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CELLULAR ENERGY METABOLISM DURING FETAL DEVELOPMENT

III. DEFICIENT ACETYL-CoA SYNTHETASE, ACETYLCARNITINE TRANSFERASE AND OXIDATION OF ACETATE IN THE FETAL BOVINE HEART

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SUMMARY

1. Fetal heart mitochondria are deficient in their capacity to oxidize acetate.

2. This has been related to lower acetyl-CoA synthetase (acetate:CoA ligase (AMP), EC 6.2.1.2.) activity in the fetal heart as compared with the calf and adult.

3. Acetylcarnitine transferase (E.C. 2.3.1.7.) activity of fetal heart mitochondria is also lower than that found in older animals.

4. Evidence is presented indicating that acetylcarnitine transferase is present in two mitochondrial compartments. Fetal heart mitochondria show oxidation of acetylcarnitine but not acetyl-CoA *plus* carnitine. This indicates that the fetal heart is functionally deficient in acetylcarnitine transferase activity outside of the membrane barrier to acetyl-CoA but that an internal compartment of the enzyme can convert acetylcarnitine to acetyl-CoA.

INTRODUCTION

Fetal heart mitochondria have efficient oxidative phosphorylation with citric acid cycle intermediates as substrates[1] but exhibit a decreased capacity to oxidize long chain fatty acids[2]. This has been attributed to the dependence of fetal tissues on glucose as an energy substrate and the enhanced capacity of fetal tissues to carry out anaerobic metabolism. In contrast to the preferred metabolism of glucose shown by the fetus, major substrates utilized by the bovine adult are acetate and other volatile short chain fatty acids generated by the microflora in the rumen of that species.

Acetate itself can permeate the mitochondrial membrane where it is activated to its coenzyme A ester prior to oxidation. COOK *et al.*[3] have previously reported that tissue levels of acetyl-CoA synthetase (acetate:CoA ligase (AMP), EC 6.2.1.2) are very high in the adult bovine heart and other tissues with active aerobic metabolism. This activity has been found in both the particle free supernatant of the cell and in the matrix fraction of the mitochondria[4]. As the mitochondrial membrane is impermeable to all acyl-CoA esters of fatty acids, extra mitochondrial acetyl-CoA must be reversibly converted to its carnitine ester by acetylcarnitine transferase (EC 2.3.1.7) in order for it to permeate the mitochondrial membrane[5]. The present

study shows that the fetal bovine heart has markedly decreased oxidation of acetate when compared to the calf and adult cow and also has diminished activities of acetyl-CoA synthetase and acetylcarnitine transferase.

METHODS

Fetal, calf and bovine adult hearts were obtained from a local slaughterhouse. Fetal age was determined by using the scale reported by WINTERS *et al.*[6]. The hearts obtained from these animals were chilled on ice within 30 min of the time of death. Mitochondria were prepared with a Brinkman P-20 homogenizer. 100 g of minced heart were suspended in 240 ml of 0.225 M mannitol, 0.075 M sucrose, 10 mM Tris–HCl (pH 7.5) and 1 mM EDTA. The preparation was homogenized for 20 sec at a rheostat setting of 2. The homogenate was then centrifuged at 900 × *g* for removing nuclei and debris, and mitochondria were isolated by centrifugation at 6000 × *g*. After 3 washes the mitochondria were suspended in 0.25 M sucrose. For some experiments 2-ml aliquots of suspended mitochondria (10 mg/ml) were exposed to sonic oscillation for three 20-second intervals using a Branson sonifier at a rheostat setting of 7. Oxidative phosphorylation was measured polarographically with a Clark electrode according to the method of CHANCE AND WILLIAMS[7]. The polarographic medium was as described previously except that bovine albumin was omitted[2].

Acetyl-CoA synthetase activity was measured by the method of STACEY *et al.*[8] as modified by PITTMAN AND MARTIN[9]. The reaction medium contained 0.1 M Tris–HCl (pH 8.2), 10 mM ATP, 0.5 mM CoA, 5 mM $MgCl_2$, 15 mM mercaptoethanol, 5 mM KF, 5 mM sodium acetate containing $2 \cdot 10^6$ counts/min of [1-^{14}C]acetate (1 mC/mmole) in a final volume of 0.2 ml. KF in the incubation medium served to inhibit the oxidation of acetyl-CoA. After incubation for 30 min at 37° the reaction was terminated by addition of 0.05 ml of 20% trichloroacetic acid. Duplicate tubes were incubated in the absence of CoA and used as blanks. After sedimentation of the denatured protein a 25 μl aliquot was transferred to a 1 cm × 5 cm strip of filter paper which was then placed over a steam source to volatilize unfixed [1-^{14}C]acetate. The filter paper strips were then dried and counted in a Beckman scintillation spectrometer.

