The Oxoacyl-Coenzyme A Thiolases of Animal Tissues

By B. MIDDLETON*

Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, U.K.

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1. The activities and relative 3-oxoacyl-CoA substrate specificities of oxoacyl-CoA thiolase were determined in a large number of animal tissues. The relative activities with different 3-oxoacyl-CoA substrates varied widely in different tissues and, in addition, the activity as measured with acetoacetyl-CoA (but not with other longer-carbon-chain acyl-CoA substrates) was activated by K^+ . 2. These properties were due to the presence, in different proportions in each tissue, of three classes of thiolase, all of which use acetoacetyl-CoA as substrate but which have different intracellular locations and substrate specificities and which differ also in kinetic and chromatographic behaviour. 3. Cytoplasmic thiolase activity was found to be widely distributed among different tissues and was due to an acetoacetyl-CoA-specific thiolase. This cytoplasmic activity was found to account for a significant proportion of the total tissue activity towards acetoacetyl-CoA in several tissues, and especially in the brain of newborn rats. 4. Mitochondrial thiolase activity towards acetoacetyl-CoA was due to two different classes of enzyme whose relative amounts varied with the tissue type. An oxoacyl-CoA thiolase of general specificity for the acyl-CoA substrate constituted one class, the other being a specific acetoacetyl-CoA thiolase that differed from its cytoplasmic counterpart in being greatly stimulated by K^+ . 5. This activation by K^+ made it possible to calculate the tissue contents of mitochondrial acetoacetyl-CoA thiolase and mitochondrial oxoacyl-CoA thiolase from measurements of activity with acetoacetyl-CoA in tissue extracts under defined conditions. 6. The properties and the different thiolases and their tissue distribution is discussed with respect to their possible roles in metabolism.

The widespread occurrence in animal tissues of thiolase activity towards acetoacetyl-CoA (Wieland *et al.*, 1956; Williamson *et al.*, 1971) has been assumed by these authors to reflect the importance of the 3-oxoacyl-CoA thiolase reaction in the β -oxidation of fatty acids and in the metabolism of ketone bodies. Two distinct types of oxoacyl-CoA thiolase have been purified from mammalian tissues, both of which can use acetoacetyl-CoA as substrate. An acetoacetyl-CoA specific thiolase (EC 2.3.1.9) has been crystallized from pig heart by Gehring *et al.* (1968) and a general oxoacyl-CoA thiolase (EC 2.3.1.16), capable of using 3-oxoacyl-CoA substrates of widely differing carbon-chain length, has been isolated from ox liver (Seubert *et al.*, 1968). The existence of these two types of thiolase, albeit in different tissues from different species, suggested that they may be involved in separate metabolic processes. Some evidence in favour of this idea came from the demonstration (Middleton, 1972b) that both these two types of oxoacyl-CoA thiolase exist in ox liver mitochondria and that another, different, acetoacetyl-CoA thiolase was present in ox liver cytoplasm. The occurrence in one tissue of several different oxoacyl-

CoA thiolases, all of which can use acetoacetyl-CoA as substrate, means that tissue activity distributions determined with this substrate alone yield little useful information about the type of thiolase present. The present work was undertaken to investigate the generality of this multiplicity of oxoacyl-CoA thiolases and thereby to gain information about the relationships between their properties, intracellular locations and possible metabolic roles. A preliminary report of this study has appeared (Middleton, 1971).

Materials

Phosphate acetyltransferase (EC 2.3.1.8), 3-hydroxyl-CoA dehydrogenase (EC 1.1.1.35), CoA (grade 1), NADH and acetyl phosphate were purchased from Boehringer Corp. (London) Ltd., London W.5, U.K. Ampholine solution [40% (w/v) in water], pH 3-10, was from LKB Produkter AB, Bromma, Sweden. DEAE-cellulose (Whatman grade DE-32) and cellulose phosphate were purchased from W. and R. Balston (Modified Cellulose) Ltd., Maidstone, Kent, U.K. Sephadex G-25 (medium grade) was obtained from Pharmacia, Uppsala, Sweden. 3-Oxohexanoyl ethyl ester was the Purum grade of Fluka A.G., Buchs, Switzerland. 5,5'-Dithiobis-(2-nitrobenzoic acid) was obtained from

* Present address: The Chemical Laboratory, University of Cambridge, Cambridge CB2 1EW, U.K.

Aldrich Chemical Co., Milwaukee, Wis., U.S.A. Thioglycolic acid was from BDH Chemicals Ltd., Poole, Dorset, U.K., and was purified before use (White, 1960) by distillation. Other chemicals were of the highest quality available.

Animals

Laboratory-bred male rats of the Wistar strain, weighing 180–220 g, were used as a routine, but there was no significant difference in tissue activity of 3-oxoacyl-CoA thiolase between male and female animals. Rat tissues and tissues from other animals were chilled in ice as soon as possible after removal from the carcass.

Methods

Acetoacetyl-CoA was prepared and purified as described previously (Middleton, 1972a). Solutions were adjusted to pH 4 with 0.1 M-NaOH and stored at -20°C . The methyl ester of 3-oxodecanoic acid was prepared by the method of Ståhlberg-Stenhagen (1945). The thioglycolic esters of 3-oxohexanoic acid and 3-oxodecanoic acid were prepared by the method of Vagelos & Alberts (1960). The CoA derivatives of these compounds were obtained by thiol-ester exchange with CoA at pH 8.5 in a solvent system composed of 2-methylpropan-2-ol and water exactly as described by Chase & Tubbs (1972). The CoA content of the 3-oxohexanoyl-CoA was kept below 10% of the final 3-oxoacyl-CoA by the use of more than a 20-fold excess of the thioglycollate ester over free CoA. 3-Oxodecanoyl-CoA was separated from any residual free CoA by precipitation with a few drops of HClO_4 after previously saturating the ice-cold solution with solid NaCl (Stewart *et al.*, 1973). The NaCl, by apparently decreasing the acid solubility of the medium-chain-length acyl-CoA derivatives, aids quantitative precipitation. The white precipitate of 3-oxodecanoyl-CoA was filtered off, washed with a little cold 0.1 M- HClO_4 , taken up in water and adjusted to around pH 4 with 0.1 M-NaOH. The free CoA content of this preparation was never more than 1% of the 3-oxodecanoyl-CoA. In view of the effect of K^+ (but not Na^+ ; see below) on oxoacyl-CoA thiolase activity the cation content of the 3-oxoacyl-CoA compounds was kept low, and restricted to Na^+ . 3-Oxoacyl-CoA derivatives were assayed by using 3-hydroxyacyl-CoA dehydrogenase by the method of Decker (1963). CoA, un-neutralized in water, was freshly prepared and was assayed as a routine by its thiol content by using the method of Ellman (1959). The purity of the CoA was checked by the use of phosphate acetyltransferase (Michal & Bergmeyer, 1963).

