Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes

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In the present study the actual role of propionic acid in the control of fatty acid and cholesterol synthesis was investigated in isolated liver cells from fed rats maintained in the presence of near-physiological concentrations of glucose, glutamine and acetate. Using ^3H_2O for lipid labelling, propionate appears as an effective inhibitor of fatty acid synthesis and to a lesser extent of cholesterol synthesis, even at the lowest concentration used (0·6 mmol/l). Butyrate is a potent activator of both synthetic pathways, and the activating effect was not counteracted by propionate. Using 1-[^{14}C]acetate, it was observed that propionate at a moderate concentration, or 1 mmol oleate/l, are both very effective inhibitors of ^{14}C incorporation into fatty acid and cholesterol. This incorporation was drastically inhibited when propionate and oleate were present together in the incubation medium. The net utilization of acetate by rat hepatocytes was impaired by propionate, in contrast to oleate. 1-[^{14}C]butyrate was utilized at a high rate for fatty acid synthesis, but to a lesser extent for cholesterol synthesis; both processes were unaffected by propionate. Intracellular citrate concentration was not markedly depressed by propionate, whereas it was strongly elevated by butyrate. In conclusion, propionate may represent an effective inhibitor of lipid synthesis when acetate is a major source of acetyl-CoA, a situation which is encountered with diets rich in readily-fermentable fibres. The present findings also suggest that propionate may be effective at concentrations close to values measured *in vivo* in the portal vein.

Fatty acid synthesis: Cholesterol synthesis: Propionate: Acetate

It has been reported consistently that dietary fibres, especially the soluble fractions, effectively lower plasma cholesterol concentrations (Truswell & Beynen, 1992). The mechanism most frequently suggested is interference with lipid digestion, or with cholesterol and bile acid absorption, in the small intestine. Depending on structural characteristics, fibres can present gel-forming properties or bile acid- and sterol-binding properties which result in accelerated faecal losses of cholesterol from the body pool. This, in turn, is considered as a major process leading to up-regulation of liver lipoprotein receptors and to depressed cholesterol concentrations. However, it has been shown also that some polysaccharides devoid of gel effects or of lipid-sequestering properties are associated with a reduction in plasma lipids as well as fatty acid synthesis (Levrat *et al.* 1994; Morand *et al.* 1994*b*).

Most soluble fibres or related compounds are broken down by the microflora of the large intestine, a process which results in the production of large quantities of short-chain fatty acids (SCFA). SCFA are almost completely absorbed into the portal vein, then metabolized by the liver. Thus, it has been proposed that SCFA may affect lipid metabolism in the liver. The hypothesis that propionate generated by bacterial fermentation of fibre could exert a

rate-controlling effect on liver cholesterol synthesis has been the subject of *in vivo* and *in vitro* investigations (Chen *et al.* 1984; Illman *et al.* 1988; Nishina & Freedland, 1990; Venter *et al.* 1990; Wright *et al.* 1990; Beaulieu & McBurney, 1992). This hypothesis is in line with the fact that propionate is already known to affect various metabolic processes such as gluconeogenesis, ureogenesis or ketogenesis (Rémésy *et al.* 1992). However, there are also findings which appear inconsistent with a physiological role for propionate in the control of carbohydrate or lipid synthesis (Cameron-Smith *et al.* 1994). It has been shown that rats fed on certain highly-fermentable carbohydrates, especially oligosaccharides, exhibit high-propionic acid fermentation together with an induction of hydroxymethylglutaryl-CoA (HMG-CoA) reductase (*EC* 1.1.1.88), the rate-limiting enzyme of cholesterol synthesis (Levrat *et al.* 1994).

The aim of the present work was, thus, to evaluate further the effect of propionate on lipid biosynthesis in isolated liver cells, under substrate conditions designed to be as close as possible to physiological conditions. The study was also performed to determine the metabolic conditions under which propionate is likely to exert control on fatty acid and cholesterol synthesis *in vivo*.

