasma arteriovenous differences. Metabolic conversions were studied using [1-¹⁴C]acetate, [1-¹⁴C]propionate and [3,4-¹⁴C]butyrate.
2. The hind-gut tissues metabolized the three VFA, although this metabolism varied with the segment studied. Butyrate was the best respiratory fuel for the colonic wall, followed by propionate; acetate participated also, but it was mainly converted to glutamate.
3. The liver was the main organ metabolizing absorbed propionate and butyrate; acetate was available for extrahepatic tissue metabolism.
4. For the rabbit, VFA represented about 40% of the maintenance energy requirement.

It is well known that the volatile fatty acids (VFA), e.g. acetate, propionate and butyrate, are the major end-products of microbial fermentation in the ruminant digestive tract and are absorbed chiefly through the rumen wall into the portal blood (Cook & Miller, 1965). These VFA are major energy sources of ruminants providing 70–80% of the animal's energy requirement (Bergman *et al.* 1965); acetate in particular contributes as much as 50% of the energy supply (Sabine & Johnson, 1964). Several groups of investigators have reported that 75–80% of the butyrate is metabolized to ketone bodies during absorption into the portal circulation, while some conversion of butyrate to acetate occurs (Stevens & Stettler, 1966; Emmanuel, 1980). In sheep, Bergman & Wolff (1971) and Weekes & Webster (1975) showed that 50% of absorbed propionate is metabolized in the mucosa. In the portal vein only acetate and propionate are present as such and are presented to extrarumen tissues for metabolism. The propionate is metabolized mainly by liver and acetate by extrahepatic tissues (Cook & Miller, 1965; Mayfield *et al.* 1966; Weigand *et al.* 1972*a, b*; Pethick *et al.* 1981). Much less information is available about short-chain fatty acid metabolism by caecal and colonic mucosa. In the pig substantial amounts (50% of total VFA) appear to be used by the tissue (Argenzio & Southworth, 1974), but in the caecum of rabbits (Henning & Hird, 1972*c*) and rats (Rémésy & Demigné, 1976) only about 12% of butyrate is converted to ketone bodies and negligible conversion occurs in the colonic wall. In man, using isolated colonocytes, Roediger (1980) showed that butyrate is an important energy source for the colonocyte, accounting for the major part of energy needs even in the presence of glucose. Bacterial fermentation of the rabbit caeco-colonic contents yields VFA. Henning & Hird (1972*a*), Vernay & Raynaud (1975) and Parker (1976) demonstrated the production of these acids. Acetate is the most abundant acid followed by butyrate and propionate and this feature appears to distinguish the domestic and wild rabbit from most other mammalian species. Absorption of VFA has been shown by Beauville *et al.* (1974), Parker (1976), Leng (1978), Bonnafous & Raynaud (1978*a*). These VFA constitute a source of energy for the rabbit calculated to provide 12% of the energy requirement by Hoover & Heitmann (1972) and 30% by Parker (1976). In the present work, we determined the extent of absorption

* Present address: Laboratoire de Biologie générale ERA-CNRS 327, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse-Cédex, France.

† For reprints.

of VFA in the large intestine in the anaesthetized rabbit by plasma arteriovenous differences in VFA concentrations, compared with VFA disappearance from the intestinal loops. Blood ketone bodies, lactate and glucose concentrations were also studied. Metabolic transformations of the three VFA were studied in the caecum, colon and liver tissues after introduction of ^{14}C -labelled VFA into the intestinal loop. The nutritional value of these fatty acids was estimated.

MATERIAL AND METHODS

Animals

Male common rabbits (*Oryctolagus cuniculus*) with an average weight of 2.5 kg were used. The animals were provided with oats and lucerne (*Medicago sativa*) and *ad lib.* water. In the present study the nature of the pellets contained in the hind-gut was not taken into account. The metabolic variations linked to the excretory cycle of the rabbit will be the purpose of a further publication.

