Rabbit Liver Acetyl-CoA Synthetase

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Acetyl-CoA synthetase (EC 6.2.1.1) was assayed in subcellular fractions of rabbit liver homogenates. The activity was located almost exclusively in the cytosol. There was no decrease in activity when butyrate or propionate (each at 5–20 mM) were added to the assay medium.

Acetate is produced by bacterial fermentation in both ruminant and non-ruminant herbivores (Elsden *et al.*, 1946; Hungate *et al.*, 1959). In ruminants, about half of the caloric needs of the animal are supplied by this metabolite (Blaxter, 1962). To be utilized by tissues, the acetate is converted into acetyl-CoA by acetyl-CoA synthetase (Beinert *et al.*, 1953; Hele, 1954). There has been considerable interest in the activity of this enzyme in the organs of a number of species and in its subcellular distribution in these organs (for reviews see Ballard, 1972; Groot *et al.*, 1976).

Since we are interested in the role of acetate in the metabolic requirements of the rabbit (a non-ruminant herbivore), we have measured the activity and the subcellular distribution of acetyl-CoA synthetase in homogenates of rabbit liver. We have also measured the effects of butyrate and of propionate (which are also produced by bacterial fermentation in rabbit caecum) on the activity of this enzyme.

Materials and Methods

Substrates and lactate dehydrogenase (EC 1.1.1.27) were obtained from Boehringer Corp. (London) W.5, U.K. Butyrate and propionate were used as their sodium salts. Sodium [^{14}C]acetate was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Gelman chromatography media (ITLC Type C. G. 20 $\times$ 20) were supplied by Gelman-Hawksley, Lancing, Sussex, U.K.

Adult New Zealand rabbits of both sexes were used. They were maintained on a diet of laboratory pellets (E. Dixon & Sons, Ware, Herts., U.K.) with water available at all times.

Preparation of homogenates and subcellular fractions

Rabbits were killed by cervical dislocation. The liver was immediately cooled on ice. The tissue was minced with scissors and then homogenized at 4°C with a Potter–Elvehjem glass homogenizer, in 9 vol./g

wet wt. of tissue in 0.25 M-sucrose, which contained 10 mM-Tris buffer, pH 7.4, and 1 mM-dithiothreitol.

Subcellular fractions were prepared by the method of Williamson *et al.* (1968) as modified by Ash & Baird (1973). Homogenates and particulate pellets, obtained after the initial centrifugation, were sonicated at 4°C. The supernatant fraction obtained after centrifugation of the sonicated particulate pellet was termed the ‘particulate fraction’. The pellet remaining after this step was resuspended and termed the ‘membrane fraction’.

Assay of acetyl-CoA synthetase

The enzyme was assayed by the method of Huang (1970) except that 10 mM-sodium acetate (total radioactivity 10 μCi), 0.15 mM-CoA and 4 mM-ATP were used. No activity was observed with homogenates in the absence of sodium acetate, ATP or CoA. Decreased activity occurred when concentrations of sodium acetate, ATP and CoA greater than 20 mM, 8 mM and 0.35 mM respectively were used, and the omission of dithiothreitol decreased the activity by about 10%. In all assays, activity was linear with respect to time and with the amount of homogenate added.

Assay of marker enzymes

The marker enzymes used to characterize the subcellular fractions were lactate dehydrogenase for the cytosol, and glutamate dehydrogenase (EC 1.4.1.3) and citrate synthase (EC 4.1.3.7) for the mitochondrial matrix. Though marker enzymes for mitochondrial membranes were not used, the pellet obtained after the final centrifugation was resuspended and assayed for the presence of the marker enzymes described above.

Lactate dehydrogenase was assayed by the method of Bergmeyer & Brent (1974), glutamate dehydrogenase by the method of Schmidt (1974) and citrate synthase by the method of Srere *et al.* (1963). In all

Table 1. *Subcellular distribution of acetyl-CoA synthetase and marker enzymes in homogenates of rabbit liver*
The results are mean values $\pm$ S.E.M. for the number of animals indicated (in parentheses under the column heading 'Enzyme'). The activities are also expressed as a percentage of those in the homogenate.

