Biochem. J. (1989) 257, 673–678 (Printed in Great Britain)

Evidence that the production of acetate in rat hepatocytes is a predominantly cytoplasmic process

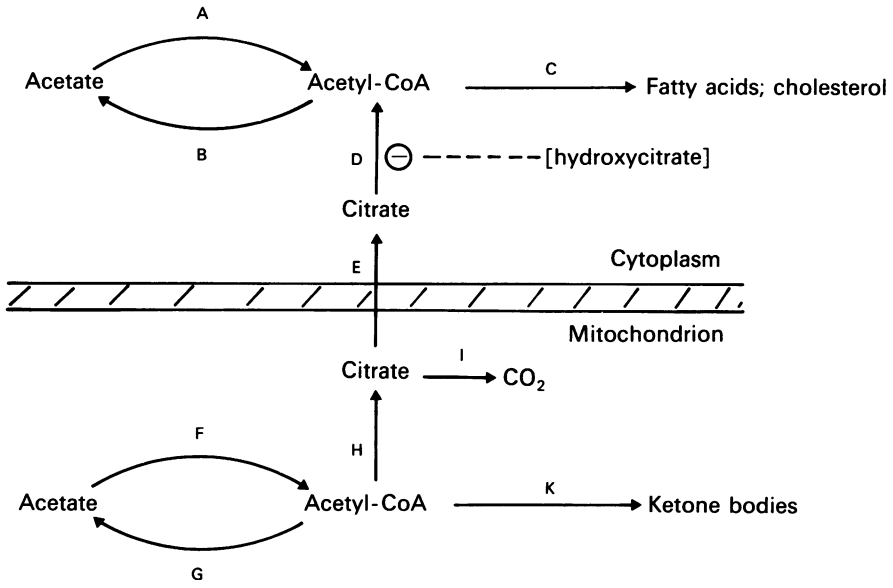
Bernard CRABTREE, Margaret-Jane SOUTER and Susan E. ANDERSON
Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, U.K.

By using [1-^{14}C]butyrate, the fluxes of butyrate to acetate and fatty acids were measured in rat hepatocytes. Both fluxes were inhibited to a similar extent by (−)-hydroxycitrate, with no significant effect on butyrate uptake. These results indicate that acetate formation takes place in the cytoplasm, presumably via ATP-stimulated acetyl-CoA hydrolase. Since acetate formation occurred despite a net uptake of acetate, the results are also consistent with the operation of a substrate cycle between acetate and acetyl-CoA, recently proposed by other workers, and suggest that this cycle is cytoplasmic.

INTRODUCTION

Mammalian tissues can produce acetate from acetyl-CoA and, in most tissues, the enzyme responsible (acetyl-CoA hydrolase, EC 3.1.2.1) is mitochondrial (Knowles *et al.*, 1974; Bernson, 1976; Robinson *et al.*, 1976). However, in the liver of some species, notably rodents, there is an additional cytoplasmic acetyl-CoA hydrolase which, in contrast with the mitochondrial enzyme, is activated by ATP and inhibited by ADP (Prass *et al.*, 1980; Matsunaga *et al.*, 1985; Söling & Rescher, 1985; N. S. Jessop, N. D. Scollan, M.-J. Souter & B. Crabtree, unpublished work). Consequently, the site of acetate formation may not be exclusively mitochondrial in liver. (A summary of the main reactions involved in liver acetate metabolism is given in Scheme 1).

However, Söling & Rescher (1985) have questioned the significance of the cytoplasmic enzyme *in vivo*. These workers carried out a thorough kinetic investigation of the mitochondrial and cytoplasmic enzymes in rat liver and calculated that, at the cellular concentrations of the salient activators and inhibitors, the activity of the cytoplasmic enzyme would be only 7% of that of the mitochondrial enzyme. These conclusions are surprising, because the cytoplasmic hydrolase is found only in liver (and to a much smaller extent in kidney; Matsunaga *et al.*, 1985), has a relatively high catalytic activity (approx. 12 μmol/min per g wet wt. at 30 °C) and possesses potentially important regulatory properties (Prass *et al.*, 1980). We have therefore investigated this problem more directly by studying the effect of the inhibitor (−)-hydroxycitrate on the flux of butyrate carbon to acetate in rat hepatocytes.