Surgical procedure

Anaesthesia of the twenty-four animals was achieved by administration, via a marginal vein of the ear, of a pentobarbital solution (20 mg/kg). After laparotomy, the intestine was removed and intestinal loops were made on the caecum, and on the ascendant, transverse and descendant colon. The contents of the loops were carefully washed out with physiological saline (9 g sodium chloride/l) at 38°. Each animal so prepared was put into a thermo-regulated chamber. The loops were filled with 5 ml of the experimental solution (Leng & Hörnicke, 1975) incubated for 20 min. A non-absorbable marker substance (2 g polyethylene glycol/l) was added to the test solution. For metabolism studies, 30 μCi ^{14}C -labelled VFA was introduced in the solution: [1- ^{14}C]acetate or [1- ^{14}C]propionate or [3,4- ^{14}C]butyrate. [1- ^{14}C]acetate enters directly into the TCA cycle, [1- ^{14}C]propionate enters the TCA cycle as succinate by the intermediate methylmalonyl CoA. The use of [3,4- ^{14}C]butyrate ensures that all the labelling enters the TCA via the Lynen spiral, without loss by decarboxylation. Samples of blood were collected by puncturing the blood vessels directly with a needle. Venous blood was removed from the caeco-colonic, portal and hepatic veins and from the inferior vena cava. Arterial blood was also collected. At the end of the experiment, loops were emptied and removed together with a portion of each lobe of the liver. These tissues were placed into ice. The choice of pentobarbital as the anaesthetic takes into account the findings of Bito & Eakins (1969) who reported that it induces little modification of blood chemistry: decrease of oxygen potential is not significant, glucose and sodium values are not affected and potassium concentration increases only slightly.

Analytical methods

Analyses on plasma were performed after deproteinization with 2 vol. 0.6 M-perchloric acid. Ketone bodies were determined enzymically by the method of Williamson & Mellanby (1963), lactate was measured by enzymic determination with L-lactic dehydrogenase (*EC* 1.1.1.27) from Boehringer, according to Hohorst's (1963) procedure, glucose was detected by the method of Werner *et al.* (1970) and VFA were determined by gas-liquid chromatography (Intersmat IGC 120 DSL) using the technique described by Rémésy & Demigné (1974). VFA were extracted from plasma with ethanol in the presence of a known quantity of internal standard (isobutyric acid). After addition of sodium hydroxide and evaporation of the ethanolic solution of the sodium salts the residue was dissolved in a dilute solution of orthophosphoric acid for analysis. In the lumen solution, VFA were detected after acidification of 1 ml of the sample with 100 μl formic acid. Polyethylene glycol was determined according to the method of Hyden (1956).

Intestinal loop and liver tissues were blotted, weighed and the area of the loops was measured by planimetry. The tissues were homogenized 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). To obtain an adequate aqueous phase it was necessary to eliminate lipid-soluble substances with chloroform (three extractions v/v) in a separating funnel. The aqueous phase was recovered and concentrated in a Rotavapor (Büchi). A portion of the extract was submitted to electrophoresis (2100 V, 40 mA, 1 h) in an acid medium (formic acid-acetic acid-water, 17:57:926 by vol., pH 2). After drying, double development ascendant chromatography was used (Schurmann, 1969; Larvergne *et al.* 1979). The autoradiographs were obtained by leaving the electrophoresis in prolonged contact (3-4 months) with photographic plates (Kodak Kodirex). The radioactive spots were removed, eluted and ^{14}C activity was determined in a liquid scintillation counter (Packard 460 C). For plasma samples the same procedure was followed but protein precipitation with sulphosalicylic acid (15 g/l) preceded chloroform extraction.

Calculations

Net transport values and ^{14}C incorporation in metabolites were expressed per g wet tissue. The mean (with SE) weight (g) of caecal, ascendant, transverse and descendant colonic loops were respectively: 6.3 (1.89), 6.1 (1.64), 6.5 (1.24), 5.1 (1.32) and the corresponding 10^{-4} mean (with 10^{-4} SE) serosal areas (m^2) were: 27 (1.4), 24 (1.7), 26 (1.4), 23 (1.1). To facilitate comparison of the various amounts of VFA and other metabolites irrespective of their concentration, percentage values were calculated. The daily contribution of the VFA absorbed to the maintenance energy requirement of the rabbits was calculated. Metabolizable energy of VFA is equal to the energy value (kJ/mol) of its heat of combustion, i.e. acetate, propionate and butyrate equal 875.3, 1534.9 and 2191.6 respectively (Hodgman *et al.* 1958). For each experimental series, the arithmetic mean and the standard error are given. The results were analysed using Student's *t* test or paired *t* test when appropriate.

RESULTS

Disappearance of VFA in intestinal loops

At 20 min after the introduction of the test solution to the intestinal loops, a marked disappearance of VFA always occurred increasing with the size of the molecule (Table 1). When the mean (with SE) initial concentration (mM) of the acetate, propionate and butyrate was 37.0 (0.52), 9.7 (0.14), 14.7 (0.27) respectively, the corresponding values for disappearance were (%) butyrate 50, propionate 43, acetate 30; moreover, the amount of VFA disappearing did not differ between sections of the intestine.