Isoelectric focusing was performed in a LKB 8101

column at 15°C by using Ampholine pH 3–10 in a 20–60% (v/v) glycerol gradient.

Protein in tissue extracts was determined by the biuret method (Gornall *et al.*, 1949) but for purified enzyme preparations the method of Warburg & Christian (1941) was used.

Preparation of subcellular fractions and extracts of tissues

Rat liver mitochondria were prepared after homogenization of chopped rat liver in 5 vol. of 0.3 M-sucrose. Conventional centrifugation methods (see Chase & Tubbs, 1972) were used and the mitochondria were twice washed in the sucrose medium, any 'fluffy' layer being carefully removed. They were finally suspended in the same medium at a concentration of about 70–100 mg of protein/ml. Extracts of mitochondria were prepared by treating frozen-thawed suspensions with Triton X-100 [$1.2\ \mu\text{l}$ of 10% (w/v) Triton (final concentration 0.5%, w/v) per mg of mitochondrial protein]. The suspension was left at 4°C for 5 min before centrifuging at 100 000g for 30 min. The supernatant fraction was retained. Breakage by freeze-thawing alone gave only partial release of thiolase activity. Sonication, although giving as good mitochondrial breakage as detergent (judged by thiolase release), was less reproducible owing to local overheating.

Rat liver cytoplasm was prepared from the post-mitochondrial fraction by centrifugation at 100 000g for 1 h.

Extracts of whole tissue were prepared from fresh samples by homogenization in a ground-glass homogenizer with a motor-driven ground-glass pestle. The tissue was dispersed into at least 5 vol. of 0.1 M-sodium phosphate, pH 7.2, containing 0.5% (w/v) Triton X-100. For tougher tissues such as skeletal muscle or stomach, the material was frozen-thawed first. For amounts of tissue larger than 5 g, disruption was effected by blending with 5 vol. of 0.1 M-sodium phosphate–0.5 mM-dithiothreitol, pH 7.2, in a Polytron overhead blender (Kinematica G.m.b.H., Lucerne, Switzerland) run at full speed for 3×1 min periods, the temperature of the homogenate being kept below 10°C . To ensure full disruption by this latter technique (particularly if the tissue had not been previously frozen-thawed), Triton X-100 was then added to the homogenate to give a final concentration of 0.5% (w/v). The suspension was then centrifuged at 100 000g for 30 min and the supernatant retained. As a routine a portion of the supernatant was then gel-filtered (Sephadex G-25) at room temperature into 100 mM-Tris-HCl buffer, pH 8.2, containing 25% (v/v) glycerol. This material was used for the separation of mitochondrial and cytoplasmic thiolase activity (see below) and the

parent tissue extract was assayed for total tissue thiolase activity.

Chromatographic separation of tissue oxoacyl-CoA thiolases

The method used was that of Middleton (1972b). The cytoplasmic oxoacyl-CoA thiolase activity is separated (after gel filtration as described above) by passing the extract down a column of DEAE-cellulose equilibrated at room temperature with the same Tris-HCl buffer. Not more than 15 mg of protein (at 25 mg/ml) is applied per ml packed volume of DEAE-cellulose. The column is washed with the same buffer and the unretarded material collected. This contains all the mitochondrial oxoacyl-CoA thiolase activity (see below). The cytoplasmic thiolase is then eluted from the column by a linear 100–500 mM-Tris-HCl gradient, pH 8.2, containing 25% (v/v) glycerol. The total volume of the gradient was 6 times that of the packed volume of the column. Alternatively the cytoplasmic thiolase may be eluted by washing the column with 300 mM-Tris-HCl buffer, pH 8.2.

The mitochondrial thiolases are separated from each other by cellulose phosphate chromatography of the unretarded material from the DEAE-cellulose step. A portion of this material is first gel filtered on Sephadex G-25 into 20 mM-potassium phosphate, pH 6.6, containing 25% (v/v) glycerol. This is then applied to a cellulose phosphate column equilibrated with the same buffer. The maximum loading used was 15 mg of protein/ml packed volume of exchanger, the protein being applied at a concentration of up to 20 mg/ml. The column is washed with the same buffer before a linear gradient is started of 25–500 mM-potassium phosphate, pH 6.6, containing 25% (v/v) glycerol. The two types of mitochondrial thiolase are eluted as sharp peaks emerging at 180–200 mM- and 250–400 mM-phosphate respectively.

The presence of glycerol in all buffers used here was found to be necessary for maintenance of thiolase activity and enabled the separations to be carried out at room temperature with activity yields of 90% or better.

Enzyme assays

3-Oxo acid CoA-transferase (EC 2.8.3.5) was assayed in the direction of acetoacetyl-CoA breakdown by the method of Williamson *et al.* (1971). Oxoacyl-CoA thiolase activity was determined at 30°C by following the stimulation of 3-oxoacyl-CoA breakdown (measured at 303 nm) caused by the addition of CoA, to a final concentration of 50 μ M, to a cuvette of 1 cm light-path containing 100 mM-Tris-HCl, pH 8.1, 25 mM-MgCl₂, 50 mM-KCl, 10 μ M-3-oxoacyl-CoA, and the enzyme, in a total volume

of 2 ml. Any thiol esterase activity present was subtracted from the apparent thiolase rate. However, thiol esterase activity towards 3-oxoacyl-CoA was, with the exception of testis and brain, less than 1% of the oxoacyl-CoA thiolase activity. In testis and brain extracts thiol esterase activity was highest with the longer-chain 3-oxoacyl-CoA substrates but was about 10% of the thiolase rate when acetoacetyl-CoA was used. Under these standard assay conditions for oxoacyl-CoA thiolase activity the apparent extinction coefficients of the 3-oxoacyl-CoA substrates were: acetoacetyl-CoA, 16.9×10^3 litre·mol⁻¹; 3-oxohexanoyl-CoA, 15.6×10^3 litre·mol⁻¹; 3-oxodecanoyl-CoA, 13.5×10^3 litre·mol⁻¹. Rates of thiolase activity are expressed as μ mol or nmol of 3-oxoacyl-CoA removed/min measured in this standard system. Tissue activities are expressed as velocities per g fresh wt. Activation by K⁺ is expressed as the observed rate of removal of acetoacetyl-CoA in the above assay system divided by the rate of removal of acetoacetyl-CoA in a system in which the KCl is replaced by an equal concentration of NaCl. It is therefore a ratio whose minimum value (no activation by K⁺) is unity. Na⁺ neither activates nor inhibits the K⁺-sensitive oxoacyl-CoA thiolase of animal tissues. When measuring K⁺ activation care was taken to ensure that neither substrates nor enzyme solutions contained K⁺, this ion being replaced by Na⁺.