MATERIALS AND METHODS

Materials

^3H_2O (5 GBq/g), [1,2-^{3}H]cholesterol (37 GBq/mmol), [9,10-^{3}H]palmitate (2·06 GBq/mmol), and [1-^{14}C]palmitic acid (2·06 GBq/mmol) were purchased from the CEA (Gif/Yvette, France), [1-^{14}C]acetate (2·18 GBq/mmol) was from Amersham (Les Ulis, France), [1-^{14}C]butyrate (370 MBq/mmol) and [4-^{14}C]cholesterol (1·9 GBq/mmol) were from DuPont NEN (Les Ulis, France). Collagenase and bovine serum albumin (BSA) were obtained from Boehringer (Meylan, France).

Liver cell isolation and hepatocyte incubations

Male Wistar rats (200–220 g) adapted to a standard chow diet were housed under temperature-controlled conditions, with a dark period from 21.00 to 09.00 hours. The animals were anaesthetized at about 08.00 hours, and liver perfusion was performed for the isolation of hepatocytes, using the collagenase perfusion technique of Berry *et al.* (1991), except that all the isolation buffers and the incubation buffer (Krebs-Ringer bicarbonate supplemented with BSA (20 g/l), pH 7·4) were supplemented with (mmol/l): glucose 10, L-glutamine 0·6 and sodium acetate 0·2. The final cell density was adjusted to 10–12 mg cells (fresh weight)/ml, after centrifuging 1·5 ml of the cell suspension at 8000 *g* for 10 s in preweighed Eppendorf microtubes. The integrity of cells was assessed by microscopic examination of the cellular suspension for trypan blue exclusion. The preparation consistently exhibited a high percentage of unstained cells ($\geqslant 90\%$), characteristic of viable preparations. The incubations were carried out at 37° in 10 ml Erlenmeyer flasks containing 2·5 ml of the hepatocyte suspension, under agitation and in an atmosphere of O_2–CO_2 (95:5, v/v). A period of equilibration of hepatocytes in the presence of the unlabelled lipogenic substrate (10 min) was allowed, then the reaction was started by addition of the radioactive substrate and of the effectors (propionate, oleate etc) as concentrated solutions (100-fold). Ethanolic KOH (300 g KOH/l ethanol (500 ml/l); 2·5 ml) was added at the end of the incubation period (30 min), together with trace amounts of labelled palmitic acid or cholesterol (^{14}C-labelled palmitate or cholesterol for incubations with ^3H_2O, ^{3}H-labelled palmitate or cholesterol for incubations with ^{14}C-labelled substrates), in order to measure the recovery yield of the extraction procedures.

Analytical methods

After 1 h of hydrolysis in ethanolic KOH at 70° the non-saponifiable fraction was extracted (three times) with light petroleum (b.p. 40–65°) and evaporated under N_2. The alkaline extract was acidified with HCl, then extracted (three times) with light petroleum. The organic phase was washed with distilled water and evaporated under N_2 before measurement of fatty acid radioactivity. Digitonin-precipitable sterols were isolated and purified from the non-saponifiable fraction according to Sendl *et al.* (1992). Fatty acid or cholesterol synthesis activities were expressed in nmol ^3H_2O or acetyl units incorporated/min per mg cell (fresh weight).

For measurement of metabolite concentrations in cell suspensions, hepatocytes were incubated under the conditions described previously, without labelled precursor. At the end of the 30 min incubation period, a 1 ml portion of the cell suspension was transferred into an Eppendorf microtube containing 0·1 ml 3 M-$HClO_4$; after agitation and centrifugation (8000 *g* for 10 s), the $HClO_4$ supernatant was withdrawn for metabolite determinations. β-Hydroxybutyrate was determined in the $HClO_4$ extract; citrate and acetoacetate were measured after neutralization of the extract with K_2CO_3 (to precipitate the majority of the perchlorate ions and to improve the stability of acetoacetate). Citrate was measured enzymically at 340 nm, using the reaction catalysed by citrate lyase (*EC* 4.1.3.6) in the presence of a mixture of malate and lactate dehydrogenases (*EC* 1.1.1.37 and *EC* 1.1.1.27 respectively, the latter to take into account some enzymic or non-enzymic decarboxylation of oxaloacetate) and of Zn^{2+} (Möllering, 1989). β-Hydroxybutyrate and acetoacetate were also determined enzymically using the reactions catalysed by the β-hydroxybutyrate dehydrogenase from *Rhodopseudomonas spheroides* (*EC* 1.1.1.30) at pH 9·0 and 6·8 respectively, according to Williamson & Mellanby (1974) and Mellanby & Williamson (1974). Acetate concentration in the incubation medium was measured using gas–liquid chromatography after ethanolic extraction (Rémésy & Demigné, 1974).