Scheme 1. Major pathways involved in hepatic acetate metabolism

Only major carbon fluxes are shown: cofactors and counter-ions (for membrane transport) are omitted. A, F, acetyl-CoA synthetase; B, G, acetyl-CoA hydrolase; C, fatty acid and sterol synthesis; D, ATP citrate lyase; E, citrate transporter; H, citrate synthase; I, tricarboxylic acid cycle; K, ketone-body synthesis; ⊖, inhibition.

EXPERIMENTAL

Sources of materials

Reagents, enzymes and radioactive compounds were obtained from the sources given previously (Crabtree *et al.*, 1981, 1987), except for the following: collagenase and bovine serum albumin (fatty acid-free) were obtained from Boehringer Corp., Lewes, East Sussex, U.K; L-carnitine, sodium butyrate and oleic acid were from Sigma, Poole, Dorset, U.K.; (−)-hydroxycitrate was a gift from Dr. P. F. Sorter, Hoffmann–La Roche, Nutley, NJ, U.S.A. Oleic acid was added to incubations as a complex with defatted albumin, prepared as described by Snoswell *et al.* (1982).

Preparation of hepatocytes

Hepatocytes were prepared by a modification of the original method of Berry & Friend (1969), adapted for tissue biopsies by Byard *et al.* (1983). A single lobe of liver was removed from a fed male rat (300–350 g body wt., anaesthetized with diethyl ether) and rinsed in Krebs–Henseleit (1932) medium minus Ca^{2+}, containing 5 mM-Hepes, 1 mM-EGTA, 10 mM-glucose and 0.1 mg of heparin/ml, at pH 7.4. The lobe was perfused with this medium, at a flow rate of 25 ml/min, for approx. 10 min at 37 °C, and then perfused, at the same temperature and flow rate, with Krebs–Henseleit medium containing 1 mM-$CaCl_2$, 5 mM-Hepes, 10 mM-glucose and 1 mg of collagenase/ml at pH 7.4. After approx. 15 min, when the lobe had become visibly swollen and tissue disruption was noticeable under the capsule, it was removed from the perfusion apparatus and dispersed in 50 ml of Krebs–Henseleit medium containing 1 mM-$CaCl_2$, 5 mM-Hepes, 10 mM-glucose and 1 mM-L-carnitine at pH 7.4. The suspension was filtered through nylon mesh (approx. 200 μm) and the cells were washed three times by centrifugation (approx. 20 ***g*** for 1 min) and resuspension in 20 ml of the dispersion medium. A preliminary estimation of cell viability was then made with Trypan Blue (Patterson, 1979), and the preparation was rejected if more than 15% of cells had taken up the dye.

Incubation of hepatocytes

Incubations were carried out in polypropylene flasks (25 ml capacity) at 37 °C, with shaking (150 cycles/min) and continuous gassing with O_2/CO_2 (19:1). Cells were incubated, at a density of 20–30 mg dry wt./ml, in 7 ml of Krebs–Henseleit medium containing 1 mM-$CaCl_2$, 5 mM-Hepes, 10 mM-glucose, 1 mM-L-carnitine, 10 mg of defatted albumin/ml, 0.4 mM-oleate and 0, 3.5 mM- or 7 mM-hydroxycitrate at pH 7.4. After approx. 10 min, 100 μl of a solution containing non-radioactive acetate (8 μmol), [1-^{14}C]butyrate (11 μmol, 6.1 μCi) and ^3H_2O (750 μCi) was added to each flask, and two 0.5 ml samples of the flask contents were taken at once (zero time) and at suitable times thereafter. One sample was mixed immediately with 0.5 ml of 6% (w/v) $HClO_4$ (extract A): the other was centrifuged for 15 s at maximum speed in an Eppendorf micro-centrifuge (model 5414) to sediment the cells, and the supernatant was mixed with 0.5 ml of 6% $HClO_4$ (extract B). (If the cells were not separated from the medium, the formation of [^{14}C]acetate from intracellular acetyl compounds during subsequent stages of the procedure interfered with the measurement of the specific radioactivity of acetate.)