Calculation of the tissue content of individual mitochondrial thiolases

The method assumes that there are only three types of oxoacyl-CoA thiolase present in significant amounts in animal tissues capable of using acetoacetyl-CoA as a substrate under the standard assay conditions. This assumption has been found to hold for rat brain, kidney, heart and liver, pig heart and ox liver. The data required for the calculation are: (i) the difference (Δ) between the tissue thiolase activity with acetoacetyl-CoA measured in the assay mixture containing K⁺ and the same activity measured in that containing Na⁺; (ii) an estimate (ρ) of the activation caused by K⁺ (measured as described above with acetoacetyl-CoA as substrate) for the isolated mitochondrial acetoacetyl-CoA thiolase of the tissue concerned; (iii) the tissue activity (C), measured with acetoacetyl-CoA, of the cytoplasmic thiolase. The tissue content (M) of the mitochondrial acetoacetyl-CoA-specific thiolase (EC 2.3.1.9) is obtained from $M = \rho\Delta/(\rho - 1)$. The formula assumes that the observed effect of K⁺, (ρ), is due to only one type of enzyme, which K⁺ activates ρ -fold under standard assay conditions. The estimate of ρ can be made by separating the K⁺-activated acetoacetyl-CoA thiolase from a given tissue by the methods described above, measuring ρ and assuming that the

K⁺-activated thiolases of other tissues from the same animal will have the same value of ρ . This is certainly valid for rat tissues, as shown in Table 4 where the mean value of ρ for purified mitochondrial acetoacetyl-CoA thiolases from rat brain, kidney, heart and liver is 4.18 with a s.d. of 0.16.

The tissue content of the general oxoacyl-CoA thiolase (EC 2.3.1.16) can now be calculated by subtracting from the total tissue thiolase activity (measured with acetoacetyl-CoA in K⁺-containing media) the sum of the mitochondrial and cytoplasmic acetoacetyl-CoA thiolase activities ($M+C$). M is calculated as described above and C is experimentally determined after DEAE-cellulose chromatography of tissue extracts (see above). This method expresses all the thiolase activities in terms of μmol of acetoacetyl-CoA removed/min per g fresh wt. of tissue measured under standard assay conditions.

Units of enzyme activity

A unit of enzyme activity is that amount of enzyme which, under the standard assay conditions

defined above, transforms 1 μmol of substrate/min. Specific activity is in units/mg of soluble protein. All graphical kinetic results in this paper have velocities corrected to 10 munits of enzyme per cuvette.

Results

Apparent oxoacyl-CoA thiolase activities of animal tissues

The oxoacyl-CoA thiolase activity of a wide variety of tissues was determined as described above with three different 3-oxoacyl-CoA substrates: acetoacetyl-CoA, 3-oxohexanoyl-CoA and 3-oxodecanoyl-CoA. In addition, the stimulation of thiolase activity by K⁺ was investigated. In agreement with previous observations (Middleton, 1972b) it was found that activation by K⁺ occurred only when acetoacetyl-CoA was the substrate. The results presented in Table 1 show that there is a large and significant variation in both the apparent thiolase specificity for 3-oxoacyl-CoA substrate and in the apparent activation by K⁺ when different tissues of the same animal are compared. These results suggest that

Table 1. *Apparent oxoacyl-CoA thiolase activities in animal tissues*

Oxoacyl-CoA thiolase activity was determined by the standard assay procedure in extracts of tissues, by using 3-oxoacyl-CoA substrates of differing acyl-carbon-chain lengths. Rat tissues were taken from at least two animals and were pooled for each observation. Results are expressed as μmol of 3-oxoacyl-CoA removed/min per g fresh wt. of tissue and are means $\pm$ s.e.m. with the numbers of observations in parentheses. Ratios of oxoacyl-CoA thiolase activities with different substrates were determined for each observation and are expressed as means $\pm$ s.e.m. Activation of oxoacyl-CoA thiolase activity by K⁺ (with acetoacetyl-CoA as substrate) was determined in the individual extracts as described in the Methods section and the results are expressed as means $\pm$ s.e.m. The following abbreviations are used in Tables 1, 2 and 4: C₄, acetoacetyl-CoA; C₆, 3-oxohexanoyl-CoA; C₁₀, 3-oxodecanoyl-CoA. C₆/C₄ and C₁₀/C₆ describe the relative rates of oxoacyl-CoA thiolase activity in tissue extracts with the appropriate pair of 3-oxoacyl-CoA substrates.

Tissue		Tissue activities with different 3-oxoacyl-CoA substrates			Relative rates of thiolase activity with different 3-oxoacyl-CoA substrates		Activation by K ⁺
		C ₁₀	C ₆	C ₄	C ₆ /C ₄	C ₁₀ /C ₆	
Rat brain (newborn)	(4)	<0.2	<0.2	4.7 $\pm$ 0.2	—	—	1.19 $\pm$ 0.04
Rat brain (adult)	(9)	<0.2	<0.2	3.6 $\pm$ 0.3	—	—	1.94 $\pm$ 0.03
Rat muscle (hind-limb)	(3)	1.1 $\pm$ 0.2	0.66 $\pm$ 0.02	2.4 $\pm$ 0.3	0.28 $\pm$ 0.02	1.59 $\pm$ 0.25	2.88 $\pm$ 0.02
Rat stomach	(4)	1.56 $\pm$ 0.1	1.0 $\pm$ 0.1	6.3 $\pm$ 0.4	0.16 $\pm$ 0.06	1.46 $\pm$ 0.03	2.77 $\pm$ 0.05
Rat heart	(6)	16.7 $\pm$ 1.7	12.5 $\pm$ 0.7	21.5 $\pm$ 2.1	0.61 $\pm$ 0.04	1.34 $\pm$ 0.10	2.87 $\pm$ 0.08
Rat kidney	(6)	10.0 $\pm$ 2.3	6.3 $\pm$ 1.4	20.6 $\pm$ 1.9	0.34 $\pm$ 0.07	1.65 $\pm$ 0.14	3.10 $\pm$ 0.07
Rat ileum	(4)	2.5 $\pm$ 0.1	1.6 $\pm$ 0.1	5.9 $\pm$ 0.3	0.28 $\pm$ 0.02	1.56 $\pm$ 0.15	1.95 $\pm$ 0.04
Rat liver	(16)	29.1 $\pm$ 1.8	26.3 $\pm$ 1.6	27.1 $\pm$ 1.6	0.95 $\pm$ 0.03	1.19 $\pm$ 0.07	1.83 $\pm$ 0.04
Rat adrenal	(6)	27.4 $\pm$ 0.6	23.0 $\pm$ 1.6	22.9 $\pm$ 2.1	1.01 $\pm$ 0.02	1.20 $\pm$ 0.07	1.64 $\pm$ 0.13
Rat testis	(4)	5.2 $\pm$ 1.1	2.9 $\pm$ 0.7	2.8 $\pm$ 0.2	1.02 $\pm$ 0.16	1.86 $\pm$ 0.04	1.50 $\pm$ 0.02
Pigeon breast muscle	(3)	23.5 $\pm$ 2.2	18.8 $\pm$ 1.6	9.3 $\pm$ 0.1	2.02 $\pm$ 0.15	1.25 $\pm$ 0.01	1.88 $\pm$ 0.18
Pig heart	(4)	11.5 $\pm$ 1.3	9.3 $\pm$ 1.4	7.4 $\pm$ 0.8	1.25 $\pm$ 0.06	1.29 $\pm$ 0.33	1.97 $\pm$ 0.03
Ox heart	(3)	12.2 $\pm$ 0.4	9.0 $\pm$ 0.7	6.1 $\pm$ 1.1	1.51 $\pm$ 0.16	1.37 $\pm$ 0.07	2.00 $\pm$ 0.01
Ox liver	(15)	73.1 $\pm$ 7.1	67.0 $\pm$ 3.2	42.1 $\pm$ 1.7	1.66 $\pm$ 0.06	1.08 $\pm$ 0.04	1.56 $\pm$ 0.04

