THE PARTIAL LATENCY AND INTRAMITOCHONDRIAL DISTRIBUTION OF CARNITINE-PALMITOYLTRANSFERASE (E.C.2.3.1.-), AND THE CoASH AND CARNITINE PERMEABLE SPACE OF RAT LIVER MITOCHONDRIA*

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Introduction. The inner of the two mitochondrial membranes has been recently correlated by Klingenberg & Pfaff (1965) with an operationally defined barrier that is impermeable to adenine nucleotides except by an exchange process which is inhibited by atractyloside, and impermeable to sucrose. The same membrane intervenes between the two palmitoyl-CoA synthetases of rat liver mitochondria, there being one enzyme within the atractyloside sensitive barrier utilising endogenous mitochondrial CoASH (Chappell & Crofts, 1965), and another enzyme outside the atractyloside sensitive barrier utilising added CoASH (Yates, Shepherd & Garland, 1966). Palmitoyl-CoA synthesised at either site requires the addition of L-carnitine to the incubation medium before β-oxidation can start. From these considerations it was anticipated that the inner mitochondrial membrane would be impermeable to CoASH but not to L-carnitine. However, space measurements (see below) indicate that the inner mitochondrial membrane is impermeable to both CoASH and L-carnitine, and palmitoyl- group transfer across this membrane possibly occurs by an anisotropic (see Mitchell, 1963) carnitine-palmitoyltransferase forming part of the membrane and catalysing

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the reaction:

$$\text{palmitoyl-L-carnitine}_{(out)} + \text{CoASH}_{(in)} \rightleftharpoons \text{L-carnitine}_{(out)} + \text{palmitoyl-CoA}_{(in)}$$

where "out" and "in" refer to the two sides of the membrane. Such an enzyme in intact mitochondria would be latent towards added palmitoyl-CoA and L-carnitine, but not latent towards added palmitoyl-L-carnitine nor added L-carnitine and endogenously generated palmitoyl-CoA, and if this were the case it could be further predicted that there is a second carnitine-palmitoyl-transferase outside the inner mitochondrial membrane and not exhibiting latency towards added palmitoyl-CoA and L-carnitine.

Methods. Preparation of mitochondria, reagents, and fluorimetric assays for CoASH and carnitine-palmitoyltransferase have been described (Garland, Shepherd & Yates, 1965; Shepherd, Yates & Garland, 1966). Separation of sonically disintegrated mitochondria into soluble and pellet (membrane) fractions was effected by centrifugation at 125,000 x g for 40 min. The penetration of L-carnitine, CoASH and sucrose into intact mitochondria was studied by incubating mitochondria (6 mg of mitochondrial protein per ml) at 1° or 25° for 10 min in a medium containing 20 mM-tris-chloride pH 7.2; 80 mM-KCl; 1 mM-EDTA; 20 mM-sucrose; 10 mg of Blue Dextran (a coloured polysaccharide of molecular weight circa 2×10^6 - Pharmacia Ltd.); 4 μC of tritiated water per ml; and either 0.5 mM-CoASH or 1.0 mM-L-carnitine. After incubation the mixture was separated into a pellet and supernatant by centrifugation at 25,000 x g for 10 min. The pellet (after extraction with 5% (w/v) perchloric acid) and supernatant were subsequently assayed for tritiated water, sucrose (Kulka, 1956), Blue Dextran (absorbancy at 610 mμ), L-carnitine (Pearson & Tubbs, 1964), and CoASH. The total water content and the extramitochondrial volume of the mitochondrial pellet were calculated from the measurements of tritiated water and Dextran Blue respectively, and the total intramitochondrial water obtained by difference. Correction was made for endogenous CoASH in calculations of the CoASH permeable space. Any endogenous L-carnitine was undetectable (< 0.1 mμMole/mg mitochondrial protein).