Statistics

The means for given variables were tested using a one-way analysis of variance and multiple-range comparisons, by Fisher's protected least-significant-differences procedures (Staview 512+; Brain Power, Calabasas, CA, USA). In all statistical tests, it was decided to reject the null hypothesis at the 5% level ($P < 0·05$).

RESULTS

Effect of short-chain fatty acids on the cellular levels of citrate and ketone bodies

Table 1 shows that cellular citrate was slightly depressed by 1·2 mM-propionate, whereas acetate had no effect. On the other hand butyrate elicited a marked rise in cellular citrate (+90%) and the butyrate effect was not noticeably altered by propionate. Under basal conditions the concentrations of ketone bodies were very low, as was the β-hydroxybutyrate:acetoacetate value (0·29). These variables were not noticeably affected by acetate or propionate; in the presence of butyrate there was a substantial rise in ketone bodies, together with a higher β-hydroxybutyrate:acetoacetate ratio.

Effect of propionate or butyrate on the rate of fatty acid and cholesterol synthesis from ^3H_2O

Fig. 1 shows that under the basal conditions presently used the incorporation of ^3H_2O into fatty acids was 0·18 nmol/min per mg fresh cell weight, as compared with 0·021 nmol/min per mg fresh cell weight in digitonin-precipitable sterols (essentially cholesterol). Propionate had an inhibitory effect on fatty acid synthesis (about −55%) and, to a lesser

Table 1. *Effect of short-chain fatty acids on intracellular concentrations (μmol/ml cell water) of citrate and ketone bodies in isolated rat hepatocytes*†

(Mean values with their standard error of the means)

Incubation conditions	Citrate		β-Hydroxybutyrate (β-HB)		Acetoacetate (Acac)		β-HB: Acac
	Mean	SEM	Mean	SEM	Mean	SEM	
Basal	0·64	0·04	0·04	0·01	0·14	0·02	0·29
2 mmol acetate/l	0·76	0·05	0·05	0·01	0·16	0·02	0·31
1·2 mmol propionate/l	0·51*	0·04	0·03	0·01	0·13	0·02	0·23
1·2 mmol butyrate/l	1·21*	0·12	0·15*	0·03	0·31*	0·03	0·48
2 mmol acetate/l + 1·2 mmol propionate/l	0·63	0·04	0·04	0·02	0·12	0·02	0·25
1·2 mmol butyrate/l + 1·2 mmol propionate/l	1·15*	0·10	0·12*	0·03	0·27*	0·03	0·44

Mean values were significantly different from those under basal conditions: * $P < 0·05$.
† For details of procedures, see pp. 210–211.