Table 2. *Oxoacyl-CoA thiolase activity in rat liver fractions*

Enzyme activity and activation by K^+ was measured as described in the Methods section. The results are expressed as means $\pm$ S.E.M. with the numbers of observations in parentheses.

Fraction		Specific activity with different 3-oxoacyl-CoA substrates (nmol/min per mg)		Relative rates with different substrates C_6/C_4	Activation by K^+
		C_6	C_4		
Cytoplasm	(6)	15.5 ± 1.8	39.9 ± 3.2	0.39 ± 0.04	1.0 ± 0.0
Mitochondrial extract	(6)	377 ± 50	282 ± 20	1.33 ± 0.09	1.95 ± 0.04

Fig. 1. *Comparison of the elution profiles on DEAE-cellulose of oxoacyl-CoA thiolase activity from rat liver cytoplasm and extracts of whole rat liver*

In both cases the material was applied to a column (7 cm $\times$ 3 cm) of DEAE-cellulose at pH 8.2 and eluted with a linear gradient of Tris-HCl as described in the Methods section. The fraction size was 8 ml. The broken line (---) represents the calculated chloride concentration in the eluate. Oxoacyl-CoA thiolase activity (Δ) was measured with acetoacetyl-CoA under standard assay conditions. (a) Oxoacyl-CoA thiolase elution profile from an extract of whole rat liver, 237 mg of soluble protein applied; (b) oxoacyl-CoA thiolase elution profile from rat liver cytoplasm, 665 mg of protein applied.

different proportions of two or more types of oxoacyl-CoA thiolase, one of which is activated by K^+ , are present in these tissues.

Apparent oxoacyl-CoA thiolase activities of rat liver subcellular fractions

Other investigations (Pette, 1965; Sauer & Erfle, 1966; Williamson *et al.*, 1968) have shown that oxoacyl-CoA thiolase activity, measured with acetoacetyl-CoA, shows a bimodal distribution in mammalian liver, being present in both mitochondria and cytoplasm. The apparent specificity for 3-oxoacyl-CoA substrate and apparent activation by K^+ of the thiolase activity in rat liver cytoplasm and mitochondrial extracts was investigated here and the results are shown in Table 2. In agreement with the results of the aforementioned investigations a higher thiolase activity in mitochondria than in cytoplasm was found. However, differences exist in the properties of the mitochondrial and cytoplasmic thiolases. Cytoplasmic thiolase activity is higher with acetoacetyl-CoA than with 3-oxohexanoyl-CoA, and K^+ has no effect on the activity with acetoacetyl-CoA. Mitochondrial activity, however, differs significantly, having a higher relative activity with the longer-chain substrate and showing a definite activation by K^+ . These different properties indicate that the cytoplasmic activity in rat liver is not derived by leakage of the higher mitochondrial activity during tissue fractionation.

Isolation of individual rat liver oxoacyl-CoA thiolases

The chromatographic methods for separating the cytoplasmic from the mitochondrial thiolases using DEAE-cellulose, and subsequently separating each mitochondrial type of thiolase on cellulose phosphate (Middleton, 1972b), had previously only been reported for the thiolases of ox liver. Fig. 1 compares the chromatography on DEAE-cellulose of thiolase activity from an extract of whole rat liver (Fig. 1a)

with that from carefully prepared rat liver cytoplasm (Fig. 1*b*). Fractions were collected from the moment of application of the enzymes to the column and were monitored for thiolase activity with acetoacetyl-CoA and 3-oxohexanoyl-CoA, and activation by K^+ was also tested. In Fig. 1*b* more than 90% of the cytoplasmic thiolase activity with acetoacetyl-CoA was retained on the column and was eluted as a single peak showing activity only with acetoacetyl-CoA. The activity with the longer-chain substrate was too low for accurate measurement. This cytoplasmic acetoacetyl-CoA-specific thiolase was unaffected by K^+ . The small amount of activity unretarded by the column in Fig. 1*b* had a different specificity, being more active with 3-oxohexanoyl-CoA than with acetoacetyl-CoA. When an extract of whole rat liver was applied to an identical column (Fig. 1*a*) the elution pattern showed two peaks of oxoacyl-CoA thiolase activity, but only 13% of the applied activity measured with acetoacetyl-CoA was retained on the column. This enzyme was clearly identical with the cytoplasmic acetoacetyl-CoA thiolase of Fig. 1*b* in its elution properties and was also found to be absolutely specific for acetoacetyl-CoA and unaffected by K^+ . In contrast to this, the unretarded oxoacyl-CoA thiolase activity showed a 2-fold activation by K^+ (with acetoacetyl-CoA as substrate) and the ratio of activity with 3-oxohexanoyl-CoA to activity with acetoacetyl-CoA was 1.4:1. All the activity with 3-oxohexanoyl-CoA that had been applied to the column was found in the unretarded peak. The oxoacyl-CoA thiolase properties of this unretarded material thus closely resemble those of mitochondrial extracts (Table 2), confirming that this chromatography on DEAE-cellulose has separated mitochondrial from cytoplasmic thiolase. The experiment shown in Fig. 1*a* has been performed three times with protein loads varying from 5 to 20 mg/ml of column packed volume and at concentrations from 5 to 30 mg/ml. The results consistently show that 12–14% of the applied thiolase activity (measured with acetoacetyl-CoA) is retained on the column and eluted as shown. The overall yields of thiolase activity are better than 95%. Therefore it appears that this technique gives quantitative isolation of the cytoplasmic acetoacetyl-CoA thiolase from rat liver and ox liver extracts.

