Biochem. J. (1974) **142**, 619–627
Printed in Great Britain

Comparative Studies on 3-Oxo Acid Coenzyme A Transferase from Various Rat Tissues

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(*Received* 9 *April* 1974)

1. Tissue activities, intracellular distribution as well as selected kinetic and molecular properties of succinyl-CoA–3-oxo acid CoA transferase (EC 2.8.3.5), which is an initiator of ketone body usage, were examined in rat kidney, heart, brain, skeletal muscle and liver. 2. The activities of the transferase in these tissues are similar to reported values and are somewhat affected by the homogenization medium. Higher recoveries of activity are obtained when a phosphate buffer is used during the homogenization; Tris solutions containing sucrose and mannitol lead to only slightly lower recoveries, but can be used in studies to determine the subcellular localization of the transferase activity. 3. A close correlation was observed between the relative activities of citrate synthase (a mitochondrial marker enzyme) and CoA transferase in the cytoplasmic, particulate and mitochondrial fractions from the five tissues. 4. The K_m values for acetoacetate (measured in two different ways), the ratio of $V_{max.}$ values for the two enzyme-catalysed half-reactions, and succinate product inhibition are quite similar for the enzyme from each tissue. 5. The enzymes are also similar in molecular weight (with an approx. mol.wt. of 100000 as determined by gel filtration). All show an active band in isoelectric-focusing studies with pI 7.6, except for the enzyme from heart (pI 6.8). 6. The results demonstrate a mitochondrial origin for CoA transferase in these rat tissues and support the proposition that CoA transferase is a ketolytic enzyme, i.e. an enzyme uniquely involved in the complete oxidation of ketone bodies. The structural and functional similarities of these transferases suggest that factors other than differences in K_m values account for differences in the utilization of ketone bodies by various tissues.

The ability of a tissue to metabolize ketone bodies is most closely associated with the presence of the enzyme succinyl-CoA–3-oxo acid CoA transferase (EC 2.8.3.5), which converts acetoacetate into the CoA derivative (Stern *et al.*, 1956; Jencks, 1973). This enzyme has been found to have the highest activity in heart and kidney tissue of various mammals, whereas the activity is lowest in liver (Williamson *et al.*, 1971). CoA transferase clearly plays a role in permitting ketone bodies to serve as fuels for respiration (Krebs, 1961); however, its reported presence in the cytoplasmic fraction of certain tissue homogenates suggests a possible function in cellular biosynthetic activities (Tildon & Sevdalian, 1972; Lowenstein, 1967). Further, the different kinetic (K_m) properties of CoA transferase from pig heart and bovine skeletal muscle have been used to explain possible differences in tissue consumption of the ketone bodies (Blair, 1969).

Our studies on CoA transferase from various adult rat tissues (kidney, heart, brain, skeletal muscle and liver) were concerned with evaluating better the physiological role of the enzyme by determining its subcellular distribution and several of its key molecular and kinetic properties. The results reported in this paper indicate that the CoA transferase from each of these rat tissues is a mitochondrial enzyme and that these CoA transferases are quite similar with respect to their K_m value for acetoacetate.

Materials and Methods

Animals and chemicals

Male adult Buffalo rats (200–250g) were obtained from Simonsen Laboratories (Gilroy, Calif., U.S.A.) and fed *ad libitum*. CoA was purchased from P-L Biochemicals (Milwaukee, Wis., U.S.A.) as the free acid. Acetoacetyl-CoA was prepared from diketen by the method of Lynen *et al.* (1958); succinyl-CoA (3-carboxypropionyl-CoA) and acetyl-CoA were similarly prepared from succinic anhydride and acetic anhydride respectively (Simon & Shemin, 1953). Lithium acetoacetate was obtained by the method of Hall (1962). LKB (Rockville, Md., U.S.A.) provided the Ampholine solutions for the isoelectric focusing studies. All other chemicals were obtained from

Sigma Chemical Co. (St. Louis, Mo., U.S.A.) and were the highest purity available. Pig heart citrate synthase (EC 4.1.3.7), rabbit muscle glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12) and pig heart 3-hydroxyacyl-CoA dehydrogenase (EC 1.1.1.35) were also purchased from Sigma Chemical Co. ICN Nutritional Biochemical Co. (Cleveland, Ohio, U.S.A.) supplied bovine serum albumin (fraction V, unesterified-fatty-acid-poor).