Each $HClO_4$ extract was centrifuged at approx. 2000 ***g*** for 10 min, and a measured sample of the supernatant was adjusted to pH 7.5 with 0.2 M-KOH containing 0.3 M-Mops. After removal of the precipitated $KClO_4$ at 0 °C, the neutralized supernatants were stored frozen until required for analysis. Acetoacetate was always assayed within 36 h of neutralization, preliminary experiments having shown no significant decarboxylation of this metabolite during this time.

Extraction and saponification of lipids

The method is based on that described and validated by Smith (1984). The pellets obtained after centrifuging $HClO_4$ extracts A were each washed with 3 × 4 ml of 50% (v/v) ethanol (Katz *et al.*, 1974) and then extracted with 4 ml of chloroform/methanol (2:1, v/v) overnight at 4 °C. The suspensions were centrifuged at 2000 ***g*** for 10 min and each residue was washed with a further 4 ml of chloroform/methanol. The washings were combined with the extracts and the solvent was removed, at 55 °C, by using a rotary evaporator. Each lipid residue was mixed with 2 ml of ethanol containing 0.2 M-KOH and heated under reflux for 90 min at 65 °C to saponify the lipids. Fatty acids were then extracted with 2 × 5 ml of light petroleum (b.p. 40–60 °C) as described by Brunengraber *et al.* (1973). The solvent was removed by evaporation under N_2 at room temperature, and each extract was redissolved in 2 ml of light petroleum and mixed with 10 ml of scintillant (Optifluor; Packard). The ^3H and ^{14}C radioactivities were measured in a Packard Tri-Carb 460CD scintillation counter by using an external standard for quench correction.

The amount of butyrate carbon (as C_2 units) converted into fatty acids was calculated from the ^{14}C radioactivity of fatty acids and the specific radioactivity (relative to C_2) of butyrate. The results, expressed per g dry wt., were plotted as a function of incubation time, and a best-fitting curve was generated by using the computer program MLP (NAG Distribution): a single exponential curve usually sufficed (Fig. 1). The initial fluxes of butyrate to fatty acids were calculated from the initial slopes of these curves (Table 1). Similar curves were fitted to plots of ^3H and ^{14}C radioactivity versus time, to obtain the initial rates of ^3H and ^{14}C labelling of fatty acids: as in Fig. 1(*b*), these showed a lag period of approx. 10 min. The initial rate of total fatty acid synthesis was calculated from the initial rate of ^3H labelling and the ^3H specific radioactivity of water in the incubations, as described by Brunengraber *et al.* (1973). Since fatty acid synthesis is an extramitochondrial process, the ^{14}C specific radioactivity of extramitochondrial (cytoplasmic) acetyl-CoA was calculated by dividing the rate of ^{14}C labelling of fatty acids by the total rate of fatty acid synthesis (see Goldberg & Brunengraber, 1980).

Separation and measurement of the specific radioactivity of acetate

The method is based on that described by Taylor (1978). A 0.5 ml portion of each neutralized supernatant from $HClO_4$ extract B (i.e. obtained after sedimenting the cells) was mixed with 100 μl of 3 M-H_2SO_4 in the centre well of a Conway micro-diffusion unit and left at room temperature for 15 min, during which most of the CO_2 was removed. Approx. 2.5 g of anhydrous Na_2SO_4 was then added to the centre well, 2 ml of 1 mM-tripotassium citrate was added to the outer well, and the