Fig. 2*a* shows the oxoacyl-CoA thiolase elution profile when the unretarded material from an experiment identical with that depicted in Fig. 1*a* is re-chromatographed on cellulose phosphate at pH 6.6. This is compared in Fig. 2*b* with the chromatography, under identical conditions, of an extract of rat liver mitochondria. In both cases fractions were monitored as before with 3-oxohexanoyl-CoA and acetoacetyl-CoA. Before testing for activation by K^+ , samples were gel-filtered on Sephadex G-25 into 10 mM-Tris-HCl buffer, pH 7.8. Both elution profiles

are extremely similar in all properties that were monitored. All the enzyme activity was retained on the columns until eluted with a steep linear phosphate gradient at pH 6.6. In both Figs. 2*a* and 2*b* the first peak of enzyme (peak I) to be eluted represented all the activity with 3-oxohexanoyl-CoA but only about 30% of the activity with acetoacetyl-CoA. Further, as shown, the ratios of activity with 3-oxohexanoyl-CoA to that with acetoacetyl-CoA were virtually constant, around 3.8:1, throughout the peak. This mitochondrial oxoacyl-CoA thiolase was not activated by K^+ . The remaining activity with acetoacetyl-CoA was eluted by higher phosphate concentrations and appeared as two peaks (IIA and IIB). This is a consistent phenomenon for the enzymes from rat liver, but the relative amount of peak IIA does appear to vary between tissues and species. In ox liver (Middleton, 1972*b*) and in rat heart it only appears as a small shoulder to the main peak of activity. Material from both peaks was not only absolutely specific for acetoacetyl-CoA but was also similarly activated by K^+ . Activation by K^+ in the experiment recorded in Fig. 2*a* was 4.25-fold for peak IIA and 4.30-fold for peak IIB. This phenomenon is discussed in a later section of this paper, but the thiolases of peaks IIA and IIB are clearly similar in type and distinct from the enzyme of peak I. It can therefore be concluded that cellulose phosphate chromatography of the rat liver thiolase activity that is not adsorbed on DEAE-cellulose at pH 8.2 yields two distinct types of enzyme, an acetoacetyl-CoA-specific K^+ -activated thiolase, and a thiolase of more general specificity for 3-oxoacyl-CoA substrate. Both these enzymes are of mitochondrial origin. Since the yields of activity (measured with 3-oxohexanoyl-CoA and acetoacetyl-CoA) are normally better than 90% for both the DEAE-cellulose and the cellulose phosphate techniques, it is clear that the apparent acetoacetyl-CoA thiolase activity observed in rat liver extracts is due to one cytoplasmic and two mitochondrial types of oxoacyl-CoA thiolase.

Tissue contents of the individual oxoacyl-CoA thiolases

The chromatographic separation techniques described above give good yields and a complete separation of the three classes of oxoacyl-CoA thiolase activity found both in ox and rat liver and can therefore be used to determine the actual content of these enzymes in animal tissues. The direct determination of cytoplasmic and mitochondrial oxoacyl-CoA thiolase activity in tissues by sub-cellular fractionation is rendered difficult or impossible by (a) the high relative activity of the mitochondrial enzymes, which could significantly raise the apparent cytoplasmic thiolase activity by leakage from the mitochondria during tissue homogenization,

Fig. 2. Separation of rat liver mitochondrial oxoacyl-CoA thiolases by chromatography on cellulose phosphate

Material was applied to a column (7 cm $\times$ 3 cm) of cellulose phosphate at pH 6.6 and eluted with a linear gradient of potassium phosphate as described in the Methods section. Fractions of 8 ml were collected. The broken line (---) represents the calculated phosphate concentration in the eluate. Oxoacyl-CoA thiolase activity (Δ) was measured with acetoacetyl-CoA under the standard assay conditions. $\blacktriangle$, Ratio of activity measured under standard assay conditions with 3-oxohexanoyl-CoA to the activity measured with acetoacetyl-CoA. (a) Oxoacyl-CoA thiolase from an extract of whole rat liver after passing unretarded through DEAE-cellulose under conditions of Fig. 1(a), 410 mg of protein applied; (b) oxoacyl-CoA thiolase activity from an extract of rat liver mitochondria, 570 mg of protein applied.

and (b) the existence of two types of thiolase activity within the mitochondria. Faced with a similar situation Thorne & Dent (1970) have reported the advantages of chromatographic methods for the determination of tissue contents of cytoplasmic and mitochondrial malate dehydrogenase.

The cytoplasmic acetoacetyl-CoA thiolase content was determined in a variety of rat tissues, in pigeon breast muscle, pig heart, and ox heart and liver, by chromatography of extracts of these tissues on DEAE-cellulose at pH 8.2. The tissue contents of the cytoplasmic acetoacetyl-CoA thiolase are given in Table 3. The activity was relatively high in tissues such as liver and adrenal glands whereas muscle tissue

contained negligible activity. In every tissue where the cytoplasmic enzyme was detected it was absolutely specific for acetoacetyl-CoA and unaffected by K^+ .

In some cases the unretarded oxoacyl-CoA thiolase from the DEAE-cellulose column was rechromatographed on cellulose phosphate to determine the tissue contents of the mitochondrial enzymes. These values are also given in Table 3, both enzymes being expressed in terms of activity with acetoacetyl-CoA. With the exception of brain, which contained virtually none of the more general oxoacyl-CoA thiolase, both mitochondrial enzymes were found in every tissue so far examined. In Table 4 are given the relative specificities for 3-oxoacyl-CoA substrates and the

Table 3. *Tissue contents of individual oxoacyl-CoA thiolases*

Oxoacyl-CoA thiolases were isolated and separated by column chromatography as described in the Methods section. For purposes of comparison all the tissue activities are expressed as μmol of acetoacetyl-CoA removed/min per g fresh wt. of tissue and, where applicable, are means $\pm$ S.E.M. with the number of observations given in parentheses. For every observation a pooled sample of tissues from at least two animals was used. The number of animals taken for the pooled sample is given in those cases where a single observation was made.

