

activity be detected though the unfractionated proteins to start with had a slight activity of the order reported in literature<sup>6</sup>.

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## The Organ and Cellular Distribution of Acetylcarnitine-CoA Acetyltransferase

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When mitochondria are incubated with carnitine and pyruvate, acetylcarnitine is formed. In the presence of catalytic amounts of succinate, the acetyl group of acetylcarnitine is completely oxidized by mitochondria<sup>1</sup>. Acetyl-CoA is the donor of the acetyl group in the acetylation of carnitine (Table 1).

Because of the impermeability of the mitochondrial membrane, acetyl-CoA formed in the mitochondria most likely cannot leave these particles. We suggest therefore that carnitine acts as a carrier of activated acetyl groups between the mitochondrial and the extramitochondrial compartments of the cell. This hypothesis requires the presence of acetylcarnitine-CoA acetyltransferase both intra- and extramitochondrially.

Table 1 shows that this enzyme indeed is found in the particle-free supernatant, but apparently in smaller amounts than in the mitochondria. This low activity in the supernatant is possibly due to the presence of inhibitory factors or to rapid inactivation of the enzyme.

*Table 1.* Acetylation of carnitine by particles and by particle-free supernatants from different rat organs. The organs were homogenized in 10 volumes 10 % sucrose containing 0.005 M Tris-buffer (pH 7.4) and 0.005 M EDTA. The particle fraction was isolated with centrifugation at  $25\,000 \times g$  for 45 min. Acetyl-CoA was generated in the incubation mixture by means of acetylphosphate, 20  $\mu$ moles; GSH, 2.5  $\mu$ moles; CoA, 0.15  $\mu$ moles, and phosphotransacetylase, 50  $\mu$ l (Transacetylase purchased from Nutritional Biochemical Co.). Other additions: Tris-maleate-buffer (pH 7.5), 20  $\mu$ moles, DL-carnitine, 10  $\mu$ moles; palmitylcarnitine, 1  $\mu$ mole; potassium chloride, 30–40  $\mu$ moles and particles or particle-free supernatant derived from 2.5–10 mg of fresh tissue. Total incubation volume was 1 ml. The acetylcarnitine formed was assayed according to Friedman and Fraenkel<sup>3</sup>. The values in the table represent  $\mu$ moles acetylcarnitine formed per 10 mg of tissue per hour at 30°.

Organ	$\mu$ moles acetylcarnitine	
	Particles	Particle-free supernatant
Kidney	0.62	0.05
Liver	0.38	0.14
Heart	2.65	0.16
Muscle	0.70	0.03
Brain	0.34	0.00

Maximum activity in isolated mitochondria is obtained when they are disintegrated by freezing and thawing or by using the pronounced lyzing ability of palmitylcarnitine<sup>2</sup>. The freezing and thawing of isolated mitochondria in 10 % sucrose does not solubilize the mitochondrial enzyme, as subsequent centrifugation at  $25\,000 \times g$  gives a completely inactive supernatant. This shows that the mitochondrial enzyme is particle-bound and excludes that the enzyme activity of the particle-free supernatant represents leakage from the mitochondria. Other experiments have shown that the microsome fraction contains no detectable acetylcarnitine-CoA acetyltransferase activity.

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