Methods

Preparation of tissue extracts and subcellular fractions. The rats were killed by cervical fracture. The tissue was rapidly removed and placed in a chilled (4°C) Tris–sucrose solution (pH7.4) containing 10mM-Tris–HCl buffer, 70mM-sucrose, 0.21M-mannitol, 0.1mM-EDTA, and (except for heart) 0.5g/litre of bovine serum albumin. After the removal of fat and connective tissue a weighed amount of the tissue was minced with scissors in the chilled Tris–sucrose solution. The minced heart tissue was incubated with Nagarse (4mg/g wet wt. of heart) (Enzyme Development Corp., New York, N.Y., U.S.A.) and homogenized by the procedure of Pande & Blanchaer (1971). Liver, kidney and heart tissue minces were homogenized at 4°C in a glass–Teflon homogenizer with a motor-driven pestle; brain and skeletal muscle minces were homogenized manually at 4°C with a glass–glass homogenizer. The final volume of medium was adjusted so as to give a 1:20 (w/v) dilution of the original weight of tissue.

The homogenates (total homogenates) from all five tissues were centrifuged at $10000g_{av.}$ at 4°C for 15min. The supernatants (cytoplasmic fractions) were set aside; the pellets were resuspended in a volume of Tris–sucrose medium equal to that of the original supernatant. These suspensions (the particulate fractions) were then centrifuged at $480g_{av.}$ at 4°C for 15min to yield supernatants (mitochondrial fractions). Portions (1–2ml) of each of these fractions (i.e. the total homogenate, cytoplasmic, particulate and mitochondrial fractions), containing 0.3% (w/v) deoxycholate, were sonicated for a total of 2min (15s sonication periods followed by 15s of cooling) at 4°C and 100W of power in a Bronwill sonicator equipped with a needle tip. The sonicated solutions were centrifuged at 4°C and $27000g_{av.}$ for 30min.

In separate studies, the total CoA transferase activity in a given tissue was determined in two different media, 0.02M-potassium phosphate buffer (pH7.0) and the Tris–sucrose solution (pH7.4). A weighed amount of the specimen tissue was minced in the appropriate medium, homogenized in a glass mortar by a motor-driven Teflon pestle and sonicated as described above; all operations were at 4°C. After centrifugation at $27000g_{av.}$ at 4°C for 30min the supernatant was assayed for CoA transferase activity.

Isolation and characterization of the mitochondria. The procedures outlined by Sordahl & Schwartz (1971) were modified as follows. Homogenates were prepared in the manner just described. All but the heart homogenate were centrifuged at $480g_{av.}$ at 4°C for 15min; the pellets were discarded and the supernatants used in the subsequent steps. The heart homogenate (containing Nagarse) was centrifuged at $10000g_{av.}$; the supernatant was discarded. The resulting pellet was suspended in the Tris–sucrose medium (lacking Nagarse) and the suspension was treated like a homogenate from the other tissues.

The supernatants from the centrifugation at $480g_{av.}$ were centrifuged at $6800g_{av.}$ for 15min. The pellets were gently washed with homogenization medium to remove any buffy-coat layer. These were resuspended in one-half of the original volume of Tris–sucrose medium, used for homogenization and then centrifuged at $10000g_{av.}$ for 15min. The resulting pellets were washed as before, resuspended in one-fourth of the original volume of homogenization medium and centrifuged as before. The pellets were then suspended in 1–2ml of homogenization medium. Mitochondrial protein was measured by the biuret method and by means of the E_{215}/E_{225} ratio.

To measure respiratory control index values, 0.050 or 0.10ml samples of the mitochondrial preparation were added to a cell equipped with a Clark oxygen electrode containing a stirred solution (at 30°C and pH7.4) of 50mM-Tris–HCl, 75mM-KCl, 5mM-$MgCl_2$, 1mM-EDTA and 12.5mM-K_2HPO_4 (final vol. 2.0ml). Succinate (5mM) alone or malate (0.5mM) plus pyruvate (5mM) were used as substrates for kidney and liver, and for heart respectively. ADP (0.25mM) was added last for determining the respiratory control index.

Mitochondrial oxidation of acetoacetate was measured in a medium (final vol. 2.0ml) containing 10mM-Tris–HCl (pH7.4), 26mM-NaCl, 58mM-KCl and 6mM-$MgCl_2$ (Alexandre *et al.* (1969). To minimize the background uptake of O_2, kidneys from rats starved for 48h were used as a source of mitochondria. Suspensions (0.15ml) of mitochondria, isolated as described above, were added to the medium at 30°C, then oxaloacetate (12μM) was introduced as a primer. Acetoacetate (2.5mM) (or an identical volume of homogenization medium as a control) was added to the incubation medium and the O_2 consumption was recorded. Maximum O_2 uptake was obtained by uncoupling the mitochondria with 2,4-dinitrophenol (22.5μM).