Plasma concentrations of VFA, ketone bodies, glucose and lactate

The plasma arteriovenous differences in VFA were greater in the caecum and ascendant colon than in the transverse and descendant colon (Table 2). The order of appearance in the blood stream was: $C_4 > C_3 > C_2$ in the first group only (Fig. 1). The liver removed about 43% of the acetate reaching it, while the uptake of propionate and butyrate was 77 and 84% respectively. The plasma arteriovenous difference in VFA was negative in peripheral blood; in this case the acetate uptake was about 18%, the other acids were not involved. For ketone bodies, glucose and lactate the arteriovenous differences in the blood were not significant (Table 3). The plasma glucose concentration in the rabbit was very high (9.99 mM).

Table 1. *Disappearance of volatile fatty acids (VFA) from intestinal loops*
(Mean values with their standard errors; no. of experiments in parentheses)

Loops	VFA	VFA disappearance (10)			
		$\mu\text{mol/g per h}$		%*	
		Mean	SE	Mean	SE
Caecum	Acetate	43	3.6	28	3.0
	Propionate	17	1.3	41	1.0
	Butyrate	28	1.4	45	3.7
Ascendant colon	Acetate	25	1.4	26	2.9
	Propionate	12	0.9	53	2.5
	Butyrate	20	1.1	60	2.7
Transverse colon	Acetate	35	1.3	36	2.9
	Propionate	14	1.9	48	2.5
	Butyrate	24	2.2	52	2.7
Descendant colon	Acetate	45	2.2	29	2.7
	Propionate	16	0.8	38	2.7
	Butyrate	28	2.1	46	3.8

The mean (with SE) weight (g) of the caecal wall was 35.4 (2.42), of the ascendant and transverse colon 22.0 (1.56) and of the descendant colon 28.0 (3.18).

* Values are expressed as a percentage of the initial concentration in the test solution.

Table 2. *Volatile fatty acids concentrations ($\mu\text{mol/l}$) in arterial and venous plasma of the rabbit*

(Mean values with their standard errors; no. of experiments in parentheses)

Plasma	Acetate (15)		Propionate (15)		Butyrate (15)	
	Mean	SE	Mean	SE	Mean	SE
Arterial	1965	305.5	135	18.5	140	25.5
Caecal vein	4555	514.5	575	56.0	1020	104.5
	***		***		***	
Ascendant colonic vein	4362	605.9	527	67.5	602	85.3
	***		***		***	
Transverse colonic vein	2465	355.5	205	41.5	220	35.5
	NS		NS		NS	
Descendant colonic vein	2381	215.0	215	24.5	240	32.0
	NS		•		*	
Portal vein	4340	712.1	637	47.3	824	101.6
	***		***		***	
Hepatic vein	2459	309.1	146	31.8	133	52.0
	NS		NS		NS	
Inferior vena cava	1605	257.3	146	18.5	127	43.6
	*		NS		NS	

NS, not significant ($P > 0.05$).

Mean values for arterial and venous plasma concentrations were significantly different: * $P < 0.05$, *** $P < 0.001$.

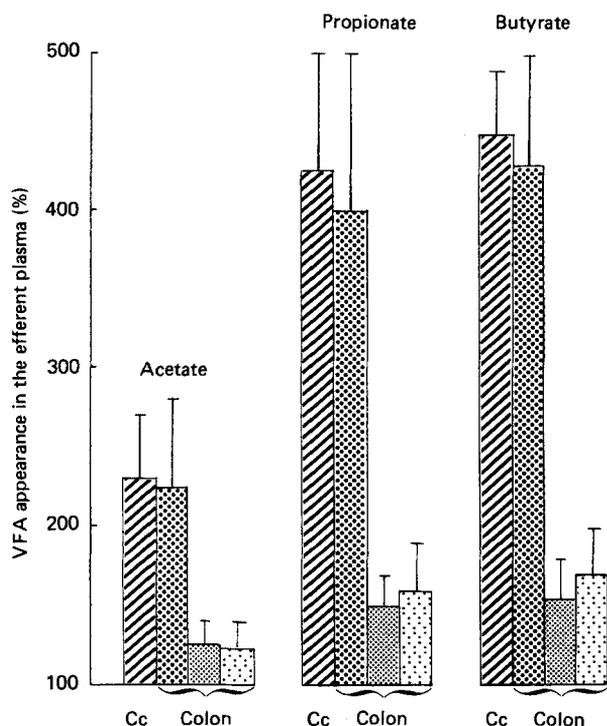


Fig. 1. Appearance of volatile fatty acids from efferent plasma. Points are mean values with their standard errors represented by vertical bars for fifteen experiments. Values are expressed as a percentage of the concentration in the corresponding artery. Cc, caecal vein (▨); colon, colonic veins (▩) ascendant, (▧) transverse, (▫) descendant.