Results. 70-75% of the total intramitochondrial water was found to be permeable to sucrose, a value in agreement with that of others. The CoASH and L-carnitine permeable spaces were identical with or slightly less than the sucrose permeable space. It is concluded that the inner mitochondrial membrane is impermeable to these compounds and that the endogenous CoASH (and presumably other CoA species) of mitochondria is confined by this membrane.

A fluorimetric assay for carnitine-palmitoyltransferase activity of intact mitochondria is shown in Fig 1 (a). The rate of CoASH release prior to the

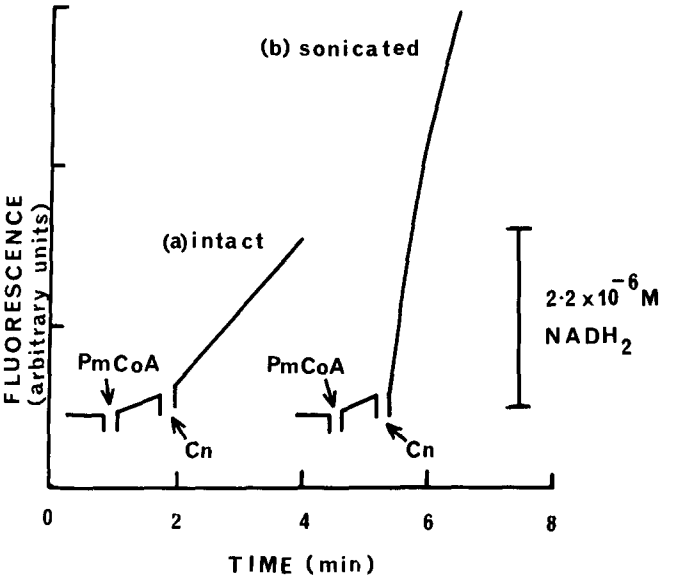

Fig. 1. Recorder tracings of fluorimetric assays for carnitine-palmitoyl-transferase. Assays at 30° in a final volume of 2.0 ml containing 20 mM-tris-chloride pH 7.2; 80 mM-KCl; 1 mM-EDTA; 2 mM-$MgCl_2$; 0.1mM-KCN; 0.4μg rotenone per ml; 1.0 mM-2-oxoglutarate; 1.0 mM-NAD; 0.06 units of oxoglutarate dehydrogenase per ml; 5 mg of bovine plasma albumin per ml; 0.16 mg of mitochondrial protein per ml; 20 μM-palmitoyl-CoA (PmCoA); and 1.25 mM-L-carnitine (Cn). Trace (a), intact mitochondria; trace (b), sonically disintegrated mitochondria.

addition of L-carnitine was probably due to a palmitoyl-CoA deacylase. Sonic disintegration of the mitochondria caused a sevenfold increase in carnitine-palmitoyltransferase activity (Fig 1 (b)), and it would appear that there is both an overt and a latent activity when assay is in the direction:

palmitoyl-CoA + L-carnitine $\longrightarrow$ palmitoyl-L-carnitine + CoASH

The pellet (after resuspension without detergents) and soluble fractions obtained by centrifugation of sonically disintegrated mitochondria were also assayed for carnitine-palmitoyltransferase activity as described in Fig 1. The total activities, K_m values for L-carnitine and K_i values for palmitoyl-D-carnitine (a competitive inhibitor against L-carnitine) are shown in Table 1. Inhibition of carnitine-palmitoyltransferase by palmitoyl-D-carnitine was predicted but not demonstrable by Fritz & Marquis (1965).