Determination of enzyme activities. The spectrophotometric procedures of Hersh & Jencks (1967) were used to measure CoA-transferase activity in both the forward and backward directions. Solutions containing the freshly prepared enzyme were used throughout. To determine the rate of acetoacetyl-CoA formation the following conditions were used:

0.067M-Tris–HCl (pH8.1), 5mM-$MgCl_2$, 2mM-iodoacetamide, 0.22mM-succinyl-CoA, 2.0mM-acetoacetate (which was added last) and the solution containing the enzyme (to give a final vol. of 1.0ml). Assays in the reverse direction used identical conditions except for the addition of 0.16mM-acetoacetyl-CoA and 50mM-succinate (which was added last). The enzyme was incubated for 2min with iodoacetamide before the substrates were added. All measurements were made in duplicate at 25°C and 310nm by using a Gilford (model 222) recording spectrophotometer. Corrections for acetoacetate production were made because of losses of acetoacetyl-CoA caused by the spontaneous hydrolysis of the substrate and acetoacetyl-CoA caused by the spontaneous hydroacetoacetyl-CoA deacylase activity. The millimolar extinction coefficient for the Mg^{2+}–acetoacetyl-CoA complex was taken to be 11.9 (at 310nm) (Hersh & Jencks, 1967). Protein concentrations were determined by the Lowry method with bovine serum albumin as standard (Bailey, 1967).

The formation of acetoacetyl-CoA was also determined by following NADH oxidation at 340nm in the coupled-enzyme reaction with 3-hydroxyacyl-CoA dehydrogenase (Hersh & Jencks, 1967). The reaction mixture (final vol. 1.0ml) contained (in addition to the transferase solution) 0.067M-Tris–HCl (pH8.1), 0.15mM-NADH, 0.22mM-succinyl-CoA (which was added last), the dehydrogenase (1.3 units) and various concentrations of acetoacetate.

Citrate synthase activity was measured at 25°C by following the decrease in absorption at 232nm (millimolar extinction coefficient of 5.4) in 0.10M-Tris–HCl (pH8.0), enzyme solution, 0.16mM-acetyl-CoA and 0.20mM-oxaloacetate (which was added last) in a final volume of 1.0ml (Srere, 1969).

Determination of kinetic parameters. The K_m values for acetoacetate were determined in two different ways by using freshly prepared particulate fractions from kidney, heart, brain, skeletal muscle and liver. These fractions were isolated in the Tris–sucrose medium in the manner described above, but the particulate pellets obtained from centrifugation were suspended in a 0.02M-potassium phosphate buffer (pH7.0) and then sonicated for a total of 2min (using sonication periods of 15s). One method for determining the K_m values used the conditions already described for directly measuring acetoacetyl-CoA formation. Replicate measurements were made at each of five different concentrations of acetoacetate (0.2, 0.3, 0.5, 1.0 and 2.0mM). A second determination was made by using the particulate fraction which had been chromatographed through Sephadex G-100 and the coupled-assay procedure with acetoacetate concentrations of 0.02, 0.03, 0.05, 0.10 and 0.5mM. The chromatographic step improved the kinetic data obtained by removing a competing, NADH-consuming enzyme activity. Saturating concentrations of succinyl-CoA in the range of 0.25–0.32mM were determined for the transferases and were used in both assay procedures. The K_m values for acetoacetate were then obtained from a Lineweaver–Burk plot of the data.

The maximum velocity for the reaction producing acetoacetyl-CoA ($V_{max.}$ in the forward direction) was also obtained from these data on the particulate fractions. In addition the maximum velocity for the reverse direction (involving acetoacetate production) was determined with 0.16mM-acetoacetyl-CoA (the saturating concentration) and succinate concentrations of 5, 15, 30, 40 and 50mM.

Portions of the particulate fractions (from all but liver tissue) that produced 3.0 ± 0.1nmol of acetoacetyl-CoA/min in a Tris–HCl solution containing acetoacetate (2.0mM) and Mg^{2+} (5mM) were used for determining product inhibition by succinate (5mM).

Determination of molecular size and pI values. In order to obtain initially a more concentrated enzyme solution, a modification of the procedure for isolating a particulate fraction from each of the tissues was used. The total homogenate was centrifuged at $48000g_{av.}$ for 15min and the pellet was suspended in 0.02M-potassium phosphate buffer (pH7.0), so that the ratio of the volume of the buffer used to the original g wet wt. of tissue was 2–3 (instead of about 20). After similar sonication treatment as described above, the suspension was centrifuged for 1h at 4°C and $48000g_{av.}$. On occasions a second centrifugation of the supernatant was needed to clarify the solution. The sonicated liver particulate fractions were concentrated an additional 4–5-fold by using Lyphogel (Gelman Instrument Co., Ann Arbor, Mich., U.S.A.). The supernatant (2–3ml), along with glyceraldehyde phosphate dehydrogenase and citrate synthase, was placed on a column (1.5cm×40cm) of Sephadex G-100 equilibrated with the same phosphate buffer at 4°C. The CoA transferase activity was eluted with this buffer. Fractions (1ml) containing CoA transferase activity were determined by assaying for the disappearance of acetoacetyl-CoA stimulated by the addition of succinate. The peak fraction of dehydrogenase activity was determined by measuring the oxidation of DL-glyceraldehyde 3-phosphate; citrate synthase activity was determined as described above.