Table 3. Glucose, lactate and ketone bodies concentrations ($\mu\text{mol/l}$) in arterial and venous plasma of the rabbit

(Mean values with their standard errors; no. of experiments in parentheses)

Plasma	Glucose (21)		Lactate (24)		Ketone bodies (12)	
	Mean	SE	Mean	SE	Mean	SE
Arterial	9990	440	3160	238	120	24
Caecal vein	9330	636	2910	300	180	11
Ascendant colonic vein	9380	679	3080	292	110	36
Transverse colonic vein	9050	524	2890	270	140	25
Descendant colonic vein	9280	669	3200	252	170	32
Portal vein	9280	669	3280	292	200	45

Mean values for arterial and venous plasma were not significantly different ($P > 0.05$).

^{14}C incorporation in metabolites present in the tissue extracts and plasma

The amount of ^{14}C recovered differed, depending on the VFA introduced and the tissues concerned. On a per g wet tissue basis the ^{14}C content was higher in the liver than in the intestinal walls for [1- ^{14}C]propionate: approximately 2.5 times more; the labelling was

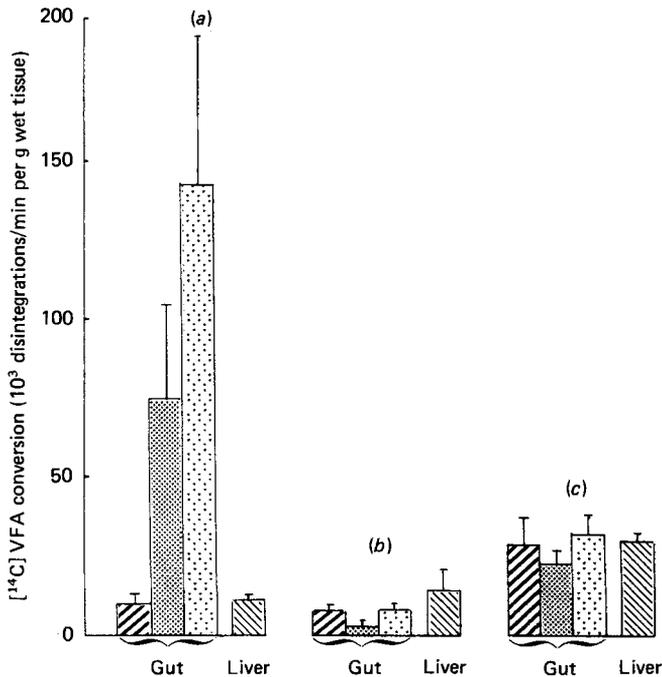


Fig. 2. Total ^{14}C incorporation into metabolites in tissue extracts after introduction of (a) $[1\text{-}^{14}\text{C}]$ acetate; (b) $[1\text{-}^{14}\text{C}]$ propionate; (c) $[3,4\text{-}^{14}\text{C}]$ butyrate in the loops. Points are mean values with their standard errors represented by vertical bars for four experiments with $[1\text{-}^{14}\text{C}]$ acetate, two experiments with $[1\text{-}^{14}\text{C}]$ propionate and four experiments with $[3,4\text{-}^{14}\text{C}]$ butyrate. Values are expressed in 10^3 disintegrations/min per g wet tissue. (/) caecal, (.) transverse colon, (x) descendant colon, (—) liver.

Table 4. Conversion (10^2 disintegrations/min per g wet tissue) of ^{14}C -labelled acetate, propionate or butyrate to free amino acids (FAA), carboxylic acids (CA) and sugars (S) in tissues of the rabbit

(Mean values with their standard errors; no. of experiments in parentheses)

Tissues		$[1\text{-}^{14}\text{C}]$ acetate (4)		$[1\text{-}^{14}\text{C}]$ propionate (2)		$[3,4\text{-}^{14}\text{C}]$ butyrate (4)	
		Mean	SE	Mean	SE	Mean	SE
Caecum	FAA	79	18.0	19	7.2	80	15.7
	CA	16	5.7	41	10.5	160	54.3
	S	10	6.4	23	10.8	52	20.6
	Total	105	29.1	83	9.5	292	88.9
Transverse colon	FAA	581	252.0	7	5.7	91	23.7
	CA	143	36.6	17	7.9	80	31.3
	S	35	9.3	1	4.6	65	19.8
	Total	759	285.6	25	16.1	236	35.4
Descendant colon	FAA	1047	376.3	33	12.7	148	30.1
	CA	322	109.3	53	21.8	107	37.7
	S	52	21.1	3	6.3	69	20.8
	Total	1421	482.9	89	13.6	324	52.9
Liver	FAA	63	7.6	129	60.2	135	22.9
	CA	35	11.5	4	8.7	48	9.5
	S	12	5.7	17	9.4	124	18.7
	Total	110	8.6	150	69.0	307	32.1