Tissue	Cytoplasmic acetoacetyl-CoA thiolase	Mitochondrial oxoacyl-CoA thiolases	
		Acetoacetyl-CoA thiolase	General oxoacyl- CoA thiolase
Rat brain (0-1 day)	4.0 (pooled, 9)	0.95 (pooled, 9)	≤ 0.1
Rat brain (adult)	1.50 ± 0.33 (3)	1.93 ± 0.16 (3)	≤ 0.1
Rat muscle (hind-limb)	0.10 ± 0.04 (3)		
Rat stomach	0.63 ± 0.05 (3)		
Rat heart	<0.05 (3)	18.6 (pooled, 6)	2.7 (pooled, 6)
Rat kidney	0.40 ± 0.12 (4)	18.2 (pooled, 5)	2.5 (pooled, 6)
Rat ileum	1.33 ± 0.07 (4)		
Rat liver	3.55 ± 0.43 (10)	15.9 ± 2.8 (3)	7.6 ± 1.7 (3)
Rat adrenal	4.46 ± 0.77 (4)		
Rat testis	0.66 ± 0.10 (3)		
Pigeon breast muscle	<0.05 (pooled, 2)		
Pig heart	<0.05 (pooled, 2)	4.5 (pooled, 2)	2.9 (pooled, 2)
Ox heart	<0.05 (pooled, 2)		
Ox liver	5.50 ± 0.57 (6)	19.8 ± 2.5 (3)	16.7 ± 2.1 (3)

Table 4. *Some properties of mitochondrial oxoacyl-CoA thiolases isolated from different tissues*

Mitochondrial oxoacyl-CoA thiolases were isolated from extracts of washed mitochondria or from whole tissue extracts after passage through DEAE-cellulose to remove any cytoplasmic thiolase. The mitochondrial thiolases were separated from each other and purified by chromatography on cellulose phosphate. All activities were determined under the standard assay conditions. For further details see the Methods section.

Source of enzyme	Acetoacetyl-CoA thiolase		General oxoacyl-CoA thiolase	
	Relative activity with different 3-oxoacyl-CoA substrates $\text{C}_4:\text{C}_6$	Activation by K^+	Relative activity with different 3-oxoacyl-CoA substrates $\text{C}_4:\text{C}_6:\text{C}_{10}$	Activation by K^+
Rat brain	1: <0.01	4.1	*	*
Rat heart	1: <0.01	4.3	1:4.2:4.1	1.0
Rat kidney	1: <0.01	4.0	1:4.0:3.2	1.0
Rat liver	1: <0.01	4.2	1:3.9:3.7	1.0
Pig heart	1: <0.01	4.8	1:3.4:3.4	1.0
Ox liver	1: <0.01	5.5	1:4.9:4.8	1.0

* Enzyme activity too low to measure.

K^+ activation properties for the mitochondrial thiolases isolated and purified on cellulose phosphate. Fig. 3 illustrates the K^+ activation of mitochondrial acetoacetyl-CoA-specific thiolases purified from pig heart and rat liver. This phenomenon is associated with mitochondrial acetoacetyl-CoA thiolases from

all tissues so far examined. With rat brain (Middleton, 1973) and rat heart and kidney, mitochondria were prepared and extracts of these were assayed for oxoacyl-CoA thiolase. This confirmed that the cellulose phosphate technique was giving information about the mitochondrial enzymes.

Some kinetic properties of the individual rat liver oxoacyl-CoA thiolases

The isolation procedures described above gave considerable purification of the individual oxoacyl-CoA thiolases. The acetoacetyl-CoA thiolase from rat liver cytoplasm was purified 11-fold to a specific activity of 0.45 unit/mg by chromatography on DEAE-cellulose as described in Fig. 1(b). The general 3-oxoacyl-CoA thiolase of rat liver mitochondria, after cellulose phosphate chromatography, had a specific activity of 4.0 μ mol of 3-oxohexanoyl-CoA removed/min per mg, a purification of 11-fold over the activity in mitochondrial extracts. The mitochondrial acetoacetyl-CoA-specific thiolase was purified 45-fold by cellulose phosphate chromatography to a specific activity of 12.6 units/mg.

To characterize these partially purified thiolases further their apparent K_m values (under the standard assay conditions) were determined for CoA and acetoacetyl-CoA. These constants are given in Table 5. Under the conditions of this investigation the cytoplasmic enzyme showed pronounced high substrate inhibition by CoA but not by acetoacetyl-CoA, but the reverse situation applied to the mitochondrial acetoacetyl-CoA-specific enzyme. This difference in kinetic behaviour is shown in Fig. 4. The mitochondrial general oxoacyl-CoA thiolase showed no sign of substrate inhibition with either substrate over the range of concentrations used in Fig. 4. The effect of K^+ concentration on the activity of the mitochondrial acetoacetyl-CoA thiolase was also investigated (Fig. 5). Under the conditions of the standard assay (only the KCl concentration being varied), a simple hyperbolic relationship was found between the extent of the activation and the K^+ concentration. The apparent K_a for K^+ is 1 mm under these conditions. These preliminary kinetic studies confirm the existence of different oxoacyl-CoA thiolases in rat liver

and further underline the need to assay for oxoacyl-CoA thiolase in systems whose cation concentration is controlled. It is also worth noting that published assays for oxoacyl-CoA thiolase activity in tissue extracts, e.g. those of McGarry & Foster (1969) and Williamson *et al.* (1971), use concentrations of acetoacetyl-CoA around 50 μ M, which, as the present study shows, must cause the specific suppression of

Fig. 3. Activation by K^+ of mitochondrial acetoacetyl-CoA thiolases

(a) Spectrophotometer trace at 303nm of an assay system containing 10 μ M-acetoacetyl-CoA and 8.8 munits of purified pig heart mitochondrial acetoacetyl-CoA thiolase (15.3 units/mg). K^+ was replaced in the assay system by 50mM- Na^+ . The additions were as indicated: CoA (50 μ M final concentration), and subsequently K^+ (as KCl, to 10mM final concentration). (b) As in (a) except that the assay system contained 5munits of purified rat liver mitochondrial acetoacetyl-CoA thiolase (12.6 units/mg). Full details are given in the Methods section.

Table 5. Kinetic properties of oxoacyl-CoA thiolases isolated from rat liver

Oxoacyl-CoA thiolases were isolated from rat liver extracts by the chromatographic techniques described in the Methods section. The apparent kinetic constants were determined under the standard assay conditions. For further details see the Methods section.

Enzyme	Apparent K_m values		Apparent K_a for activation by K^+ (mM)
	Acetoacetyl-CoA (μ M)	CoA (μ M)	
Cytoplasmic acetoacetyl-CoA thiolase	50	3*	—
Mitochondrial general oxoacyl-CoA thiolase	10	18	—
Mitochondrial acetoacetyl-CoA thiolase	7*	21	1

* Substrate inhibition occurs.

