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Inhibition of Palmitoyl-L-Carnitine Oxidation in Rat Liver Mitochondria by Pent-4-enoyl-L-carnitine

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Some fatty acids with a methylene group separated by two carbon atoms from a carboxyl group possess hypoglycaemic activity (Anderson *et al.* 1958). Of these, pent-4-enoic acid (Senior, Robson & Sherratt, 1968) and methylenecyclopropylacetic acid (Holt, Holt & Böhm, 1966) are known to inhibit strongly the formation of $^{14}\text{CO}_2$ from [$1\text{-}^{14}\text{C}$]palmitate in rat liver mitochondria. By contrast, the non-hypoglycaemic compounds *n*-pentanoic acid, cyclobutanecarboxylic acid and cyclopropanecarboxylic acid do not.

Oxidations in mitochondria were measured polarographically at 30° and pH 7.0 in the presence of 20mM-malonate. Pent-4-enoic acid (0.1mM) inhibited the oxidation of palmitoyl-L-carnitine by 80% and this inhibition was abolished under uncoupling conditions (addition of 0.02mM-dinitrophenol and 10mM-arsenate), indicating that an activated derivative is responsible. Preincubation of uncoupled mitochondria with pent-4-enoyl-L-carnitine for 3min. inhibited palmitoyl-L-carnitine oxidation (80% at 0.1mM; 50% at 0.01mM), when the maximum remaining rate of oxidation was only reached 2–3min. after the addition of palmitoyl-L-carnitine, in contrast with the linear rate immediately obtained in the absence of inhibitor. *n*-Pentanoyl-L-carnitine, cyclobutanecarbonyl-L-carnitine and cyclopropanecarbonyl-L-carnitine only inhibited slightly under these conditions.

Intramitochondrial pent-4-enoyl-CoA is probably formed from exogenous pent-4-enoyl-L-carnitine and this might inhibit long-chain fatty acid oxidation, either directly or by binding CoA. Alternatively, possible products of β -oxidation of pent-4-enoyl-L-carnitine may be inhibitory; although any acryloyl-CoA formed may not inhibit strongly as suggested by Williamson, Scholz & Fukami (1969), since the oxidation of palmitoyl-L-carnitine is only inhibited weakly by acrylic acid in coupled mitochondria (50% at 2mM, 10% at 0.1mM). There was a pulse of oxygen uptake of 30ng.atoms/mg. of protein when 0.1mM-pent-4-enoyl-L-carnitine was added to uncoupled mitochondria, which may be compared with the amount of pent-4-enoyl-CoA that could be formed assuming sequestration of the total mitochondrial CoA content of about 2nmoles/

mg. of protein (Garland, Shepherd & Yates, 1965). It is therefore not yet possible to explain how pent-4-enoic acid or methylenecyclopropylacetic acid inhibits long-chain fatty acid oxidation (see Senior *et al.* 1968).

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The Chain Length of Fatty Acids Synthesized by Fatty Acid Synthetase

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The triglycerides of many tissues contain predominantly long-chain fatty acids, and the synthesis of these acids by fatty acid synthetase has been extensively studied in a number of tissues. In pigeon and rat liver, the product of fatty acid synthetase at optimum substrate concentrations is predominantly free palmitic acid (Wakil, 1961; Hsu, Wasson & Porter, 1965; Burton, Haavik & Porter, 1968).

The triglycerides of milk from a number of species contain significant amounts of short-chain and medium-chain fatty acids (Garton, 1963; Glass, Troolin & Jenness, 1967). In rabbit milk over 70% of the esterified fatty acids are octanoic acid and decanoic acid, and these are not of dietary origin (Smith, Watts & Dils, 1968). Particle-free extracts of lactating rabbit mammary gland synthesize a wide range ($\text{C}_{4:0}$ – $\text{C}_{18:0}$) of even-numbered saturated fatty acids from acetate by the malonyl-CoA pathway (Smith & Dils, 1964, 1966). Increasing the activity of acetyl-CoA– CO_2 ligase (ADP) in these extracts increased the rate of synthesis and the proportion of long-chain fatty acids synthesized. A high proportion of butyric acid was always synthesized, though none occurs in rabbit milk (Smith *et al.* 1968).

Fatty acid synthetase has now been isolated from rabbit mammary gland and its properties have been compared with rat liver fatty acid synthetase. At