The chromatographed CoA transferase from each tissue was placed in a solution containing sucrose (0.67-M, 0.23g/ml) and LKB carrier ampholytes (approx. 12μl/ml) for developing a gradient of pH7–9, which was later introduced at the midpoint in filling an LKB 110ml-capacity isoelectric-focusing apparatus. A voltage of 400V was initially applied (0.6W initially) and after 16h this was increased to 600V. Electrofocusing was terminated approx. 48h later and 1ml fractions were collected. The pH gradient was determined (by using fractions de-aerated

Table 1. *Total CoA transferase activity of various rat tissues measured in different homogenization media*

Total activity is expressed in terms of μmol of acetoacetate formed/min per g wet wt. of tissue. The values shown are means ±S.E.M. with relative percentage of activity shown in parentheses. The tissue sample was homogenized and sonicated in 0.02M-potassium phosphate buffer (pH 7.0) or in a buffer (at pH 7.4) containing 0.01M-Tris–HCl, 0.07M-sucrose and 0.21M-mannitol, to which deoxycholate (0.3%, w/v) was added before sonication. See the Materials and Methods section for experimental details.

Tissue (no. of animals)	Total activity: Phosphate buffer	Total activity: Tris–sucrose medium plus deoxycholate
Kidney (3)	97.1 ± 5.9 (100)	89.2 ±2.6 (100)
Heart (3)	81.5 ±11.1 (84)	54.5 ±9.4 (61)
Brain (3)	13.3 ± 1.7 (14)	11.5 ±0.97 (13)
Skeletal muscle (3)	5.5 ± 0.64 (5.7)	5.0 ±0.36 (5.6)
Liver (3)	0.50± 0.07 (0.51)	0.58±0.08 (0.65)

Table 2. *Intracellular distribution of CoA transferase activity in various rat tissues*

The actual enzyme activity, given in parentheses for the total homogenate, is expressed as units/min per g wet wt. of tissue. For CoA transferase 1 unit is defined as 1 μmol of acetoacetate formed; for citrate synthase, 1 μmol of citrate formed. The activity found in the total homogenate is expressed as 1.0 and the activities in the other fractions are expressed relative to this.

Tissue (no. of animals)	Relative enzyme activity: Total homogenate: CoA transferase	Total homogenate: Citrate synthase	Cytoplasmic fraction: CoA transferase	Cytoplasmic fraction: Citrate synthase	Particulate fraction: CoA transferase	Particulate fraction: Citrate synthase
Kidney (3)	1.00 (91.7)	1.00 (24.5)	0.09	0.06	0.88	0.91
Heart (3)	1.00 (58.5)	1.00 (75.1)	0.21	0.23	0.75	0.71
Brain (3)	1.00 (12.7)	1.00 (28.9)	<0.01	<0.01	1.05	1.06
Skeletal muscle (3)	1.00 (6.26)	1.00 (14.3)	0.41	0.31	0.62	0.83
Liver (3)	1.00 (0.24)	1.00 (7.53)	<0.01	0.03	1.01	0.97

with N_2) and the presence of CoA transferase was detected as described for the chromatographic studies.

Results

The total CoA transferase activity in kidney, heart, brain, skeletal muscle and liver was found to be slightly dependent on the homogenizing media (Table 1), but the activities were comparable with and in the same order as reported values (Williamson *et al.*, 1971; Tildon & Sevdalian, 1972; Bässler *et al.*, 1973). Similar results were obtained if the activity was measured in the reverse direction (i.e., the conversion of acetoacetate into the CoA derivative) or if the results were expressed in terms of mg of protein in the total sonicated material instead of g wet wt. of tissue. Highest activities were obtained with phosphate buffer (which is used for storing frozen solutions of the purified enzyme with little loss in activity) (Hersh & Jencks, 1967). If the tissue is homogenized first in Tris–sucrose medium (as is used in the subsequent studies for the intracellular localization of CoA transferase) and then sonicated with 0.3% (w/v) deoxycholate, slightly lower activities (decreased by 8–33%) were observed. Further, after approx. 3h the activity in the heart enzyme preparation decreased 15–30% from the initial measurement (made within 30min after centrifugation). If no deoxycholate was used with the Tris–sucrose solution, the lower activities were still obtained, but the heart preparation was more stable. However, for the subsequent studies with citrate synthase, consistent results were only obtained when deoxycholate was added to the fractions before sonication. The addition of deoxycholate (0.3% w/v) to the phosphate medium or of Triton X-100 (0.1 and 0.3%) to the phosphate or Tris–sucrose medium neither increased the yield of activity nor the stability of the enzyme. The freezing–thawing technique for homogenization (Tildon & Sevdalian, 1972) gave consistently lower activities than the procedures described in the Materials and Methods section. The results indicate that no unusual effects on enzyme activity result from using the Tris–sucrose solution.