Fig. 4. Effect of varying the substrate concentration on reciprocal plots of the velocity of the reaction catalysed by purified rat liver cytoplasmic and mitochondrial acetoacetyl-CoA thiolases

Cytoplasmic acetoacetyl-CoA thiolase (Δ) had a specific activity of 1.8 units/mg; mitochondrial acetoacetyl-CoA thiolase ($\blacktriangle$) had a specific activity of 12.6 units/mg. (a) and (c), Reciprocal velocity as a function of reciprocal CoA concentration; acetoacetyl-CoA at $10\mu\text{M}$. (b) and (d), Reciprocal velocity as a function of reciprocal acetoacetyl-CoA concentration; CoA at $50\mu\text{M}$. Conditions, except for the variable substrate, were those of the standard assay (see the Methods section).

Fig. 5. Effect of varying the concentration of K^+ on the activation of the rat liver mitochondrial acetoacetyl-CoA thiolase

The reciprocal of the activated velocity (velocity in the presence of K^+ minus the velocity in the absence of K^+) is expressed as a function of the reciprocal K^+ concentration. All other variables were held constant at standard assay values. For details see the Methods section.

the mitochondrial acetoacetyl-CoA thiolase relative to any cytoplasmic acetoacetyl-CoA thiolase or mitochondrial general oxoacyl-CoA thiolase present in the extracts.

The difference between the assay conditions used in the present study and those used by the above-

mentioned investigators accounts for any discrepancies between their results and the absolute or relative thiolase activities in Table 1. Also the existence of the substrate inhibition of acetoacetyl-CoA-specific thiolases prevents the maximum velocities of these enzymes being measured by a simple assay. Therefore the tissue activities quoted in Tables 1 and 3 do not measure maximal tissue thiolase capacities. Comparisons of relative tissue thiolase activities are, however, perfectly valid.

Isoelectric focusing of isolated rat liver oxoacyl-CoA thiolase

This was carried out on the cytoplasmic acetoacetyl-CoA thiolase after purification on DEAE-cellulose and on the mitochondrial general oxoacyl-CoA thiolase after purification on cellulose phosphate. The results are shown in Fig. 6. The former enzyme gave a single peak of activity with a pI of 5.8. The recovery of activity was 68% and the peak fractions were purified to a specific activity of $1.8\mu\text{mol/min per mg}$. Although the general oxoacyl-CoA thiolase ran as a single peak on cellulose phosphate at pH 6.6, it was separated by isoelectric focusing into two peaks with pI values of 6.95 and 8.15. However, the relative substrate specificities (measured with acetoacetyl-, 3-oxohexanoyl- and 3-oxodecanoyl-CoA) were the same for enzyme focusing at either pH value. The apparent K_m for acetoacetyl-CoA was also measured and was identical for the enzymes focusing at pH 6.95 or 8.15. Multiple peaks of the ox liver mitochondrial general oxoacyl-CoA thiolase were also found on isoelectric focusing (Middleton, 1972b).

Fig. 6. Isoelectric focusing of purified rat liver oxoacyl-CoA thiolases

Focusing was carried out in Ampholine (pH3–10) for 2 days at 15°C and 500 V. Oxoacyl-CoA thiolase activity is expressed as a function of the eluate pH. Δ , Activity measured with acetoacetyl-CoA under standard assay conditions; $\blacktriangle$, ratio of the activity measured with 3-oxohexanoyl-CoA to the activity measured with acetoacetyl-CoA, both being under standard assay conditions. (a) Rat liver cytoplasmic acetoacetyl-CoA thiolase after purification (Fig. 1b) on DEAE-cellulose; (b) rat liver mitochondrial general oxoacyl-CoA thiolase after purification (Fig. 2b) on cellulose phosphate.

forms of both types of mitochondrial oxoacyl-CoA thiolase may exist in rat liver.

Discussion

The results reported in the present paper confirm the generality of the existence of different types of oxoacyl-CoA thiolase in animal tissues. By using the chromatographic methods developed for the isolation of the oxoacyl-CoA thiolases from ox liver (Middleton, 1972b), it has been possible to isolate these enzymes from other tissues and determine their properties and relative contributions to the observed tissue oxoacyl-CoA activities. The striking variation of the apparent substrate specificity and activation by K^+ noted in Table 1 for oxoacyl-CoA thiolase activities in a wide variety of tissues can now be explained in terms of three classes of oxoacyl-CoA thiolase differing in substrate specificity and activation by K^+ and present in different proportions in each tissue. Thus the large apparent activation by K^+ and the relatively low ratio of activity with 3-oxohexanoyl-CoA to activity with acetoacetyl-CoA that is observed in rat heart and kidney (Table 1) is due primarily to the high ratio of the mitochondrial acetoacetyl-CoA thiolase to the general oxoacyl-CoA thiolase (Table 3). In the brain of newborn rats the general oxoacyl-CoA thiolase activity is absent, and here the slight activation by K^+ reflects a low ratio of mitochondrial to cytoplasmic acetoacetyl-CoA thiolase. The large variation in relative amounts of these two acetoacetyl-CoA thiolases in rat brain during postnatal development is reported in the following paper (Middleton, 1973).

There is, however, some evidence for the existence of yet another type of oxoacyl-CoA thiolase. The ratios in Table 1 showing the relative tissue activity with 3-oxodecanoyl-CoA compared with that with 3-oxohexanoyl-CoA are consistently higher than predicted by the specificity properties of the isolated general oxoacyl-CoA thiolases in Table 4. This is particularly noticeable for rat kidney. Since the chromatographic separation methods used here gave very good recoveries of oxoacyl-CoA thiolase activity when measured with acetoacetyl-CoA and 3-oxohexanoyl-CoA, these compounds must be very poor substrates for the postulated enzyme. This additional type of oxoacyl-CoA thiolase must show a preference for long-chain 3-oxoacyl-CoA substrates.

The thiolase whose relative tissue activities have been most thoroughly investigated in this study is the cytoplasmic acetoacetyl-CoA specific enzyme. As shown in Table 3 this enzyme makes a significant contribution to total tissue acetoacetyl-CoA thiolase rates in several tissues. The relative tissue activity is highest in liver, the brain of newborn rats, and in adrenal

The double-peaking phenomenon observed in Fig. 2 for the cellulose phosphate chromatography of the mitochondrial acetoacetyl-CoA thiolase could not be investigated by isoelectric focusing because of the complete loss of the enzyme activity during the process. However, when material from either peak was re-run separately on cellulose phosphate only a single peak of activity was observed, whereas combined fractions from both peaks gave, once more, two peaks of activity. It thus appears that multiple

Table 6. *Calculated tissue contents of mitochondrial oxoacyl-CoA thiolases*

Values are calculated from the whole-tissue oxoacyl-CoA thiolase activities given in Table 1, by using the K^+ activation properties of the purified, isolated mitochondrial enzymes of Table 4 according to the procedure described in the Methods section. Results are expressed in terms of the common 3-oxoacyl-CoA substrate, acetoacetyl-CoA, as $\mu\text{mol removed/min per g fresh wt. of tissue}$.