Enzyme	Fraction ...	Enzyme activity (μ mol of substrate converted/min per g wet wt. of tissue)			
		Homogenate	Cytosol	Particulate	Membrane
Acetyl-CoA synthetase (6)		0.13 $\pm$ 0.01 (100%)	0.11 $\pm$ 0.02 (85%)	0.007 $\pm$ 0.004 (5%)	0.006 $\pm$ 0.004 (5%)
Lactate dehydrogenase (6)		101 $\pm$ 8 (100%)	98 $\pm$ 9 (97%)	9 $\pm$ 4 (9%)	2 $\pm$ 1 (2%)
Glutamate dehydrogenase (6)		21 $\pm$ 2 (100%)	1.4 $\pm$ 0.5 (7%)	20 $\pm$ 2 (95%)	2 $\pm$ 1 (10%)
Citrate synthase (4)		17 $\pm$ 3 (100%)	1.3 $\pm$ 0.4 (8%)	16 $\pm$ 3 (96%)	0.4 $\pm$ 0.1 (3%)

of these assays, activity was linear with respect to time and amount of homogenate added.

The activities of all of the enzymes assayed are expressed as μ mol of substrate transformed/min per g wet wt. of tissue so that the results could be compared with those of other workers.

Results and Discussion

The apparent K_m values of acetyl-CoA synthetase in the homogenate for sodium acetate, ATP and CoA were 2.61 ± 0.22 , 1.90 ± 0.18 and 0.12 ± 0.02 mM (mean values $\pm$ S.E.M. for four independent experiments) respectively. These are similar to values reported for rat liver homogenates (Aas & Bremer, 1968), and lie within the ranges quoted for other species (see review by Groot *et al.*, 1976).

The activity of acetyl-CoA synthetase (based on that measured in rabbit liver homogenates) was 0.13 ± 0.01 μ mol of acetyl-CoA formed/min per g wet wt. of tissue (mean value $\pm$ S.E.M. for 30 independent experiments). Corresponding values, with 10 mM-sodium acetate, for rat, guinea pig and bovine liver are 1.22, 0.18 and 0.83 respectively (Ash & Baird, 1973; Groot *et al.*, 1976).

The subcellular distribution of acetyl-CoA synthetase and the activities of the enzyme markers used (Table 1) show clearly that the synthetase is predominantly cytosolic in this tissue. By contrast, Ash & Baird (1973) found little synthetase activity in the cytoplasmic fraction of bovine liver, the majority of the activity being particulate. Ballard (1972) has shown that most of the synthetase of rat liver is cytoplasmic, whereas 87% of that in rat heart is mitochondrial. The enzyme is absent in guinea-pig liver mitochondria where acetate utilized in the mitochondria is activated by a distinct propionyl-CoA synthetase (Groot *et al.*, 1976).

However, Scholte *et al.* (1971) have reported that the acetyl-CoA synthetase activity recovered in the particulate-free supernatant fraction of homogenates of guinea-pig heart can be attributed to leakage from the mitochondria during the preparation. Activity of mitochondrial origin could be underestimated when glutamate dehydrogenase is used as a marker

Table 2. *Effects of butyrate and propionate on the activity of acetyl-CoA synthetase in homogenates of rabbit liver*

The results are mean values $\pm$ S.E.M. with the number of animals used shown in parentheses. Butyrate or propionate was added to the assay medium as shown.

Butyrate or propionate added (mM)	Activity of acetyl-CoA synthetase (μ mol/min per g wet wt. of tissue) in the presence of:	
	Butyrate	Propionate
0	0.13 $\pm$ 0.01 (30)	0.13 $\pm$ 0.01 (30)
5	0.15 $\pm$ 0.04 (3)	0.12 $\pm$ 0.04 (3)
10	0.13 $\pm$ 0.02 (9)	0.14 $\pm$ 0.02 (9)
15	0.16 $\pm$ 0.05 (3)	0.19 $\pm$ 0.05 (3)
20	0.16 $\pm$ 0.04 (3)	0.19 $\pm$ 0.06 (3)

enzyme as the latter is bound to the inner mitochondrial membrane. Leakage of enzymes from the matrix would be unaccounted for. In the present results, however, good recoveries of the activities of marker enzymes were obtained. Over 90% of the activities detected in the homogenate were recovered in the appropriate fractions. In addition, we have used both citrate synthase (which is not bound to the inner mitochondrial membrane and so would be readily lost into the cytoplasmic fraction if disruption of the mitochondria occurs) and glutamate dehydrogenase as markers for the mitochondria matrix.