Table 3. *Comparison of CoA transferase and citrate synthase activities in mitochondrial and in particulate fractions from various rat tissues*

The isolation of particulate and of mitochondrial fractions, the determination of the respiratory control index values (RCI) for the mitochondrial fraction, and measurement of enzyme activities are described in the Materials and Methods section. Results are given as means±S.E.M.

Tissue (no. of animals)	RCI of the mitochondrial fraction	Ratio of enzyme activities in the mitochondrial and particulate fractions	
		CoA transferase	Citrate synthase
Kidney (3)	7.9±1.2	0.38±0.03	0.41±0.06
Heart (3)	4.8±1.3	0.42±0.04	0.41±0.06
Brain (3)	—	0.26±0.02	0.24±0.02
Skeletal muscle (4)	—	0.69±0.02	0.50±0.05
Liver (3)	15.1±2.1	0.37±0.05	0.42±0.05

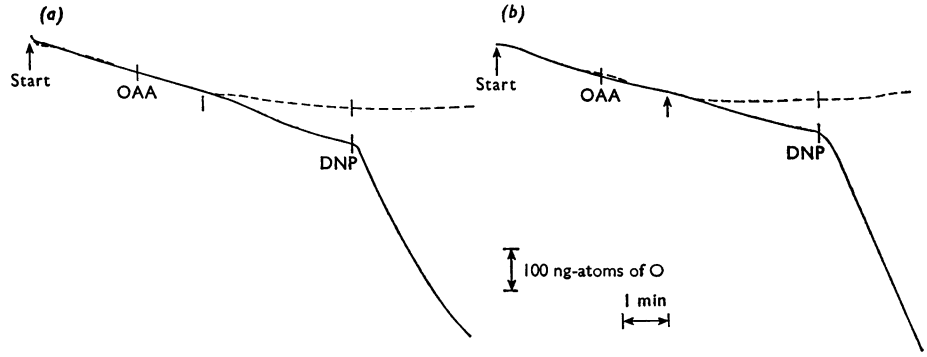

Fig. 1. *Acetoacetate oxidation by rat kidney and heart mitochondria*

The assay conditions used are described in the Materials and Methods section. In trace (*a*) kidney mitochondria (4.1 mg of total protein) and in trace (*b*) heart mitochondria (2.5 mg of total protein) were used. Additions to the stirred mitochondrial suspensions of oxaloacetate (OAA) and 2,4-dinitrophenol (DNP) are indicated by lines across the traces. Arrows indicate that either acetoacetate (——) or additional incubation medium (----) was added. Respiratory control indexes are 6.7 and 4.8 for kidney and heart respectively.

The intracellular distribution of CoA transferase was then determined in the Tris–sucrose medium, which in general allows isolation of mitochondria from various tissues with increased activity and structural intactness (Sordahl & Schwartz, 1971). Measurement of citrate synthase activity was used to determine the extent of mitochondrial rupture during the course of isolation (Srere, 1969). The activities of both CoA transferase and citrate synthase in the total homogenate, cytoplasmic fraction and particulate fraction for each of the five tissues are compared in Table 2. The results indicate that the transferase from these tissues, with the possible exception of the skeletal muscle enzyme, is not localized in the cytoplasm. Considerable activity, comparable with that reported here, has been observed in heart and muscle cytoplasmic fractions (Tildon & Sevdalian, 1972; Lowenstein, 1967). However, our data for the heart preparations indicate that this activity is presumably due to mitochondrial contamination of the cytoplasmic fraction, as shown by the ratios of cytoplasmic to total activity of 0.23 for citrate synthase and 0.21 for CoA transferase. Except for the citrate synthase activity in skeletal muscle, the recovery of enzyme activity in the cytoplasmic and particulate fractions was excellent (94–106% of the activity in the total homogenate). The higher recovery of citrate synthase from the muscle fractions (114% of the total activity in the crude homogenate) makes equivocal any statements about the exclusive localization of the transferase activity in the particulate fraction. Various attempts to clarify this situation proved unsuccessful. For example, elimination of deoxycholate during sonication did not alter the results shown in Table 2, except for the relative amount of citrate synthase in the muscle particulate fraction.

The transferase activity in the particulate fraction containing deoxycholate was decreased to about 40% of the activity in the same fraction lacking deoxycholate; the activity of the crude homogenate decreases to about 60% of that obtained with added deoxycholate. Deoxycholate had no effect on the activity in the cytoplasmic fraction. Although no explanation can be offered for these effects, the conclusion that CoA transferase is predominantly associated with the particulate fractions even in skeletal muscle still seems likely.