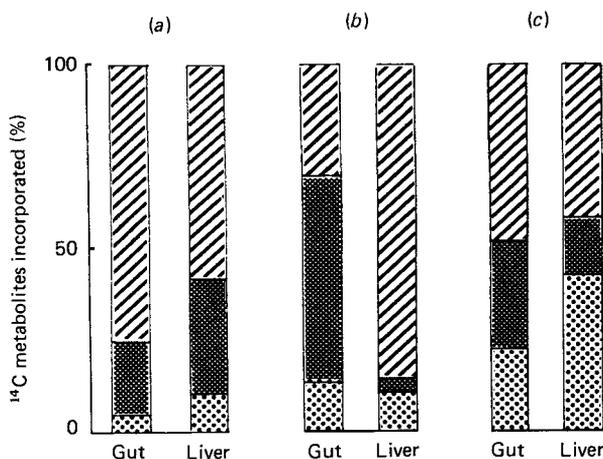


Fig. 3. ^{14}C incorporation into metabolites, (/) free amino acids, (■) carboxylic acids, (●) sugars, in gut and liver, after introduction of (a) $[1-^{14}\text{C}]$ acetate; (b) $[1-^{14}\text{C}]$ propionate; (c) $[3,4-^{14}\text{C}]$ butyrate in the loops. Points are mean values for four experiments with $[1-^{14}\text{C}]$ acetate, two experiments with $[1-^{14}\text{C}]$ propionate and four experiments with $[3,4-^{14}\text{C}]$ butyrate. Values are expressed as a percentage of the total ^{14}C incorporation. Gut percentages are the mean values for the three regions.

identical for $[3,4-^{14}\text{C}]$ butyrate and seven times less for $[1-^{14}\text{C}]$ acetate (Table 4). In the intestinal tissues, the ^{14}C content with acetate increased as the distance from the caeco-colonic junction increased. The concentration of ^{14}C in the descendant colon was about thirteen times more than in the caecum. This pattern was not found with the other acids (Fig. 2).

The radioautochromatograms of intestinal extracts showed ^{14}C in numerous products of intermediary metabolism such as free amino acids, carboxylic acids and sugars (Table 4). The labelling balance of the three groups was dependent on the VFA introduced in the loop. A considerable percentage of the radioactivity was found in amino acids which accounted for 75% of the ^{14}C recovered after the injection of acetate; this value decreased for the other acids, i.e. 23–36% with propionate and 27–46% with butyrate. The percentage of the radioactivity found in carboxylic acids accounted for 15–22% of the ^{14}C content with acetate, 50–60% with propionate and 30–55% with butyrate (Fig. 3). Irrespective of differences in the ^{14}C content of the tissues, the percentage of free amino acids, carboxylic acids and sugars were remarkably similar, both in the intestinal and hepatic tissues, with acetate and butyrate. In the case of propionate, a high percentage of the ^{14}C in the liver was recovered in amino acids (87%) compared with 30% in amino acids in intestinal walls ($P < 0.01$).

For all three VFA, the radioactivity in amino acids was mainly associated with glutamate (20–80%), aspartate (7–40%), alanine (2–17%), asparagine (1–18%) and glutamine (3–10%). ^{14}C -labelling was also recovered in glycine–serine (8%) and cysteine (25%) with propionate, valine (1%) with acetate and basic amino acids (18%) with butyrate. After the introduction of $[1-^{14}\text{C}]$ acetate the greatest labelling found in colonic tissues was due to a strong accumulation of glutamate (77%), the concentration in the descending colon was about fourteen times greater than that in the caecum. With acetate and butyrate, ^{14}C was found in TCA cycle acids, citrate (12–30%), ketoglutarate (1%), succinate (20–30%), fumarate (5–12%) and malate (3–20%); with propionate ^{14}C was only found in succinate (3%), fumarate (12%), malate (3%) and oxaloacetate (4%). There was no labelling of citrate and ketoglutarate. With the three VFA, other ^{14}C -labelled carboxylic acids were detected (glycerate, glycolate, lactate and uronates); with butyrate and propionate ^{14}C was also

recovered in adipic and sebacic acids. In gut tissues, radioactivity was regularly detected in glucose and phosphorylated compounds (10–40%) and sometimes an equivalent labelling was found in fructose, maltose and sucrose. Of the ^{14}C content in sugars, 74% was detected in glucose with propionate and 11–25% with acetate and butyrate. For each ^{14}C -labelled VFA introduced into the loops, there was no difference in intestinal and liver radioautographs. In the case of acetate the great accumulation of glutamate observed in intestinal tissues was not so intense in hepatic tissues. Most of the labelled metabolites found in gut tissues were recovered in portal plasma, this was not the case in arterial plasma where radioactivity was only detected in citrate, lactate and glucose. The total radioactivity measured in portal vein plasma ($10^6 \times$ disintegrations/min per l) for acetate, propionate and butyrate was 5.2, 3.8 and 4.4 respectively; meanwhile the radioactivity found in arterial plasma ranged between 1.1 and 1.3.