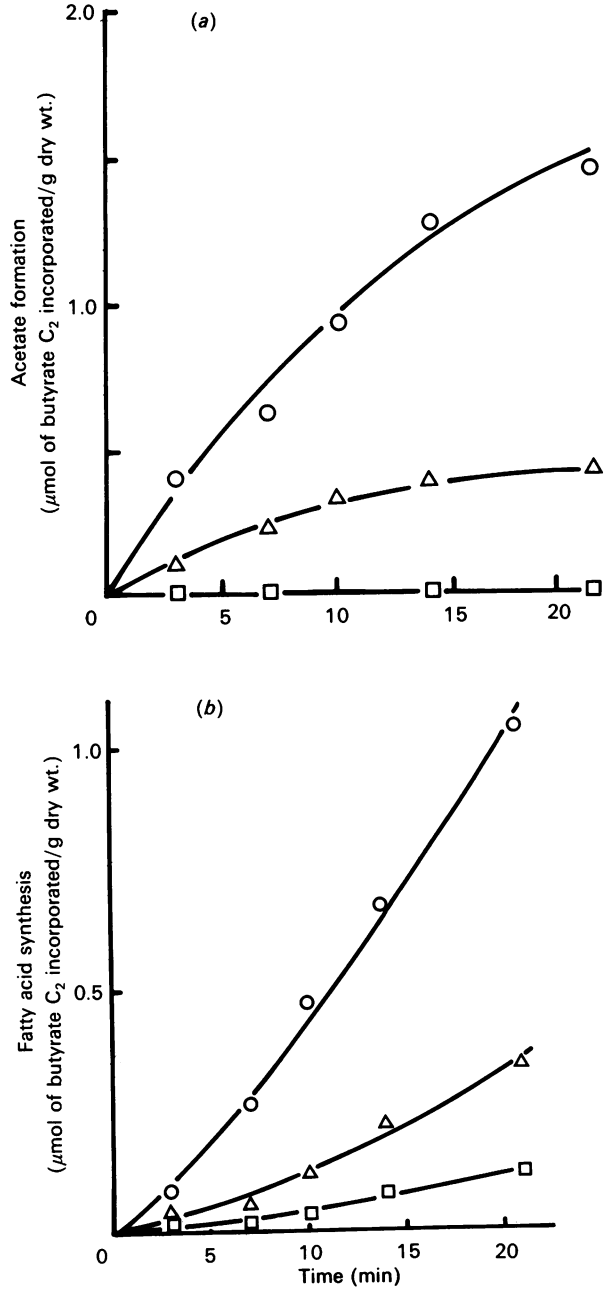


Fig. 1. Effect of hydroxycitrate on the flow of butyrate carbon to acetate and fatty acids in rat hepatocytes

The data illustrate the results of one of three experiments, together with the best-fitting curves obtained as described in the Experimental section. The initial rates of these curves, and those obtained from similar plots of the other two experiments, were used to calculate the fluxes given in Table 1. (*a*) Acetate formation, and (*b*) fatty acid synthesis, each expressed as μmol of C_2 per g dry wt. Key: ○, no inhibitor; △, 3.5 mM-hydroxycitrate; □, 7 mM-hydroxycitrate.

unit was sealed with a glass lid greased with soft white paraffin. After approx. 24 h at room temperature, each unit was opened and as much as possible of the outer solution was removed, adjusted to pH 9 (approx.) with 0.2 M-KOH containing 0.3 M-Mops and freeze-dried. Each residue was dissolved in 0.5 ml of 1 mM-tri-

Table 1. Effect of hydroxycitrate on the initial rates of fatty acid synthesis, ketone-body formation, acetate and butyrate uptake and acetate production in rat hepatocytes

Values represent means ± S.E.M. for three separate experiments: a negative value denotes a percentage activation. Since the fluxes varied by as much as 3-fold between experiments, the significance testing (*t* test) refers to the percentage inhibitions by hydroxycitrate, which showed a much smaller variation: ***$P < 0.001$; **$P < 0.01$. All fluxes are initial rates, calculated as described in the text. The initial ^{14}C specific radioactivity of cytoplasmic acetyl-CoA (see the Experimental section) was 0.17 ± 0.05 (expressed as a fraction of butyrate C_2 specific radioactivity) and was decreased significantly ($P < 0.01$) by 45 ± 8% and 69 ± 2% at 3.5 mM- and 7 mM-hydroxycitrate respectively.

Flux	Rate in the absence of hydroxycitrate (μmol of C_2/min per g dry wt.)	Percentage inhibition by hydroxycitrate	
		3.5 mM	7 mM
Acetate net uptake	0.26 ± 0.14	−36 ± 26	−84 ± 69
Butyrate uptake	11.86 ± 2.8	17 ± 6	30 ± 19
Ketone-body production	4.6 ± 0.8	−18 ± 12	−30 ± 22
Butyrate → acetate	0.24 ± 0.09	68 ± 4***	100***
Butyrate → fatty acids	0.034 ± 0.005	62 ± 6***	93 ± 2***
Total fatty acid synthesis	0.17 ± 0.02	47 ± 4***	78 ± 10**
Total cytoplasmic acetyl-CoA hydrolysis	1.8 ± 0.9	39 ± 7**	100***

potassium citrate, and a 100 μl sample was assayed for acetate by the enzymic method of Guynn & Veech (1974). At the end of the assay, the contents of each spectrophotometric cuvette were transferred quantitatively to plastic scintillation vials, acidified with 100 μl of 1 mM-HCl and freeze-dried: each residue was dissolved in 1 mM-sodium butyrate, acidified once more with 1 mM-HCl and freeze-dried. Each residue was finally dissolved in 1 ml of water, mixed with 10 ml of Optifluor scintillant, and the ^{14}C radioactivity was measured as described above. The specific radioactivity of acetate was obtained as the ratio of the ^{14}C radioactivity to the amount of acetate measured enzymically in each sample.