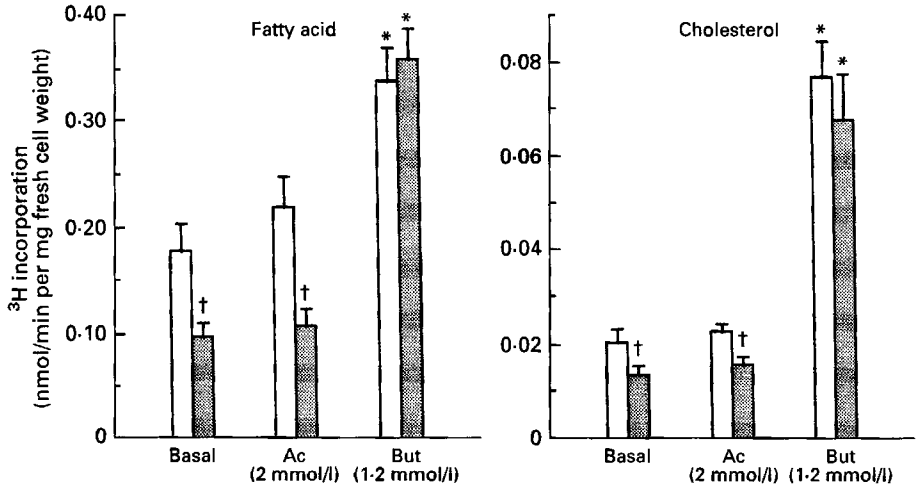

Fig. 1. Effect of propionate on fatty acid and cholesterol synthesis from ^3H_2O in isolated hepatocytes under basal conditions or in the presence of acetate (Ac; 2 mmol/l) or butyrate (But; 1·2 mmol/l). For each substrate, a comparison was made between control (□) and 1·2 mmol propionate/l (▨). Values are means with their standard errors represented by vertical bars for three experiments. Mean values were significantly different from those under basal conditions: * $P < 0·05$. The effect of propionate was significant: † $P < 0·05$. For details of procedures, see pp. 210–211.

extent, on cholesterol synthesis (about −30%). It must be noted that the inhibitory effect of propionate was practically maximal at the lowest concentration studied (0·6 mmol/l) and the effect was not noticeably higher at 2·5 mmol/l (values not shown). Acetate addition led to marginally higher rates of fatty acid and cholesterol synthesis; propionate inhibition was similar to that observed under basal conditions. Butyrate addition led to a dramatic acceleration in the rate of ^3H_2O incorporation into fatty acids and cholesterol but, in contrast to findings with the other experimental conditions, 1·2 mmol propionate/l had no significant effect on this process.

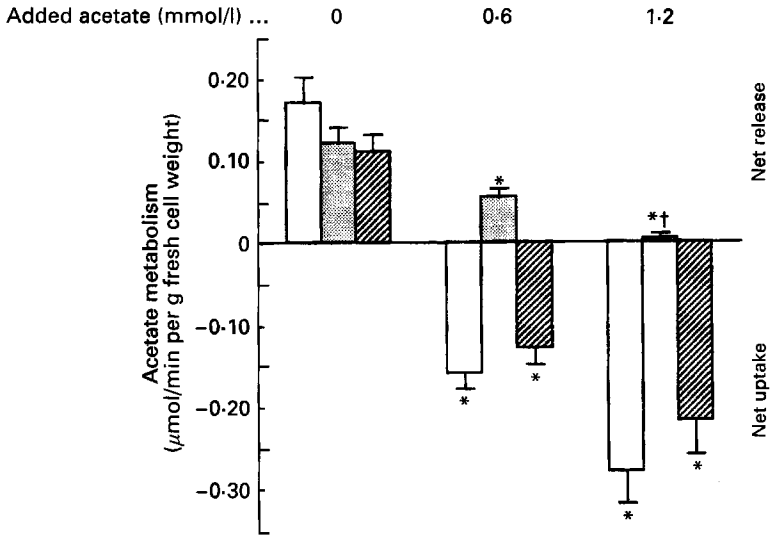

Fig. 2. Effect of propionate (1·2 mmol/l) or oleate (1 mmol/l) on the net balance of acetate in isolated rat hepatocytes when acetate was present at 0, 0·6 or 1·2 mmol/l. The positive values correspond to a net release of acetate by liver cells, and the negative values to a net uptake. For each acetate concentration, a comparison was made between basal conditions (□), 1·2 mmol propionate/l (▨) or 1 mmol oleate/l (▩). Values are means with their standard errors represented by vertical bars for four experiments. Mean values were significantly different from incubation conditions where no acetate was added: * $P < 0·05$. The effect of propionate was significant compared with basal conditions: † $P < 0·05$. For details of procedures, see pp. 210–211.

Effect of propionate on acetate metabolism and the rate of fatty acid and cholesterol synthesis from [1-^{14}C]*acetate*

Fig. 2 depicts the effect of propionate (1·2 mmol/l) and of a long-chain fatty acid (1 mmol oleate/l) on acetate metabolism by isolated hepatocytes. Under the present basal conditions there was a release of acetate into the medium by liver cells which was not significantly altered by propionate or oleate. In the presence of higher concentrations of acetate in the medium (within the physiological range: 0·6 or 1·2 mmol/l) a net uptake of acetate was observed, which was concentration-dependent. Oleate had no significant effect on this process, whilst propionate appeared as a potent inhibitor of acetate uptake since acetate balance was practically nil whatever its concentration.