Evaluation of metabolizable energy derived from VFA absorption

The quantity of VFA that disappeared in the intestinal loops enabled us to evaluate the energy supplied by these fatty acids (Table 1). The flux of VFA through the hind-gut, equivalent to 244 kJ/24 h, represented 37.7% of the maintenance energy requirement of the animals.

DISCUSSION

A review of the literature suggests that the values for volatile fatty acids, glucose and lactate in the blood of the rabbit are a function of the animal's food supply and physiological state. In lactating rabbits, blood glucose levels range between 5 and 5.6 mM (Jones & Parker, 1977; Jouglar, 1983); in animals fed on commercial food the concentrations (mM) of VFA, lactate and glucose are 0.7–1.1, 1.1–1.6 and 7.1–7.9, respectively (Kozma *et al.* 1974; McMillan *et al.* 1975; Parker & Mould, 1977; Gisberti *et al.* 1981; Jouglar, 1983; M. Vernay, unpublished results); in animals provided with oats and lucerne the amounts are two to three times higher: VFA 1.7–1.9 (Beauville *et al.* 1974; Bonnafeous & Raynaud, 1978*a, b*), lactate 3.9–8.8 (Alexander & Chowdhury, 1958; Kinsey, 1961) and glucose 8–9 mM (Duke-Elder, 1961; Jouglar, 1983). These facts and the use of different experimental procedures (e.g. conscious or anaesthetized rabbits, surgery) may explain some of the discrepancies found in the literature, since it is recognized that anaesthesia affects the blood flow rate (Katz & Bergman, 1969).

The absorption capacity of the caecum and colon did not diminish with increasing distance from the caeco-colonic junction, as the variations in plasma VFA might suggest. We showed that irrespective of the intestinal loops the net flux of VFA was always important and increased with the size of the molecule. It is known that absorption of VFA is also linked with electrolyte transport (Leng, 1978) and is under hormonal control (M. Vernay and J. Marty, unpublished results). In ruminants, the metabolic activity of the rumen epithelium explains the variations in plasma VFA concentrations. This epithelium is most active on butyrate, less active on propionate and least active on acetate (Cook *et al.* 1969; Weigand *et al.* 1972*a, b*; Weekes & Webster, 1975). Butyrate is oxidized to ketone bodies (80–90%) and carbon dioxide (Hird & Symons, 1961; Emmanuel, 1980). Bergman & Wolff (1971) and Weekes & Webster (1975) found 50% of the propionate produced in the rumen was metabolized during absorption. Much of this acid is oxidized into CO_2 , pyruvate and lactate. Weigand *et al.* (1972*a, b*), Weekes (1972) and Weekes & Webster (1975) concluded that the true conversion of propionate into lactate averaged only 2.3% and can be formed from glucose by the glycolytic pathway. Acetate is converted to CO_2 or free fatty acids (Hanson & Ballard, 1967; Hood *et al.* 1972; Pethick *et al.* 1981). In the rabbit the striking VFA decrease in colonic veins probably can be explained by a greater metabolism by the

gut tissue, although arteriovenous differences of ketone bodies, glucose and lactate did not change significantly. This is not in good agreement with Henning & Hird (1972c) and McMillan *et al.* (1975) who reported that fatty acids pass into the bloodstream by simple diffusion, with little metabolism in the wall of the large intestine. In gut extracts the radioactivity found in various metabolites suggests that VFA are implicated in the metabolism of the caeco-colonic wall, but the amount of radioactivity recovered in these tissues is small compared with the quantities of fatty acids absorbed. It appears that a large proportion is metabolized into CO₂, free amino acids, carboxylic acids and sugars; some of the metabolites can be stored or transported away from the gut, since most of the labelled metabolites present in gut tissues are recovered in portal blood.