Preliminary experiments showed that the above procedure removed all the ^{14}C radioactivity present as butyrate and other volatile compounds (e.g. ketone bodies) that were transferred, along with acetate, to the outer solution during micro-diffusion. (Since acetate was converted into non-volatile citrate in the enzymic assay, its radioactivity was retained, whereas that of butyrate and the other compounds was removed, by freeze-drying under acid conditions.) This removal of contaminating radioactivity, together with the determination of both acetate concentration and radioactivity in the same sample, enabled the specific radioactivity of acetate to be determined with greater accuracy.

The amount of butyrate carbon converted into acetate was calculated by multiplying the specific radioactivity of

acetate by the amount of acetate present in the incubation and dividing the result by the specific radioactivity (relative to C_2) of butyrate. The results, expressed per g dry wt. of hepatocytes, were plotted as a function of incubation time (Fig. 1*a*) and a best-fitting curve was generated as described in the previous section. The initial rates of acetate production were calculated from the initial slopes of these curves and assumed to represent the flux of butyrate to acetate via acetyl-CoA hydrolase. [At subsequent times, these slopes will not represent the rate of acetyl-CoA hydrolase if a significant proportion of [^{14}C]acetate has been removed via acetyl-CoA synthetase (EC 6.2.1.1) activity; this is a possible reason for the non-linearity of Fig. 1(*a*).]

Measurement of metabolite concentrations

The neutralized supernatants from $HClO_4$ extracts A were used to measure the concentrations of ATP (Lamprecht & Trautschold, 1974), acetate (Guynn & Veech, 1974) and ketone bodies (Williamson *et al.*, 1962). Butyrate was extracted from these neutralized supernatants by the method of Patience & Thomas (1982), with isopentanoic acid as an internal standard: the bicarbonate extracts were freeze-dried, each residue was redissolved in approx. 10 μl of water, and the concentration of butyrate was measured, by g.l.c., as described by Crabtree *et al.* (1987).

RESULTS AND DISCUSSION

Incubation conditions

The ATP content of the cells, which is a good and convenient indicator of viability (see Cornell, 1983; Pogson *et al.*, 1984), was 8.1 ± 0.7 and 8.3 ± 0.8 μmol/g dry wt. at the beginning and end, respectively, of the incubations without hydroxycitrate. Assuming a wet-wt./dry-wt. ratio of 4 (Brunengraber *et al.*, 1972), these values are similar to those reported previously (Brunengraber *et al.*, 1973; Panek *et al.*, 1977; Cornell, 1983). At the highest concentration of hydroxycitrate (7 mM) the corresponding values were 8.6 ± 0.9 and 7.6 ± 0.9 μmol/g dry wt. Therefore the hepatocytes were not deficient in ATP, nor did they become so during incubations with or without the inhibitor. In preliminary investigations, the ATP content of some hepatocyte preparations was found to decrease significantly after approx. 15 min incubation, but this did not occur if 1 mM-L-carnitine was included in the preparation and incubation media. Since hepatocytes prepared with collagenase are deficient in carnitine (Christiansen & Bremer, 1976; Sandor *et al.*, 1985), these results suggest that, in some cases, this deficiency may be severe enough to affect the production of ATP.