Data in Table 3 support the contention that CoA transferase is a mitochondrial enzyme. There is a close correlation between the ratios of citrate synthase and CoA transferase activities in the mitochondrial and particulate fractions from the five tissues. The integrity of the mitochondrial preparations can be correlated with the respiratory control index, which is high for kidney and heart preparations and is essentially unity for the brain and muscle preparations. The difficulties in obtaining mitochondria with good respiratory control from these two tissues have been observed by others (Chappel & Hansford, 1969; Max *et al.*, 1972). Also, both kidney and heart mitochondria (either coupled or uncoupled with dinitrophenol) show the expected uptake of O_2 in the presence of acetoacetate (Fig. 1), clearly indicating its ability to serve as a fuel of respiration.

Various kinetic and molecular properties of the CoA transferase from these tissues were then examined by using either the sonicated particulate material directly or the pooled active fractions after chromatography of this material on Sephadex G-100. The ability to recover much of the CoA transferase activity in the particulate fraction permits the amount of starting protein to be decreased without considerable loss in enzymic activity. Table 4 shows the K_m values for acetoacetate measured in two ways; either the particulate sonicates were assayed spectrophotometrically by measuring the formation of acetoacetyl-CoA (as its Mg^{2+} complex), or the chromatographed material was used in a coupled assay with 3-hydroxyacyl-CoA dehydrogenase. These results show a close agreement between the K_m values determined by these two procedures and also (which is more important) the close similarity of these values for the transferase from all five tissues. Further, if sonicated mitochondrial fractions from all of the tissues except liver are used to determine these K_m values, no significant deviations are found. Even though these preparations vary with respect to the purity of the CoA transferase, they displayed quite similar values (47–58) for the ratio of the maximum velocities in the reverse and forward directions ($V_{max.}$ for acetoacetate formation to $V_{max.}$ for acetoacetate consumption). Likewise, when portions of the particulate fractions from these tissues that display the same amount of activity were assayed in the presence of 5mM-succinate, the extent of product inhibition was almost identical (59–68% inhibition).

The approximate molecular weight of CoA transferase from each of these tissues was estimated by co-eluting through a Sephadex G-100 column the particulate fraction and two molecular weight marker enzymes, citrate synthase and glyceraldehyde phosphate dehydrogenase. The chromatogram for the kidney enzyme preparation (Fig. 2) is typical for all the enzyme preparations. These results suggest that the CoA transferase molecule is intermediate in size between citrate synthase (87000 daltons) (Srere,

Table 4. *Comparison of selected kinetic properties of CoA transferase from various rat tissues*

The following data were collected for particulate sonicates, except for the K_m determinations by the coupled-assay procedure (which used chromatographed materials). The forward reaction, in which acetoacetyl-CoA is formed, and the reverse reaction were followed by the direct spectrophotometric assay involving the formation and decomposition respectively of the Mg^{2+} complex (as described in the Materials and Methods section). The formation of acetoacetyl-CoA was also coupled to the oxidation of NADH by using 3-hydroxyacyl-CoA dehydrogenase (see the Materials and Methods section for details). To determine succinate inhibition samples of the sonicated particulate fractions from the different tissues were used that displayed the same activity (3.0±0.1 nmol of acetoacetate used/min) in 2.0mM-acetoacetate by using the direct spectrophotometric assay. The inhibition of acetoacetate consumption was then measured in the presence of 5.0mM-succinate. The activity in the liver preparations was too low to permit measurement of a K_m value by the coupled assay or the succinate inhibition. The numbers in parentheses refer to the number of times the measurement was made; a separate animal was used for each measurement. Results are given as means±S.E.M.

Tissue	K_m values for acetoacetate (mM)		$V_{max.}^{reverse}/V_{max.}^{forward}$	Succinate inhibition
	Direct assay	Coupled assay		
Kidney	0.60±0.09 (3)	0.21±0.07 (2)	47± 5 (2)	0.59±0.07 (2)
Heart	0.59±0.10 (3)	0.20±0.07 (2)	48± 7 (2)	0.64±0.06 (2)
Brain	0.55±0.14 (3)	0.31±0.13 (2)	48± 5 (2)	0.68±0.05 (2)
Skeletal muscle	0.64±0.09 (4)	0.21±0.06 (2)	57±10 (2)	0.67±0.08 (2)
Liver	0.22±0.15 (2)	—	58±14 (2)	—