FRACTION	Activity $\left(\frac{\text{E.C. units} \times 10^{-3}}{\text{mg mitochondrial protein}}\right)$	K_m for L-carnitine ($M \times 10^{-5}$)	K_i for Palmitoyl-D-carnitine ($M \times 10^{-5}$)
Intact Mitochondria	2.7	10.0	27
Sonicated Mitochondria	18.7	-	-
Soluble Enzyme	2.4	9.8	20
Pellet Enzyme	16.6	3.5	2.5

Table 1. Properties of Carnitine-Palmitoyltransferase Activities of Rat Liver Mitochondria

Discussion. These data (Table 1) show that the overt and latent carnitine-palmitoyltransferase activities of rat liver mitochondria correspond to the soluble and pellet (membrane) fractions respectively. The pellet (membrane) activity is associated with the inner mitochondrial membrane, which confines the endogenous CoA of mitochondria, and the overt, soluble activity is outside that membrane. Since the addition of palmitoyl-carnitine to intact mitochondria causes rapid and extensive acylation of endogenous CoASH (Garland, Shepherd & Yates, 1965), and since palmitoyl- groups are transferred from endogenously generated palmitoyl-CoA to added L-carnitine (Bremer, 1963), it appears that the membrane-bound enzyme is not latent towards added palmitoyl-carnitine or endogenous palmitoyl-CoA. The scheme of Fig 2 is consistent with the properties of the two carnitine-palmitoyltransferase activities, the permeability properties

of the inner membrane, the distribution of the two palmitoyl-CoA synthetase activities, and the low level of intermediates of β-oxidation in rat liver mitochondria (Garland, Shepherd & Yates, 1965). The operationally defined β-oxidation compartment may have an organised complex of the enzymes of β-oxidation as its structural counterpart, and this concept is not incompatible with the finding that the β-oxidation of octanoate and butyrate is carnitine dependent when proceeding via added CoASH and carnitine independent when proceeding via endogenous CoASH (Yates & Garland, unpublished observations).

The partial latency of carnitine-palmitoyltransferase activity of rat liver mitochondria does not alter our previous conclusion (Shepherd, Yates & Garland,

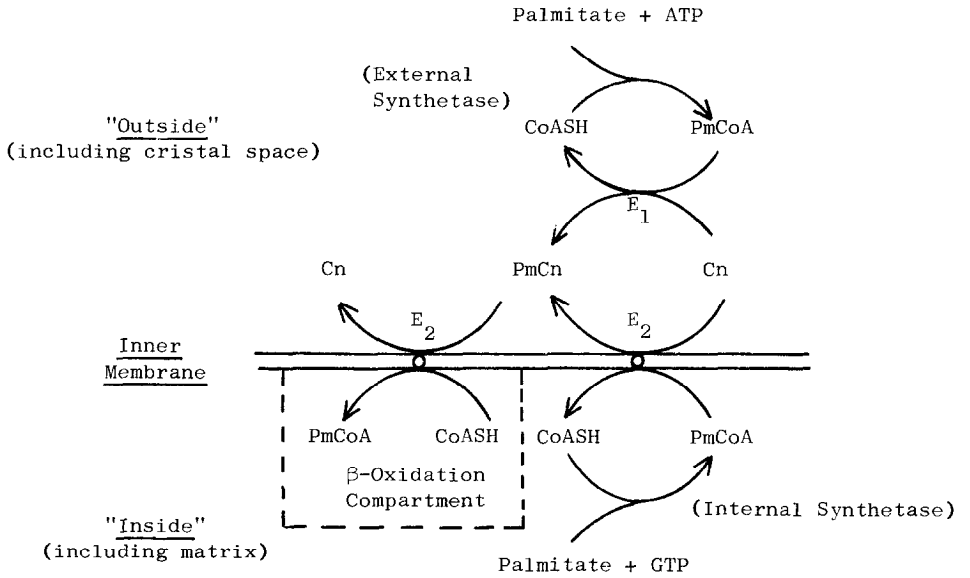

Fig. 2. Function of carnitine-palmitoyltransferase activities in palmitate oxidation by rat liver mitochondria. Cn, L-carnitine; Pm, palmitoyl-; E_1, "overt" or soluble enzyme; E_2, "latent" or membrane-bound enzyme.

1966) that this enzyme is rate-limiting for the oxidation of palmitoyl-CoA synthesised outside the inner mitochondrial membrane, but indicates that this is not so for palmitoyl-CoA synthesised inside the inner mitochondrial membrane.

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