In the presence of [1-^{14}C]acetate (Fig. 3); it appears that the rate of ^{14}C incorporation into fatty acid and cholesterol was proportional to acetate concentration in the medium. Under the present conditions the value for fatty acid labelling (FAL):cholesterol labelling (CL) was about 3·9, which was lower than that obtained with $^{3}H_2O$ (FAL:CL 7·7). Propionate appeared to be an effective inhibitor of the incorporation of ^{14}C from labelled acetate into both fatty acid and cholesterol, with practically no difference between the 0·6 or 1·2 mmol/l concentrations. Oleate (1 mmol/l) was also a potent inhibitor of both metabolic processes, and when oleate was present together with propionate (both in physiological concentrations) a drastic inhibition of [^{14}C]acetate incorporation into fatty acid was observed (up to 90–95%). However, the percentage inhibition of label incorporation into fatty acid or cholesterol by propionate or oleate was moderately influenced by the acetate concentration in the medium.

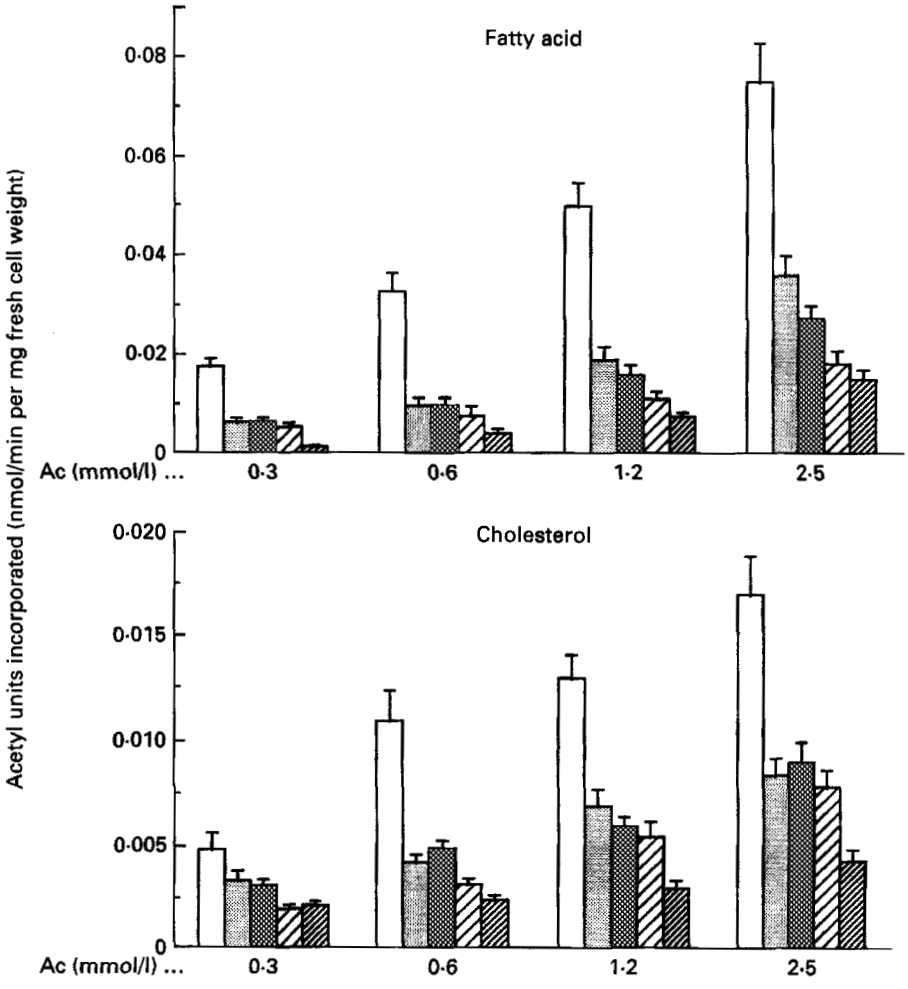

Fig. 3. Effect of propionate or oleate on fatty acid and cholesterol synthesis from 1-[^{14}C]acetate (Ac; present at different concentrations, from 0·3 to 2·5 mmol/l) in isolated rat hepatocytes. Values are means with their standard errors represented by vertical bars for three experiments. For each acetate concentration, a comparison was made between control (□), 0·6 mmol propionate/l (▨), 1·2 mmol propionate/l (▩), 1 mmol oleate/l (▨) and 1 mmol oleate/l together with 1·2 mmol propionate/l (■). Mean values were all significantly different from those for control incubations for all acetate concentrations ($P < 0·05$). For details of procedures, see pp. 210–211.