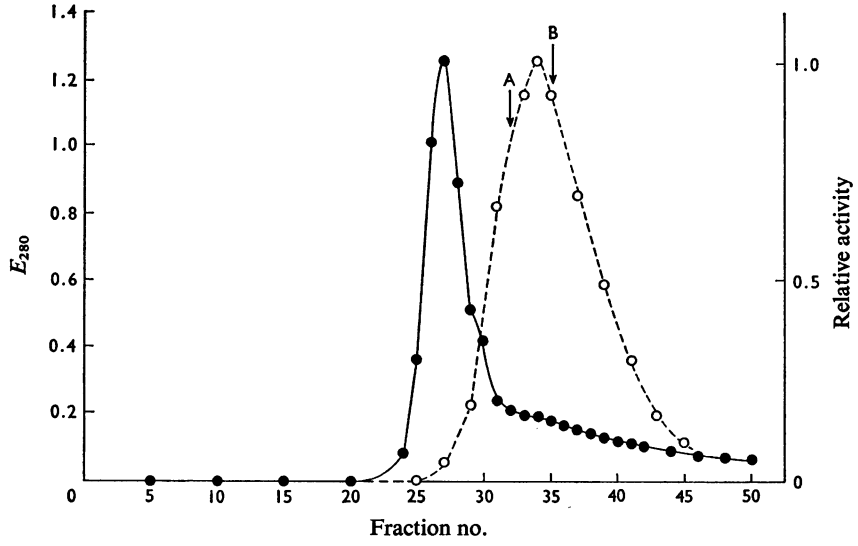

Fig. 2. *Elution diagram of rat kidney CoA transferase activity from a gel-filtration column*

Sonicated kidney particulate fractions (mixed with glyceraldehyde phosphate dehydrogenase and citrate synthase) were placed on a column of Sephadex G-100 equilibrated at 4°C with 0.02M-potassium phosphate buffer (pH 7.0). CoA transferase activity (○) and E_{280} (●), measured against a column buffer control, were determined on the 1ml fractions. Fractions containing maximum glyceraldehyde phosphate dehydrogenase (A) and citrate synthase (B) activities are noted at the arrows. Identical results were obtained from a replicate chromatographic experiment. For details see the Materials and Methods section.

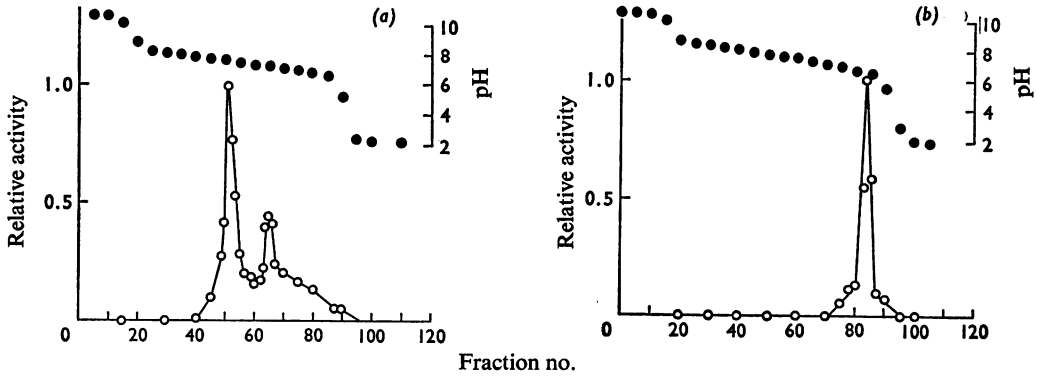

Fig. 3. *Isoelectric-focusing patterns for rat kidney and heart CoA transferase*

Freshly chromatographed preparations of kidney (*a*) and heart (*b*) CoA transferase were focused in a 110ml apparatus for 64h by using a pH gradient of 7–9. CoA transferase activity in the 1ml fractions was measured. Both kidney and heart preparations have been electrofocused three times with results similar to those shown. See the Materials and Methods section for experimental details.

1969) and glyceraldehyde phosphate dehydrogenase (144000 daltons) (Harrington & Karr, 1965). Thus the approximate mol. wt. of 100000 for the different rat tissue CoA transferases is similar to the values reported for the pig heart enzyme (Jencks, 1973; Edwards *et al.*, 1973).

The pI value of each of the transferases was determined by using the Sephadex-chromatographed

material for isoelectric-focusing studies. Fig. 3 shows the activity patterns for the kidney and heart preparations. Only one band of activity having a pI value of 6.8 was noted for the heart enzyme preparation, whereas the kidney preparation generally contained two active bands; a major band with pH value of 7.6 and a lesser one at 7.2. The peak in transferase activity in the brain and muscle preparations was found only at pH7.6. Thus all but the heart preparation show a peak of activity at approximately pH7.6 and all but the kidney preparation contain only one active component. An explanation for these observations, e.g. the presence of isoenzymes, must await further studies on purer material.