In the case of acetate, ¹⁴C incorporation is particularly important in colonic tissues. The great accumulation of glutamate indicated that acetate is introduced into the TCA cycle and converted into ketoglutarate (Marty *et al.* 1981). The accumulation of glutamate may be due to a difference in rates between acetate incorporation in the cycle and the oxidation capacity of the implicated cells. The difference between the caecum and descendant colon may be due also to the extent to which glutamine is metabolized beyond glutamate. As far as glutamine metabolism is concerned, the caecum and the beginning of the colon appear to resemble the small intestine, as described by Watford *et al.* (1979). Glutamine is extensively oxidized and converted to alanine. The present finding in the rabbit hind-gut is in line with that of Pinkus & Windmueller (1977) in the rat and of Roediger (1980) in the human colon who showed that there is an aborally-decreasing gradient of glutamine utilization from jejunum to rectum associated with decreasing glutaminase (*EC* 3.5.1.2) and glutamate dehydrogenase (*EC* 2.3.2.1) activities in intestinal mucosa. One can also consider glutamate as a reserve form which can be diverted towards either oxidative metabolism or protein synthesis. A kinetic study involving a longer absorption time might confirm this hypothesis. Our results may explain the observations of Sabine & Johnson (1964) in the ruminant, who showed that a constant specific activity of blood acetate is reached rapidly during continuous infusion of ¹⁴C acetate whereas the specific activity of expired CO₂ does not reach equilibrium for 4 h. They supposed that acetate is rapidly converted into other metabolites which are more slowly oxidized into CO₂. Substantial labelling of glutamate was also found by Mayfield *et al.* (1966) in *in vitro* incubations of various tissue homogenates of sheep with ¹⁴C acetate.

It appears that propionate enters the metabolic pool via the methylmalonyl pathway. This is confirmed by the presence of certain labelled amino acids, i.e. aspartate, glutamate, alanine, glycine and serine. This pathway has been demonstrated in the sheep in the liver and rumen epithelium mitochondria by Smith (1971) and in the tissues of the rats by Gaitonde *et al.* (1977). Therefore propionate enters the metabolic pool as intramitochondrial succinyl CoA, the succinate is oxidized to oxaloacetate, which is the precursor of labelled amino acids recovered in the present study, and the substrates for gluconeogenesis. A proportion of ¹⁴C also appears in lactate and glucose in ruminant, rat and guinea-pig tissues (Bergman & Wolff, 1971; Weekes & Webster, 1975; Mottaz & Worbe, 1979; Deacius *et al.* 1979).

The major metabolic pathway for butyrate is not the production of ketone bodies. There seems to be agreement between different authors that there is little variation in the concentration of ketone bodies in the venous blood of the hind-gut of the rabbit (Henning & Hird, 1972b, c; Bonnafous & Raynaud, 1978a; Woodnutt & Parker, 1981). This low ketogenesis is in relation with the reduced activity of the 3-hydroxy-3-methylglutaryl-CoA synthetase (*EC* 4.1.3.5) in the caeco-colonic tissues (Henning & Hird, 1972b). For the ruminant, Stevens & Stettler (1966) reported that approximately 90% of the butyrate is metabolized into ketone bodies with only 10% in other metabolites such as acetate and CO₂.

In the rabbit, the relative proportions are reversed in the colonic tissues; butyrate is wholly metabolized and can reach ultimate stages of oxidation. This important metabolism of butyrate in the colon may also be applicable to other species, thus Roediger (1980), using isolated human colonocytes, reported that this acid is the best respiratory fuel for the distal colonic mucosa.

In contrast to the ruminant, the plasma glucose concentration in the rabbit is high (10 mM) but the energy substrate utilized by the hind-gut tissues is not glucose because arteriovenous differences are not significant. Respiratory fuels for maintaining cellular function can be derived from either the bowel lumen or the circulation (Hanson & Parsons, 1977). The present study shows that intestinal tissues preferentially metabolized intraluminal substances such as VFA, end-products of bacterial metabolism. Readily available carbohydrates are absorbed in the small intestine. In the hind-gut, carbohydrates produced by bacteriolysis of vegetable and endogenous mucopolysaccharides are rapidly used up at a rate in proportion to the bacterial metabolism (Bonnafous & Raynaud, 1978*b*). In addition there is no glucose absorption in the large intestine (Long *et al.* 1967), consequently this metabolite is not an important source of energy for intestinal tissues as in ruminants (Hood *et al.* 1972) and in the rat (Windmueller & Spaeth, 1978). In the rabbit colon, the various mechanisms involved in the absorption of electrolytes (Frizzell *et al.* 1976; Yorio *et al.* 1977; McCabe *et al.* 1982) and perhaps also of amino acids liberated by the lytic factor (Bonnafous & Raynaud, 1968, 1969, 1973) require considerable energy. Fatty acids yield energy for these metabolic activities. This is in good agreement with the findings of Roediger (1979) who showed that for the rat colonocyte, lumen VFA are preferred fuels in comparison with circulating respiratory fuels; the order of preference is VFA > ketone bodies > amino acids > glucose. However, the labelling of essential amino acids and sucrose in caeco-colonic extracts implies the participation of microbial enzymes, as was observed previously in a preliminary study (Marty *et al.* 1981).