Tissue	Mitochondrial oxoacyl-CoA thiolase activity	
	Acetoacetyl-CoA thiolase	General oxoacyl-CoA thiolase
Rat brain (newborn)	1.0	0
Rat brain (adult)	2.3	0
Rat muscle (hind-limb)	2.1	0.2
Rat stomach	5.2	0.5
Rat heart	18.3	3.2
Rat kidney	18.3	2.1
Rat ileum	3.8	0.8
Rat liver	16.1	7.4
Rat adrenal	11.8	6.6
Rat testis	1.2	0.9
Pigeon breast muscle*	5.3	3.8
Pig heart	4.6	2.8
Ox heart*	3.7	2.4
Ox liver	18.3	18.3

* Calculated by using the K^+ -activation properties of the ox liver mitochondrial acetoacetyl-CoA thiolase.

glands; it is of lower activity in kidney and testis and it is absent from muscle tissue. The pattern of its relative activities broadly parallels the known abilities of these tissues to synthesize steroid from acetate (Srere *et al.*, 1950; Dietschy & Siperstein, 1967; McGarry & Foster, 1969). It is therefore proposed that a major function of the cytoplasmic acetoacetyl-CoA thiolase is to provide acetoacetyl-CoA from acetyl-CoA in the cytoplasm for subsequent conversion into steroid. In liver the enzyme may also play a part in the cytoplasmic pathway for ketone-body synthesis reported by Sauer & Erfle (1966) and Williamson *et al.* (1968).

Although the tissue contents of the mitochondrial types of thiolase have not been extensively determined, it is possible to calculate these from the apparent thiolase activities in Table 1 measured with acetoacetyl-CoA in Na^+ - and K^+ -containing media, together with the experimentally determined cytoplasmic acetoacetyl-CoA thiolase contents for the tissue concerned. The details are given in the Methods section. The calculated tissue contents of these mitochondrial oxoacyl-CoA thiolases are given in Table 6 for all the animal tissues investigated in this study. A comparison between the observed tissue contents (Table 3) and the calculated values in Table 6 indicates the validity of the calculation procedure and therefore enables a comprehensive review of the

relative tissue activities of the mitochondrial thiolases to be made.

The acetoacetyl-CoA-specific thiolase (EC 2.3.1.9) crystallized from pig heart by Gehring *et al.* (1968) must be identical with the mitochondrial enzyme of the same specificity, which is activated by K^+ . Inspection of Table 3 shows that there is no other acetoacetyl-CoA-specific thiolase in pig heart. This mitochondrial K^+ -activated acetoacetyl-CoA thiolase (EC 2.3.1.9) has the widest tissue distribution of all the thiolases (Tables 3 and 6), being present even in tissue such as rat hind-leg muscle, which contains little other thiolase activity. A possible role for this widespread enzyme could be in extrahepatic acetoacetate utilization. This would fit well with its relative abundance (when compared with other thiolases) in rat hind-leg muscle, a tissue in which Ruderman *et al.* (1971) have demonstrated a high and preferential use of acetoacetate as a metabolic fuel *in vivo*. Williamson *et al.* (1971) have reported that rat tissue activities of 3-oxo acid CoA-transferase (EC 2.8.3.5; the first enzyme involved in acetoacetate metabolism) and thiolase are roughly parallel. This present work shows that these investigators were not measuring a single type of thiolase activity and so this possible relationship was reinvestigated here. By using the assay of Williamson *et al.* (1971) for the 3-oxo acid CoA-transferase this activity was determined in rat

Table 7. 3-Oxo acid CoA-transferase activity in extrahepatic tissues of the adult rat

Activities were the means of three observations, expressed as μmol of acetoacetyl-CoA removed/min per g fresh wt. of tissue, and were determined by the method of Williamson *et al.* (1971).

Tissue	3-Oxo acid CoA-transferase activity	
	Present work	Williamson <i>et al.</i> (1971)
Kidney	26.0	25.5
Heart	—	24.8
Stomach	13.3	—
Ileum	7.1	—
Adrenal	—	5.7
Brain	2.5	2.1
Testis	1.9	—
Muscle (hind-limb)	—	0.5

Fig. 7. Relationship between the activities of 3-oxo acid CoA-transferase and the mitochondrial acetoacetyl-CoA thiolase in eight tissues of the adult rat

The tissues are as follows: 1, kidney; 2, heart; 3, stomach; 4, ileum; 5, adrenal; 6, brain; 7, testis; 8, skeletal muscle (hind limb). $\circ$, Acetoacetyl-CoA thiolase content determined by chromatographic separation; $\bullet$, acetoacetyl-CoA thiolase content calculated from the total tissue activity with acetoacetyl-CoA (Table 1). For further details see the text.

testis, stomach and ileum (Table 7) and the results, together with those published by Williamson *et al.* (1971), were compared with the tissue activity of the

mitochondrial acetoacetyl-CoA thiolase (Tables 3 and 6). Fig. 7 shows that a significant correlation exists ($r = 0.89$, $P < 0.005$) between the activity of 3-oxo acid CoA-transferase and mitochondrial acetoacetyl-CoA thiolase in eight extrahepatic tissues of the rat, thus supporting the role proposed for this thiolase. No significant correlation can be seen between the transferase activity and the activities of the other types of thiolase present in the same tissues. The presence of high activities of the mitochondrial acetoacetyl-CoA thiolase in liver, a tissue from which 3-oxo acid CoA-transferase is absent (Williamson *et al.*, 1971), must reflect another role, namely the production of acetoacetyl-CoA for conversion into acetoacetate.

The metabolic function of the general oxoacyl-CoA thiolase is indicated by both its mitochondrial location and its wide specificity to be the thiolase step of β -oxidation. Seubert *et al.* (1968), who have crystallized this enzyme from ox liver, showed that 3-oxopalmitoyl-CoA was a good substrate as well as the shorter-chain compounds used in the present study. Therefore this enzyme alone is capable of carrying out the thiolysis of all the possible 3-oxoacyl-CoA intermediates of β -oxidation. It is not known whether its decreased relative ability to thiolys acetoacetyl-CoA is at all significant under the conditions of very low 3-oxoacyl-CoA concentration that must prevail *in vivo* during β -oxidation (Greville & Tubbs, 1968). In summary, therefore, it is proposed that the three different types of oxoacyl-CoA thiolase demonstrated here to exist in animal tissues have different roles in metabolism, these roles being tailored to their catalytic properties and intracellular locations.

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