Effect of propionate on the rate of fatty acid and cholesterol synthesis from 1-[^{14}C]*butyrate*

Fig. 4 shows the incorporation of ^{14}C into fatty acid and cholesterol obtained with 1·2 mmol 1-[^{14}C]butyrate/l, compared with 2·4 mmol 1-[^{14}C]acetate/l. Due to its very high rate of uptake and metabolism by hepatocytes, butyrate was studied at a concentration of 1·2 mmol/l in the medium. The incorporation of ^{14}C into fatty acids under basal conditions was about 1·7-fold faster with butyrate than with acetate; in contrast to acetate (cf. Fig. 3), butyrate metabolism was not inhibited by propionate. As shown previously, the rate of cholesterol synthesis from labelled acetate was lower (about 0·02 nmol acetyl unit/min per mg fresh cell weight) than that of fatty acid synthesis, and it was reduced by propionate. The rate of incorporation of ^{14}C from 1-[^{14}C]butyrate into cholesterol was very low, less

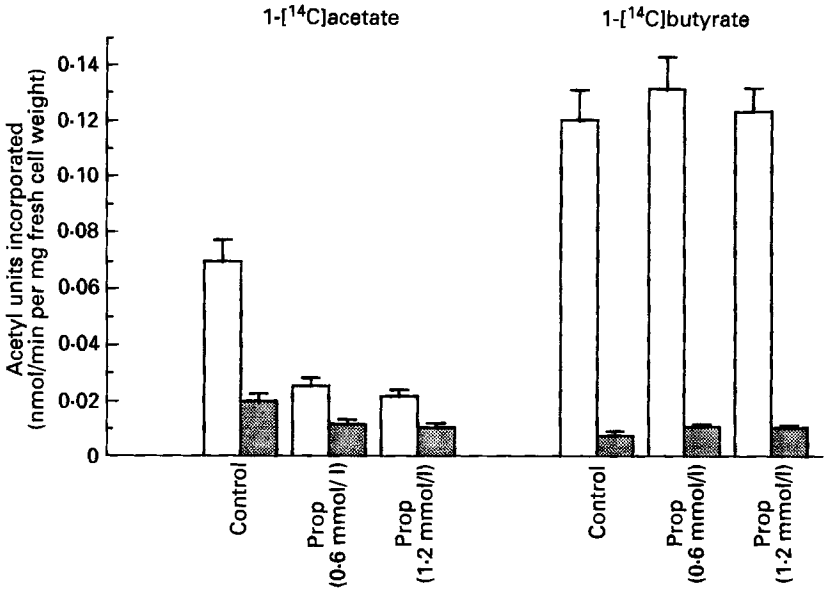

Fig. 4. Effect of propionate (Prop; 0·6 or 1·2 mmol/l) on fatty acid synthesis (□) and cholesterol synthesis (▨) from 1·2 mmol 1-[^{14}C]butyrate/l, compared with 2·4 mmol 1-[^{14}C]acetate/l. Values are means with their standard errors represented by vertical bars for three experiments. Mean values in the presence of Prop were all significantly different from control values (no Prop) with acetate as a substrate ($P < 0·05$), whereas no significant effect of Prop was observed with butyrate as a substrate.

than 0·01 nmol acetyl unit/min per mg cell, and as a result there was a very high lipogenesis:cholesterogenesis ratio (approximately 16) compared with that obtained with acetate as substrate. Cholesterol synthesis from butyrate was not significantly depressed in the presence of propionate.