With our experimental conditions, propionate and butyrate are largely removed from the portal blood during its passage through the liver, leaving acetate as the only VFA present in a significant concentration in the peripheral blood of the rabbit. Similar results have been obtained in sheep (Reid, 1950; Leng & Annison, 1963) and in the rat (Rémésy *et al.* 1980). Liver tissue metabolizes propionate by the methylmalonate pathway also, but the labelling of amino acids in the liver is greater than in intestinal tissue. This is in agreement with the work reported by Gaitonde *et al.* (1977) in the rat, which showed that ¹⁴C propionate is a better source of amino acids in the liver than in the other tissues. Butyrate appears to be utilized readily by liver tissue *in vivo*, its uptake being consistently high (84%). In the portal blood of the rabbit butyrate represents 14% of the total VFA, this value is characteristic of this animal (Henning & Hird, 1972*a*; Beauville *et al.* 1974). In the ruminant and other monogastric animals, butyrate represents only 5–6% of total VFA in portal vein plasma (McClymont, 1951; Friend *et al.* 1964; Cook & Miller, 1965). In the rabbit, hepatic tissue has a greater capacity for removing and metabolizing butyrate than in other species. Hepatic tissue preferentially converts butyrate and propionate, these observations can be explained with reference to ruminant experiments: Cook *et al.* (1969), Smith (1971) and Ash & Baird (1973) showed that liver mitochondria of sheep do not metabolize acetate in the presence of propionate and butyrate. McGarry *et al.* (1971*a, b*, 1975, 1977) reported the possibility of a direct penetration of propionate and butyrate across the mitochondrial membranes, without intervention of the carnityl acyl transferase system, which constitutes the primary site of free fatty acid metabolism regulation. They reported also that hepatic activation of butyrate is promoted by the existence of an active intramitochondrial acyl CoA synthetase, while propionate activation would depend on a distinct specific propionyl CoA synthetase with possible kinetic properties (Groot, 1975). In the livers of sheep (Snoswell

& Koundakjian, 1972), rat (Hanson & Ballard, 1967) and rabbit (Woodnutt & Parker, 1978), acetyl CoA synthetase is mainly localized in the cytosol fraction. This location suggests that in rabbit liver as in the livers of sheep and rats, acetate is principally converted to fatty acids and CO₂ since the enzymes involved in fatty acid synthesis are cytosolic. However, a part of the radioactive metabolites recovered in hepatic extracts are absorptive forms of the fatty acids, since most of the metabolites that we attribute to colonocyte metabolism are recovered in portal blood. This fact does not exclude an hepatic metabolism based on the fatty acid uptake, but indicates the complexity of the metabolic pathways that lead the ¹⁴C of VFA from the hind-gut lumen to the hepatocytes.

Acetate is the major fatty acid present in the hepatic venous and arterial plasma (93%). The metabolism of this acid occurs in extrahepatic tissues because arteriovenous differences were negative. For the rabbit, as for the ruminant, the acetate is mainly oxidized into CO₂, and it is the predominant fatty acid precursor as compared to glucose (Beauville *et al.* 1974; Smith, 1975; Vézinhét & Nougues, 1977).

VFA, fermentation products of the intestinal microflora, are largely metabolized in the caeco-colonic tissues and liver. The nature and the partition of the ¹⁴C labelling show that these bacterial metabolism waste products are utilized by the host as a source of energy and of protein synthesis. In fact the flux of VFA through the hind-gut represents 37.7% of the maintenance energy requirement of the rabbits. This result is considerably higher than that obtained in *in vitro* incubations by Hoover & Heitmann (1972), but it is of the same order as that obtained by Parker (1976) in an *in vivo* isotope dilution experiment.

Thus, in the rabbit, metabolic utilization of VFA has been established. These fatty acids are involved in hind-gut metabolism: butyrate is the best respiratory fuel for the colonic wall, followed by propionate and acetate. They are also metabolized by the liver and extrahepatic tissues. In our experiment the theoretical energy contribution could be as much as 40% of the maintenance energy requirement of the rabbit. This finding could probably be extended to the other mammals who have a fermentative compartment inhabited by an anaerobic microbial population.

Scintillation counting and analysis of amino acids, carboxylic acids and sugars were performed at the Centre de Physiologie Végétale LA CNRS 241-UPS. The authors wish to thank Mr Abravanel for his invaluable help in initiating them in the different techniques.

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