The rates of acetate and butyrate uptake (Table 1) were similar to those reported by Jessop *et al.* (1986) and Demigné *et al.* (1986) respectively, and there was no significant net uptake or release of glucose (results not shown). However, the rates of ketogenesis were higher, and those of total fatty acid synthesis (approx. 0.6 μmol of C_2/min per g at the end of the 'lag' period) lower, than those measured in perfused liver (approx. 0.7 and 3.5 μmol of C_2/min per g dry wt. respectively: Mayes & Topping, 1974; Snoswell *et al.*, 1982). These differences probably result from the presence of carnitine and butyrate in the incubations. Carnitine promotes fatty acid oxidation rather than esterification in hepatocytes (Agius *et al.*, 1986), and butyrate, in addition to being a good oxidizable substrate (Table 1), can produce ketone bodies without entering the mitochondrial acetyl-CoA pool (Pahl-Wostl & Seelig, 1986). Indeed, in similar incubations without butyrate, the rate of ketogenesis was only 1.19 ± 0.14 μmol of C_2 units/min per g dry wt. (mean $\pm$ S.E.M. for six determinations).

Effect of hydroxycitrate on the flux of butyrate carbon to fatty acids and acetate

(−)-Hydroxycitrate is a specific inhibitor of ATP citrate lyase (EC 4.1.3.8) and consequently inhibits the transfer of acetyl groups from mitochondria to cytoplasm (Watson & Lowenstein, 1970; Lowenstein, 1971; Brunengraber *et al.*, 1972). Its inclusion in the present incubations (where up to 7 mM was required to obtain a near-maximal effect) did not significantly affect ATP content (see above), acetate or butyrate uptake or ketone-body production (Table 1).

In contrast, the fluxes of butyrate carbon to both fatty acid and acetate were considerably inhibited by hydroxycitrate and, most significantly, to a similar extent (Fig. 1; Table 1). Indeed, the percentage inhibition of the butyrate-to-acetate flux divided by the percentage inhibition of the butyrate-to-fatty-acid flux in the three experiments was 1.12 ± 0.13 and 1.07 ± 0.03 at 3.5 mM- and 7 mM-hydroxycitrate respectively. Since butyrate is activated only in the mitochondria of rat liver (Groot *et al.*, 1976), and hydroxycitrate did not significantly inhibit butyrate uptake (Table 1), this parallel inhibition of the flux of butyrate to both acetate and fatty acids strongly suggests that the production of acetate occurs in the cytoplasm, that is via the activity of the ATP-stimulated hydrolase. Hydroxycitrate would not be expected to inhibit the production of acetate via the mitochondrial hydrolase: indeed, it would be likely to increase this process as a result of the consequent accumulation of mitochondrial citrate and acetyl-CoA.

Therefore these results do not support the enzymological predictions of Söling & Rescher (1985), that the cytoplasmic hydrolase is unimportant, and suggest that further activators (or de-inhibitors) of this enzyme await discovery.

A possible complication with the above results is that, at the concentrations of (−)-hydroxycitrate used, there may also be some inhibition of citrate synthase (Sullivan *et al.* 1977; see Scheme 1). If this resulted in a decreased uptake of butyrate and/or deflection of acetyl-CoA towards ketone-body formation, it would decrease the flux of butyrate carbon to acetate independently of the effect on ATP citrate lyase in the cytoplasm. Indeed, in one experiment there was a small (approx. 10%) decrease in butyrate uptake and increased ketone-body output in response to 7 mM-(−)-hydroxycitrate. However, with the pooled data from three separate experiments, there was no significant effect of (−)-hydroxycitrate on either butyrate uptake or ketone-body output (Table 1), so that interference by inhibition of citrate synthase appears to be insignificant.

Effect of hydroxycitrate on the total rates of acetyl-CoA hydrolysis and fatty acid synthesis

Since the present results indicate that acetate production in rat hepatocytes is predominantly, if not exclusively, cytoplasmic, the total rate of acetyl-CoA hydrolysis can be calculated by dividing the initial rate of

^{14}C labelling of acetate by the specific radioactivity of cytoplasmic acetyl-CoA (which, in turn, can be calculated from the ^{3}H and ^{14}C labelling of fatty acids: see the Experimental section). The resulting calculations (Table 1) give rates similar to those predicted by Rabkin & Blum (1985), but somewhat lower than those reported by Jessop *et al.* (1986), who estimated hydrolase activity from the rate of decrease in [^{14}C]acetate specific radioactivity.