DISCUSSION

In vivo investigations of the effects of propionate have used diets supplemented with propionate, or containing polysaccharide liable to yield high propionic acid fermentations. Isolated hepatocytes represent an alternative model to study the effectiveness of SCFA as precursors or effectors of lipid metabolism in liver cells. Hepatocytes from post-absorptive rats tend to release small amounts of acetate under basal conditions; the equilibrium between release and uptake fluxes corresponds to acetate concentrations in the range of 0·4–0·5 mmol/l, a value slightly higher than that observed *in vivo* (Buckley & Williamson, 1977; Rémésy *et al.* 1992). Liver cells present capacities for both acetate activation and acetyl-CoA hydrolysis; thus, a non-significant acetate balance may be the result of substantial opposing fluxes (Crabtree *et al.* 1990). Propionate is an effective inhibitor of acetate uptake *in vitro* (Gordon & Crabtree, 1992; Demigné & Rémésy, 1994) as well as *in vivo* (Rémésy *et al.* 1992). In the present model the addition of propionate elicited relatively small differences in the acetate balance when no acetate was added; however, propionate was an effective inhibitor of acetate utilization when its concentration in the medium was 0·6 or 1·2 mmol/l. Butyrate is taken up and metabolized at a very high rate by rat hepatocytes and, in contrast to acetate or lactate, its utilization by liver cells is virtually unaffected by propionate (Demigné *et al.* 1986).

In accordance with previous investigations it was found that the basal rate of fatty acid synthesis was higher (about 8-fold) than that of cholesterol, using ^3H_2O incorporation;

however, when both processes were activated in the presence of butyrate there was only a 4–5-fold difference between the two pathways. When 0.6 mmol [^{14}C]acetate/l (a near-physiological value) was used as a substrate there was a smaller difference between the rate of labelling of fatty acids or cholesterol (a value of 3–4 was generally observed). The reasons for the difference between these two types of labelling are certainly complex, such as a possible discrepancy between fatty acid and cholesterol in the intrinsic labelling by $^{3}H_2O$. A preferential channelling of acetate towards microsomal cholesterol rather than to fatty acids seems unlikely, particularly since it has been established that acetyl-CoA synthase (*EC* 6.2.1.1) activity is not present in rat microsomes (values not shown).

Labelling of fatty acid or cholesterol was approximately proportional to the concentration of [^{14}C]acetate in the medium, but it was markedly inhibited by propionate or oleate. It is noteworthy that the percentage inhibition of [^{14}C]acetate incorporation into fatty acids or cholesterol by propionate was independent of the acetate concentration. Determination of intracellular citrate in previous investigations has not provided conclusive data on the role of this metabolite (Nishina & Freedland, 1990), whereas the present findings suggest that citrate is responsive to SCFA, especially butyrate. It must be noted that a large part of the citrate pool in liver cells is mitochondrial (Soboll *et al.* 1980). Conceivably, in the presence of propionate, a substantial lowering of the cytosolic concentration of citrate could be masked by an elevation of its production in mitochondria. Long-chain fatty acids are physiological inhibitors of lipogenesis; in the presence of propionate and oleate a marked inhibition of fatty acid synthesis (up to 90%) and of cholesterol labelling (50–60%) was observed. Oleate may represent a major source of sterol-C (Gibbons *et al.* 1986) and it could compete with acetate for acetyl-CoA provision for HMG-CoA synthesis, although acetate uptake was not significantly depressed by oleate (see Fig. 2).