The results shown in Table 1 indicate that leakage from the mitochondria was only 8% and 6.5% for citrate synthase and glutamate dehydrogenase respectively. This indicates that mitochondrial integrity was maintained during tissue preparation and that the acetyl-CoA synthetase activity measured was primarily located in the cytosol of rabbit liver.

The addition of butyrate or of propionate in the assay medium had no significant effect on the activity of acetyl-CoA synthetase (Table 2). This contrasts with the results of Ash & Baird (1973), who reported that either propionate (10 mM) or butyrate (10 mM) inhibited acetyl-CoA synthetase activity in bovine liver by 50% and to a lesser extent in rumen epithelium. A decreased rate of oxidation of acetate by sheep liver has also been reported in the presence of

either propionate or butyrate (Leng & Annison, 1963; Smith, 1971), although this effect may be mediated at a point other than activation of acetate to acetyl-CoA. Pritchard & Tove (1960) have demonstrated with sheep liver slices that the decrease in acetate oxidation is not paralleled by any change in the synthesis from acetate of either protein or lipid. This suggests that activation of acetate was maintained, but that oxidation of acetate was specifically inhibited. The present results indicate that in rabbit liver (unlike bovine tissue) acetyl-CoA synthetase is specific for acetate and does not use the other short-chain fatty acids tested.

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References

- Aas, M. & Bremer, J. (1968) *Biochim. Biophys. Acta* **164**, 157–166
- Ash, R. & Baird, G. D. (1973) *Biochem. J.* **136**, 311–319
- Ballard, C. J. (1972) *Am. J. Clin. Nutr.* **25**, 773–779
- Beinert, H., Green, D. E., Hele, P., Hift, H., Von Korff, R. W. & Ramakrishnan, C. V. (1953) *J. Biol. Chem.* **203**, 35–45
- Bergmeyer, H. U. & Brent, E. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 575–579, Academic Press, New York, San Francisco and London
- Blaxter, K. L. (1962) *The Energy Metabolism of Ruminants*, pp. 185–216, Hutchinson, London
- Elsden, S. R., Hitchcock, M. W. S., Marshall, R. A. & Phillipson, A. T. (1946). *J. Exp. Biol.* **22**, 191–200
- Groot, P. H. E., Scholte, H. R. & Hulsmann, W. C. (1976) *Adv. Lipid Res.* **14**, 75–126
- Hele, P. (1954) *J. Biol. Chem.* **206**, 671–676
- Huang, K. P. (1970) *Anal. Biochem.* **37**, 98–104
- Hungate, R. E., Phillips, G. D., McGregor, A., Hungate, D. P. & Buechner, H. K. (1959), *Science* **130**, 1192–1194
- Leng, R. A. & Annison, E. F. (1963) *Biochem. J.* **86**, 319–327
- Pritchard, G. I. & Tove, S. B. (1960) *Biochim. Biophys. Acta* **41**, 130–136
- Schmidt, E. (1974). in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.) pp. 650–656, Academic Press, New York, San Francisco and London
- Scholte, H. R., Wit-Peters, E. M. & Bakker, J. C. (1971) *Biochim. Biophys. Acta* **231**, 479–486
- Smith, R. M. (1971) *Biochem. J.* **124**, 887–881
- Srere, P. A., Brazil, H. & Gonen, L. (1963) *Acta Chem. Scand.* **17**, S129–S134
- Williamson, D. H., Bates, M. W. & Krebs, H. A. (1968) *Biochem. J.* **108**, 353–361