The initial rates of total fatty acid synthesis and acetyl-CoA hydrolysis were also inhibited by hydroxycitrate (Table 1). However, at 3.5 mM-hydroxycitrate, the percentage inhibitions of these total activities were significantly smaller ($P < 0.01$) than those of the corresponding fluxes of carbon from butyrate. Moreover, the specific radioactivity of cytoplasmic acetyl-CoA was also significantly lowered at this concentration of hydroxycitrate (Table 1). These differences could be explained if a significant proportion of the flux through cytoplasmic acetyl-CoA is derived from extramitochondrial sources and is therefore relatively unaffected by the inhibitor. These sources would include acetate activation via extramitochondrial acetyl-CoA synthetase and fatty acid oxidation in the peroxisomes (see Mannaerts & Debeer, 1981; Masters & Crane, 1984). Indeed, Goldberg & Brunengraber (1980) calculated that mitochondrially generated acetyl-CoA accounted for only approx. 36% of the total rate of fatty acid synthesis from acetate in rats *in vivo*.

Metabolic role of cytoplasmic acetyl-CoA hydrolase

Although the present results demonstrate the importance of cytoplasmic acetyl-CoA hydrolase, they do not allow any firm conclusions about its metabolic role. It may be involved in a substrate cycle between acetate and acetyl-CoA (Rabkin & Blum, 1985; Jessop *et al.*, 1986), which could help to dissipate excess fuel. Indeed, since acetate formation (via hydrolase activity) occurred despite a net uptake of acetate by the cells (Table 1), a substrate cycle is strongly indicated. However, in contast with muscle, where acetyl-CoA hydrolase and hence the acetate/acetyl-CoA cycle is predominantly mitochondrial (Matsunaga *et al.*, 1985; Crabtree *et al.*, 1987), the present results indicate that in liver the cycle is cytoplasmic, as predicted by Rabkin & Blum (1985). Moreover, the results in Table 1 show that, when acetyl-CoA hydrolase activity was almost totally inhibited, there was no corresponding increase in net acetate uptake (Table 1). This suggests that the activity of acetyl-CoA synthetase is also inhibited under these conditions; and, if this supposition is correct, it would support the hypothesis that these two enzymes act together to produce a substrate cycle between acetate and acetyl-CoA in liver cytoplasm.

However, several other roles for cytoplasmic ATP-stimulated acetyl-CoA hydrolase are possible. For example, it may be involved in controlling cytoplasmic CoA concentrations by serving as a 'safety valve' to prevent excessive accumulations of cytoplasmic acetyl-CoA, thereby deflecting excess fuel from the liver to the extrahepatic tissues (Prass *et al.*, 1980; Matsunaga *et al.*, 1985). However, this hypothesis is difficult to reconcile with the formation of acetate when there is a net uptake of acetate by the liver. The hydrolase may also serve to convert peroxisomally generated acetyl-CoA into acetate for subsequent oxidation in the mitochondria (Mannaerts & Debeer, 1981); it may be important for hydrolysing toxic acyl-CoA derivatives (Prass *et al.*, 1980); and it may enable ATP citrate lyase to provide a continuous supply of oxaloacetate for gluconeogenesis (Prass *et al.*, 1980), which would explain why this hydrolase is found only in liver and, to a lesser extent, kidney (Matsunaga *et al.*, 1985). These possibilities, including substrate cycling, are not mutually exclusive, and work is required to determine which, if any, of them applies *in vivo*.

REFERENCES

Agius, L., Chowdhury, M. H. & Alberti, K. G. M. M. (1986) Biochem. J. **239**, 593–601

Bernson, V. S. M. (1976) Eur. J. Biochem. **67**, 403–410

Berry, M. N. & Friend, D. S. (1969) J. Cell Biol. **43**, 506–520

Brunengraber, H., Sabine, J. R., Boutry, M. & Lowenstein, J. M. (1972) Arch. Biochem. Biophys. **150**, 392–396

Brunengraber, H., Boutry, M. & Lowenstein, J. M. (1973) J. Biol. Chem. **248**, 2656–2669

Byard, J. L., Reese, J. A. & Knadle, S. A. (1983) in Isolation, Characterization, and Use of Hepatocytes (Harris, R. A. & Cornell, N. W., eds.), pp. 69–76, Elsevier, Amsterdam

Christiansen, R. Z. & Bremer, J. (1976) Biochim. Biophys. Acta **448**, 562–577

Cornell, N. W. (1983) in Isolation, Characterization, and Use of Hepatocytes (Harris, R. A. & Cornell, N. W., eds.), pp. 11–20, Elsevier, Amsterdam