Lipid labelling with 1-[^{14}C]butyrate was definitely different from that obtained with 1-[^{14}C]acetate. That 1·2 mmol butyrate/l is a more effective acetyl-CoA supplier than 2·5 mmol acetate/l is not surprising since the rate of butyrate metabolism is faster than that of acetate (Demigné *et al.* 1986; Crabtree *et al.* 1989; Bergman, 1990). Thus, butyrate appears to be a particularly efficient precursor of long-chain fatty acids, in accordance with the potent stimulation of fatty acid synthesis observed using $^{3}H_2O$, and it is noteworthy that this process was not affected by propionate. Butyrate can be metabolized to acetoacetate, which is liable to be activated in the cytosol and to yield acetoacetyl-CoA, then HMG-CoA (Bergstrom *et al.* 1984). This might have resulted in a particularly effective labelling of cholesterol with butyrate, but this was not observed in the present study. Intracellular citrate is markedly elevated by butyrate (Morand *et al.* 1994*a*); this was very effective in stimulating fatty acid synthesis since, in contrast to long-chain fatty acids, it does not yield inhibitory acyl-CoA in the cytosol. Under such conditions a high rate of fatty acid synthesis may be expected if large quantities of acetyl-CoA are generated in mitochondria (from butyrate, for example) and transferred to the cytosol, probably via citrate. Recent investigations suggest that the extramitochondrial acetyl-CoA pool is not isotopically homogeneous and that subpools of acetyl-CoA, derived from different sources, may be channelled without full mixing towards fatty acid synthesis, cholesterol synthesis or other processes (Zhang *et al.* 1994). Propionate did not inhibit lipid labelling by butyrate, in contrast to Nishina & Freedland's (1990) data; this is consistent with the fact that butyrate activation and metabolism is also poorly affected by propionate (Demigné *et al.* 1986; Rémésy *et al.* 1992).

Initially it had been postulated that propionate could be a link between digestive fermentation and the cholesterol-lowering effect frequently observed with fibre diets. The present *in vitro* data show that propionate may be effective in depressing fatty acid synthesis

as well as cholesterol synthesis. It is noteworthy that the propionate effect was near-maximum at 0·6 mmol/l, which is close to the values observed *in vivo* in rats adapted to high-fibre diets (Rémésy *et al.* 1992; Levrat *et al.* 1994). This tends to rule out, as causative factors, metabolites which accumulate when the capacity for propionate metabolism is overloaded, e.g. methylmalonyl-CoA or, to a lesser extent, propionyl-CoA (Corkey *et al.* 1982). It is difficult to suggest a direct effect of propionate itself or its mitochondrial CoA derivatives on the microsomal cholesterol metabolism. For example, the demonstration that propionate had an inhibitory effect on HMG-CoA synthase (*EC* 4.1.3.5; Lowe & Tubbs, 1985) was relevant for the mitochondrial enzyme involved in the ketogenesis pathway, but not for the microsomal enzyme (which is not rate-limiting in the pathway of cholesterol synthesis). A direct impact of propionate, or of its CoA derivatives, on HMG-CoA reductase is questionable, due to the respective subcellular locations of cholesterogenesis and propionate metabolism. *In vitro* experiments on solubilized HMG-CoA reductase have shown that short-chain acyl-CoA thioesters, including propionyl-CoA, are rather weak inhibitors of this enzyme (Roitelman & Shechter, 1989). Propionate, when absorbed in large quantities *in vivo*, seems unable to counteract stimulation of HMG-CoA reductase which results from extensive losses of bile acids and sterols in the faeces (Favier *et al.* 1994; Moundras *et al.* 1994).

In fact, propionate appears to be an effective inhibitor of cholesterol synthesis under some conditions, when acetyl-CoA provision depends chiefly on pyruvate (lactate utilization by liver cells is impaired by propionate; Morand *et al.* 1994*a*), or when acetate is a major substrate for lipid synthesis. The latter situation is encountered with diets rich in readily-fermented fibres, which are associated with large quantities of both acetate and propionate in the portal vein (Bergman, 1990; Rémésy *et al.* 1992). Under such conditions acetate is probably a major source of acetyl-CoA, other potential precursors (lactate, free fatty acids) being taken up in relatively small quantities by the liver during the absorptive period. Conditions leading to a high production and absorption of SCFA are characterized by metabolic adaptations such as depressed lipogenesis, frequently concomitant to stimulation of liver HMG-CoA reductase and cholesterogenesis (Arjmandi *et al.* 1992; Morand *et al.* 1994*b*). *In vitro*, stimulation of cholesterogenesis has been reported in hepatocytes isolated from rats fed on a pectin diet compared with those from rats on a fibre-free diet; also, there was a considerable acceleration (9-fold) of the rate of [^{14}C]acetate incorporation into cholesterol (Stark & Madar, 1993). Thus, propionate could be involved in the cholesterol-lowering effect of fibres by impairing acetate utilization (and, to some extent, pyruvate metabolism), especially when cholesterol synthesis is activated to compensate for enhanced faecal losses of steroids.

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