

recent finding that blue-green-algae, like bacteria, resist lysis by polyene anti-fungal antibiotics¹², agents which are believed to bind selectively to sterols in cell membranes¹³. The structural involvement of sterols in intracellular organelles such as the nuclear membrane has been suggested as a feature distinguishing procaryotic and eucaryotic organisms¹⁴.

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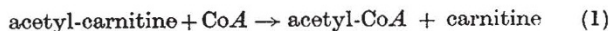
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Acetyl-carnitine in Heart and Liver

Friedman and Fraenkel¹ showed that the reversible acetylation of L-carnitine by acetyl-coenzyme A could be catalysed by extracts from pigeon liver. Carnitine acetyl transferase (E.C.2.3.1.7) has recently been partly purified from pig heart². The existence of a carnitine palmitoyl transferase, distinct from the acetyl transferase, has been shown^{3,5}, and it has been suggested^{4,6} that acyl-carnitine compounds might play a part in the transfer of activated acyl-groups across the mitochondrial membrane. Intracellular levels of acyl-carnitine compounds might be expected to be influenced by conditions known to affect the tissue levels of acyl-coenzyme A compounds⁷.

Acetyl-carnitine has been assayed by combining reaction (1), catalysed by carnitine acetyl transferase, with an assay for acetyl-coenzyme A (reaction (2)).



Reaction (2) is catalysed by the combined action of malate dehydrogenase and citrate synthase. Acetyl-carnitine thus gives rise to an equivalent amount of reduced NAD.

The assay system contained, in 2 ml., tissue extract containing up to 0.16 μ moles of acetyl-carnitine and the following components (final concentrations shown): *tris*-hydrochloride, pH 7.8, 50 mM; L-malate, 15 mM; NAD, 0.25 mM; CoA, 0.15 mM; GSH, 1 mM; EDTA, 1 mM; excess malate dehydrogenase. On addition of 10 μ l. of citrate synthase suspension, an increase in extinction at 340 m μ resulted, corresponding to the amount of acetyl-coenzyme A in the extract added. The addition of 25 μ l. of carnitine acetyl transferase suspension then

Table 1. MEAN VALUES OBTAINED FOR THE L-ACETYL-CARNITINE CONTENT OF VARIOUS TYPES OF RAT LIVER AND HEART

Tissue	No. of rats	μ m.moles of L-acetyl-carnitine per g wet weight of tissue
Rat liver (fed controls)	4	61
Rat liver (40 h fasted animals)	4	115
Rat heart (normal fed animals)		
Non-perfused	3	364
Perfused with glucose (1 mg/ml.) and insulin (0.05 units/ml.)	10	28
Perfused with glucose (1 mg/ml.), insulin (0.05 units/ml.) and acetate (4 mM)	8	809
Perfused with 1 mM pyruvate	6	214
Perfused with 1 mM pyruvate and 4 mM butyrate	4	420
Perfused with 1 mM pyruvate and 5.5 mM β -OH-butyrate	4	689
Rat heart (40 h fasted animals)		
Non-perfused	3	472
Perfused with glucose (1 mg/ml.) and insulin (0.05 units/ml.)	3	23
Perfused with 1 mM pyruvate	4	43
Rat heart (alloxan diabetic animals)		
Non-perfused	3	420
Perfused with glucose (1 mg/ml.) and insulin (0.05 units/ml.)	5	460

caused a further increase in the extinction at 340 m μ corresponding to the amount of acetyl-carnitine present. Preliminary results for the acetyl-carnitine content of rat heart and liver extracts are shown in Table 1.

The tissue extracts used were prepared from rat hearts and livers removed immediately after killing the animal, or from rat hearts perfused by drip-through as described by Newsholme and Randle⁸. The material was rapidly frozen by immersion in acetone containing solid carbon dioxide, and powdered in a percussion mortar. The tissue powder was homogenized in ice-cold 5 per cent (w/v) perchloric acid (about 1 g tissue in 3 ml.). After centrifuging, the supernatant was adjusted to pH 7.0.

The acetyl-carnitine content of rat heart was found to be about five times higher than that of rat liver. This is in contrast to acetyl-coenzyme A levels, which are higher in rat liver (about 20 μ m.moles/g) than in rat heart (< 10 μ m.moles/g) under normal conditions. It appeared that in fasted rats acetyl-carnitine levels are raised slightly in liver and possibly also in heart. Perfusion with a medium containing glucose and insulin greatly lowered the level in heart from normal and fasted animals but had little effect with hearts from alloxan diabetic animals. Perfusion with pyruvate caused much more marked lowering of acetyl-carnitine levels in hearts from fasted animals than in hearts from fed controls. Amount of acetyl-carnitine in hearts from normal rats were raised above normal by perfusing with a medium containing pyruvate and β -hydroxy-butyrate, or by adding acetate to the glucose and insulin perfusion medium.

In general it appears that acetyl-carnitine levels fluctuate in a similar way to those of acetyl-coenzyme A; however, particularly in heart, acetyl-carnitine is present in much larger amounts.

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