Crabtree, B., Taylor, D. J., Coombs, J. E., Smith, R. A., Templer, S. P. & Smith, G. H. (1981) Biochem. J. **196**, 747–756

Crabtree, B., Marr, S. A., Anderson, S. E. & MacRae, J. C. (1987) Biochem. J. **243**, 821–827

Demigné, C., Yacoub, C., Rémésy, C. & Fafournoux, P. (1986) Biochim. Biophys. Acta **875**, 535–542

Goldberg, R. P. & Brunengraber, H. (1980) in Alcohol and Aldehyde Metabolizing Systems IV (Thurman, R., ed.), pp. 413–418, Plenum Press, New York

Groot, P. H. E., Scholte, H. R. & Hülsmann, W. C. (1976) Adv. Lipid Res. **14**, 75–126

Guynn, R. W. & Veech, R. L. (1974) Anal. Biochem. **61**, 6–15

Jessop, N. S., Smith, G. H. & Crabtree, B. (1986) Biochem. Soc. Trans. **14**, 146–147

Katz, J., Wals, P. A. & van de Welde, R. L. (1974) J. Biol. Chem. **249**, 7348–7357

Knowles, S. E., Jarrett, I. G., Filsell, O. H. & Ballard, F. J. (1974) Biochem. J. **142**, 401–411

Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66

Lamprecht, W. & Trautschold, I. (1974) in Methods of Enzymatic Analysis, 2nd edn. (Bergmeyer, H. U., ed.), pp. 2101–2110, Academic Press, New York

Lowenstein, J. M. (1971) J. Biol. Chem. **246**, 629–632

Mannaerts, G. P. & Debeer, L. J. (1981) in Short Term Regulation of Liver Metabolism (Hue, L. & van de Werve, G., eds.), pp. 273–290, Elsevier, Amsterdam

Masters, C. & Crane, D. (1984) Trends Biochem. Sci. **9**, 314–317

Matsunaga, T., Isohashi, F., Nakanishi, Y. & Sakamoto, Y. (1985) Eur. J. Biochem. **152**, 331–336

Mayes, P. A. & Topping, D. L. (1974) Biochem. J. **140**, 111–114

Pahl-Wostl, C. & Seelig, J. (1986) Biochemistry **25**, 6799–6807

Panek, E., Cook, G. A. & Cornell, N. W. (1977) Lipids **12**, 814–818

Patience, R. L. & Thomas, J. D. (1982) J. Chromatogr. **234**, 225–230

Patterson, M. K. (1979) Methods Enzymol. **58**, 141–152
Pogson, C. I., Carpenter, W. R., Cook, J. S., Fisher, M. J., Lomax, M. A., Salter, M. & Stanley, J. C. (1984) Proc. Nutr. Soc. **43**, 119–132
Prass, R. L., Isohashi, F. & Utter, M. F. (1980) J. Biol. Chem. **255**, 5215–5223
Rabkin, M. & Blum, J. J. (1985) Biochem. J. **225**, 761–786
Robinson, J. B., Mahan, D. E. & Koeppe, R. E. (1976) Biochem. Biophys. Res. Commun. **71**, 959–965
Sandor, A., Kispal, G., Melegh, B. & Alkonyi, I. (1985) Biochim. Biophys. Acta **835**, 83–93
Smith, R. A. (1984) Ph.D. Thesis, University of Leeds
Snoswell, A. M., Trimble, R. P., Fishlock, R. C., Storer, G. B. & Topping, D. L. (1982) Biochim. Biophys. Acta **716**, 290–297
Söling, H.-D. & Rescher, C. (1985) Eur. J. Biochem. **147**, 111–117
Sullivan, A. C., Singh, M., Srere, P. A. & Glusker, J. P. (1977) J. Biol. Chem. **252**, 7583–7590
Taylor, D. J. (1978) Ph.D. Thesis, University of Leeds
Watson, J. A. & Lowenstein, J. M. (1970) J. Biol. Chem. **245**, 5993–6002
Williamson, D. H., Mellanby, J. & Krebs, H. A. (1962) Biochem. J. **108**, 353–361

Received 5 May 1988/19 August 1988; accepted 27 September 1988