Discussion

The studies by Krebs, Williamson and their colleagues have helped to undermine the notion that the ketone bodies, acetoacetate and 3-hydroxybutyrate, are useless metabolic products by demonstrating that they serve as major fuels of respiration for all tissues except liver (for reviews see Krebs *et al.*, 1971; Williamson & Hems, 1970). The function of succinyl-CoA–3-oxo acid CoA transferase in this metabolism, namely to produce the activated acetoacetyl-CoA derivative, has been amply demonstrated by these and other investigators (Jencks, 1973; Tildon & Sevdalian, 1972). An inference of these previous studies is that the transferase is a mitochondrial enzyme, since acetoacetyl-CoA is unable to cross the mitochondrial membrane and the complete oxidation of the ketone body substrates involves tricarboxylic acid-cycle and respiratory-chain enzymes. However, the presence of a significant fraction of the total cellular transferase activity in the cytoplasmic fractions from rat heart, kidney and leg muscle has been reported (Tildon & Sevdalian, 1972; Lowenstein, 1967). Our present studies (Tables 2 and 3) substantiate these observations for heart and muscle, but indicate that most, if not all, of the cytoplasmic activity has a mitochondrial origin. This conclusion emerges from a comparison of citrate synthase (a mitochondrial marker enzyme) and CoA transferase activities in the various subcellular fractions, which had not been done previously. Thus our results support the proposition that CoA transferase is a ketolytic enzyme, i.e. an enzyme uniquely involved in the complete oxidation of ketone bodies. Although the enzyme can function in the ketogenic direction [by coupling with acetoacetyl-CoA thiolase (EC 2.3.1.9) to convert acetyl-CoA into acetoacetate], conditions that would favour net production of ketone bodies in non-hepatic tissue probably do not pertain *in vivo* (as noted by Weidemann & Krebs, 1969). Further, the recent detection of an acetoacetyl-CoA synthase activity in the cytoplasm of rat liver, brain and adipose tissue implicates this alternative means for activating acetoacetate for biosynthetic (as opposed to bioenergetic) purposes (Buckley & Williamson, 1973).

Kinetic studies on CoA transferases from pig heart and ox skeletal muscle have led to the suggestion that the lower K_m value for acetoacetate of the heart enzyme can account in part for the preferential utilization of ketone bodies at normal physiological concentrations by heart tissue (compared with the muscle tissue) (Blair, 1969). The results of the limited kinetic studies on these two tissues from the same species, carried out by Tildon & Sevdalian (1972), support our own results with respect to the kinetic similarities of enzyme from various tissues. Unfortunately their studies did not include measurement of the physiologically important K_m value for acetoacetate. This value (Table4), which was determined by two different methods for the transferase from five tissues, does not vary sufficiently (e.g., by a factor of 10 or more) to confirm the earlier suggestion about the importance of the K_m value in preferential tissue consumption of ketone bodies. In addition, product inhibition by succinate, which could play a significant role in the regulation *in vivo* of the transferase owing to the relatively low K_i value (1–2mM for the pig and rat heart enzymes compared with the K_m value for succinate of about 30mM), is comparable in all five tissues examined (Table 4). Finally, although we did not determine the equilibrium constants for the CoA transferase-catalysed reaction, the values for the ratio of the $V_{max.}$ in the reverse direction to the $V_{max.}$ in the forward direction are sufficiently close to one another and to the value of 25 for the same ratio determined with the purified pig heart enzyme (Hersh & Jencks, 1967) to indicate that these equilibria all favour acetoacetate formation (and not its utilization). This means that the position of the equilibrium for the thiolase-catalysed reaction (which normally lies in favour of acetyl-CoA formation) and the concentration of acetyl-CoA are important factors in controlling acetoacetate utilization by all of these tissues. Our results are consistent with the generally held thesis that blood concentrations of ketone bodies primarily determine the extent of ketone body contribution to cellular metabolism (Krebs *et al.*, 1971). Other factors, such as the tissue content of CoA transferase, tissue size, tissue blood flow and possibly (and presently unknown) different regulatory properties of the transferase, can account for known differences in tissue utilization of ketone bodies (Williamson, 1973). The importance of these factors is demonstrated by noting that skeletal muscle, which has less than 6% of the transferase content of kidney (Table 1), can and does consume more ketone bodies than the other tissues studied combined owing to the fact that muscle accounts for 40% of the rat body wet weight (Bässler *et al.*, 1973).

The studies on the molecular characteristics of CoA transferase from each of the five tissues reinforce the

view that these enzymes comprise a set of structurally and functionally similar (but not identical) proteins. In addition to having similar molecular weights and isoelectric points, these enzymes display similar chromatographic behaviour with the specific substrate-elution techniques described by Edwards *et al.* (1973). At present we are using this method to purify the CoA transferase to homogeneity so as to better determine any structural differences and their possible significance in enzyme functioning.

We acknowledge helpful discussions on methodology with Dr. B. Reynafarje and Dr. P. Pedersen. This study was supported by research grants from the National Science Foundation (GM-40233), the American Cancer Society (BC-141) and a U.S. Public Health Service Institutional grant. A. F. is a recipient of a Research Career Development Award (GM-70423) from the National Institute of General Medical Sciences.

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