Acetylcarnitine transferase activity was determined spectrophotometrically by the method of FRITZ *et al.*[10]. The assay medium contained 10 mM NAD^+, 20 mM potassium malate, 1 mM KCN, 5 mM EDTA, 1 mM CoA, 50 mM Tris–HCl (pH 7.8), 4 μg of malate dehydrogenase, 4 μg of citrate synthetase, mitochondrial protein and water to a final volume of 1 ml. The medium was preincubated at 37° for 2 min and the reaction started by the addition of 5 mM acetylcarnitine. Reduction of NAD^+ was followed at 340 nm using a Gilford recording spectrophotometer. The steps in this reaction sequence are as follows:

$$\text{Acetylcarnitine} + \text{CoA} \leftrightharpoons \text{acetyl-CoA} + \text{carnitine} \quad (1)$$

$$\text{Acetyl-CoA} + \text{oxaloacetate} \longrightarrow \text{citrate} + \text{CoA} \quad (2)$$

$$\text{Malate} + \text{NAD}^+ \leftrightharpoons \text{oxaloacetate} + \text{NADH} \quad (3)$$

$$\text{Acetylcarnitine} + \text{malate} + \text{NAD}^+ \longrightarrow \text{carnitine} + \text{citrate} + \text{NADH}$$

Protein was determined by a modification of the biuret method[11] or by the

method of LOWRY *et al.*[12]. Reagents were obtained from commercial sources. (−)-Acetylcarnitine and (−)-carnitine were generously provided by Dr. Umehara of the Otsuka Parmaceutical Factory, Osaka, Japan.

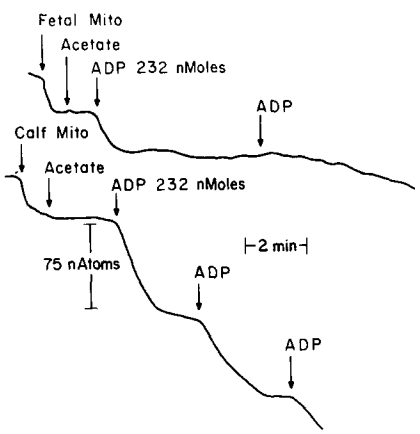
RESULTS

Oxidation of acetate by fetal and calf heart mitochondria

The oxidation of acetate by fetal heart mitochondria was very slow when compared to that of the calf. Deficient acetate oxidation by the fetal material was seen over a wide gestational range and in very young calves (1–2 weeks old) was also less than the value seen with older animals. Fig. 1 shows a representative polarographic experiment with acetate as the substrate for both fetal and calf heart mitochondria. The calf mitochondria showed efficient acceptor controlled respiration (ADP-stimulated) with the ADP/O ratio in the expected range of 3 (232/75). In contrast, acetate oxidation by fetal mitochondria was extremely slow upon addition of ADP. The transient burst of oxidation upon the first addition of ADP is probably due to the oxidation of endogenous substrates.

Acetyl-CoA synthetase and acetylcarnitine transferase activities of fetal and calf heart mitochondria

Fig. 2 compares the acetyl-CoA synthetase activity of heart mitochondria from a 7-month bovine fetus, 5–6-week-old calf and an adult cow. Activity was linear with protein concentration and was uniformly lowest in the fetal material. Sonic disruption of the mitochondria increased activities of fetal, calf and adult acetyl-CoA synthetases about 2-fold There was no further increase in activity when the mitochondria were treated with Triton X-100 (0.1%). With early fetal hearts (3–4 months gestation), acetate activation was reduced by as much as 90% when compared



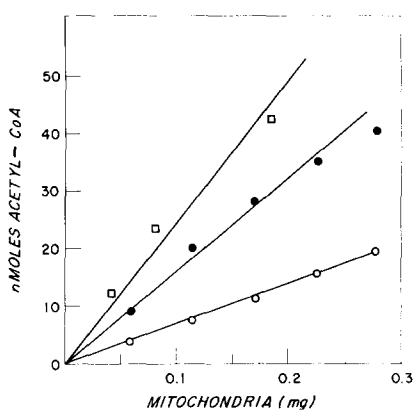


Fig. 1. Polarographic tracings of bovine fetal and calf heart mitochondria with acetate as the substrate. Mitochondria were isolated from a 7-month bovine fetus and 6-week-old calf. The concentration of acetate was 10 mM. The assays contained 0.75 mg of mitochondrial protein.

Fig. 2. Acetyl-CoA synthetase activities of fetal, calf and adult heart mitochondria as a function of mitochondrial protein. ○—○, fetal; ●—●, calf; □—□, adult. The conditions are as in the text.

with values obtained for the adult. These results suggest that the lower rate of oxidation of acetate observed with fetal mitochondria is due to low acetyl-CoA synthetase activity.

Although the mitochondrial membrane is permeable to acetate, the oxidation of external acetyl-CoA requires that it be converted to acetylcarnitine which is the transport intermediate. Fig. 3 compares aetylcarnitine transferase activity of mitochondria from fetal, calf and adult hearts. The activity of all samples was markedly enhanced (about 10-fold) following sonic disruption of the mitochondria. Acetylcarnitine transferase activities of intact fetal, calf and adult mitochondria were 4.2, 7.0 and 9.6 nmoles/min per mg, respectively. Activity was lowest in the fetal samples but was not decreased to the same extent relative to the calf and adult as was the acetyl-CoA synthetase activity of fetal heart mitochondria.

Table I compares the rates of oxidation of glutamate–malate, acetate, acetyl-CoA *plus* carnitine and acetylcarnitine by fetal, calf and adult heart mitochondria. The oxidation of both acetate and acetyl-CoA *plus* carnitine by the fetal mitochondria was quite limited. In view of the decreased activities of acetyl-CoA synthetase and acetylcarnitine transferase, these results were not unexpected. These data suggest

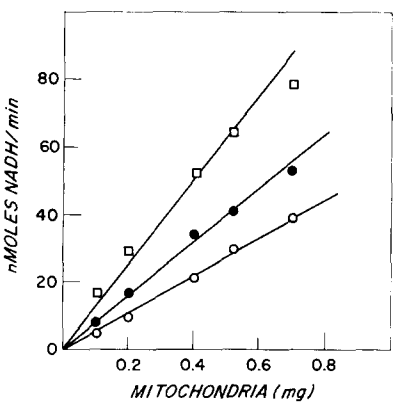


Fig. 3. Acetylcarnitine transferase activities of sonically disrupted fetal calf and adult heart mitochondria as a function of mitochondrial protein. ○—○, fetal; ●—●, calf; □—□, adult. The conditions are as in the text.

TABLE I

OXYGEN CONSUMPTION BY FETAL, CALF AND ADULT HEART MITOCHONDRIA

Oxygen consumption was measured polarographically with an excess of ADP in the reaction-medium. Each experiment contained 0.6 mg of mitochondrial protein. Substrate concentrations were as follows: glutamate–malate, 10 mM; acetate, 10 mM; acetyl-CoA, 5 mM; (−)-carnitine, 1 mM; (−)-acetylcarnitine, 5 mM.

Mitochondria	*Oxygen consumption (natoms O/min per mg)*			
	Glutamate–malate	*Acetate*	*Acetyl-CoA + carnitine*	*Acetylcarnitine*
Fetus	123	6	6	46
Calf	158	65	62	68
Adult	140	52	54	58

that fetal mitochondria can reconvert acetylcarnitine to acetyl-CoA once transport into the mitochondria has been effected but are deficient in the initial formation of acetylcarnitine external to the membrane barrier. This functional evidence for an internal and external transferase is reminiscent of our previous observations concerning palmitylcarnitine transferase showing that fetal heart mitochondria were deficient in the oxidation of palmityl-CoA in the presence of carnitine but readily oxidized long chain acylcarnitines[3].

DISCUSSION

The data reported here show bovine fetal heart mitochondria to be almost totally deficient in the oxidation of acetate. This information correlates with the fact that acetate and other volatile short chain fatty acids produced by the rumen are the major substrates removed from the circulation of the bovine adult. The deficiency of acetate oxidation observed with fetal heart mitochondria probably relates to the fact that glucose is the preferred substrate of the fetal heart. HANSON AND BALLARD[13] have shown that the oxidation of glucose to carbon dioxide is considerably greater in the fetus than in the adult. In contrast to monogastric mammals, the ruminant fetus has an active gluconeogenesis[14] so glucose can be made available to the fetus by that pathway as well as through placental transport.

It has been shown previously that long chain fatty acid oxidation is much less active in the fetal heart than in the adult[15,16]. Our own previous work[3] has shown that fetal heart mitochondria have deficient carnitine dependent oxidation of palmityl CoA. As fetal tissues have active anaerobic metabolism, the oxidation of both acetate and long chain fatty acids may become important only after birth. The shift to acetate oxidation by the heart after birth also probably relates to the development of the rumen microflora which generate the volatile short chain fatty acids which are then absorbed into the circulation.

Our previous work has also shown that fetal heart mitochondria can oxidize medium chain fatty acids (caprylic, caproic, capric). Thus, fetal heart mitochondria should have activating enzymes for those fatty acids. It has been established by others[4] that the activating enzyme for medium chain fatty acids is distinct from that which activates acetate and propionate. Our results could be explained theoretically by an increased activity of acetyl-CoA hydrolase in the developing heart. However, in view of the substrate concentrations used (5–10 mM) and the short duration of the assays, this possibility is unlikely.

CASILLAS AND NEWBURGH[17] have reported developmental increases in acetylcarnitine transferase activity in the chick embryo. These changes paralleled an increase in tissue concentrations of acetylcarnitine. These investigators suggested that this enzyme facilitated the transfer of acyl groups from the yolk sac to the embryo. These results are not surprising if one considers the total dependence of the chick embryo on the lipid laden yolk. As noted above, the mammalian fetus is much more dependent on carbohydrate metabolism as an energy source.

Acetylcarnitine transferase has been shown to be a wholly mitochondrial enzyme by NORUM AND BREMER[18] and also by BARKER *et al.*[19]. The latter investigators suggested that acetylcarnitine transferase is largely inaccessible to external acetyl-CoA in liver and manmmary gland mitochondria of the guinea pig and goat.

Our own data with bovine heart mitochondria also suggests that most acetylcarnitine transferase activity is internal to the membrane barrier to acetyl-CoA. Sonic disruption of the mitochondria resulted in a 10-fold increase in activity presumably due to labilization of the internal acetylcarnitine transferase.

Our observations concerning acetylcarnitine transferase activity also lend further support to the hypothesis that the enzyme is present in two functional sites in the mitochondrion (Fig. 4). The oxidation of acetyl-CoA *plus* carnitine by the fetal heart was very slow whereas acetylcarnitine was oxidized at a significant rate. This suggests that a low activity of the external acetylcarnitine transferase in the fetal heart is rate limiting for acetyl-CoA transport and oxidation. However, an inside acetylcarnitine transferase can still convert acetylcarnitine to acetyl-CoA which then participates in the citric acid cycle. BRDICZKA *et al.*[20] have also reported data indicating that acetylcarnitine transferase is present in two different mitochondrial compartments. As noted above, these data indicating compartmentation of acetylcarnitine transferase activity support the earlier distinction of a similar localization of palmitylcarnitine transferase activity inside and outside of the membrane barrier to palmityl-CoA (Fig. 4).

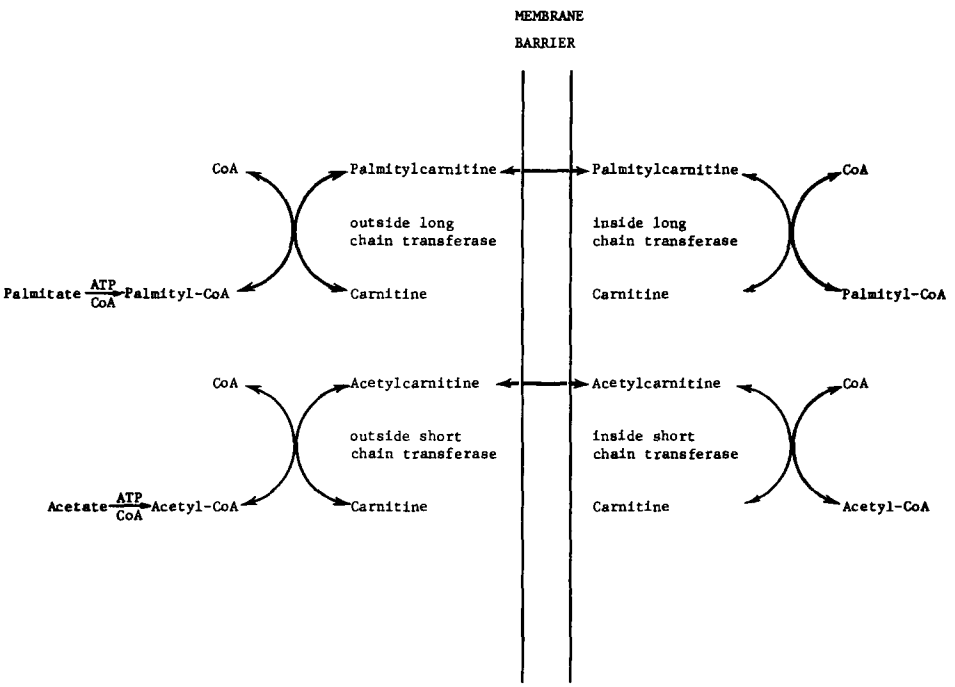


Fig. 4. Scheme of internal and external acylcarnitine transferases. The short chain transferase (acetylcarnitine tranferase) and long chain transferase (palmitylcarnitine transferase) are shown to be situated on both sides of the membrane barrier to acyl-CoA.

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