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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

optimum acetyl-CoA and malonyl-CoA concentrations both enzymes synthesized predominantly free palmitic acid as well as small proportions of C_{6:0}-C_{14:0} fatty acids. A significant proportion (10-15%) of butyric acid was also formed. Lowering the concentration of malonyl-CoA relative to acetyl-CoA decreased the rate of fatty acid synthesis and the proportion of palmitic acid formed. The proportion of medium-chain fatty acids synthesized increased slightly, whereas that of butyrate increased to about 50%. Hence the chain length of the fatty acids formed by these fatty acid synthetases can be controlled by the relative concentrations of acetyl-CoA and malonyl-CoA available. The enzyme from rabbit mammary gland does not possess a specific mechanism for the synthesis of medium-chain fatty acids. Alternative mechanisms of control of chain length, such as the interaction of fatty acid synthetase with other enzymes of lipogenesis, will be discussed.

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The Polyenoic Acids and their Elongation Products in the Muscle Tissue of *Phacochoerus aethiopicus*: a Re-evaluation of 'Animal Fat'

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We have reported that the muscle tissue lipids from bovids free to select their own food were rich in polyenoic acids (30% of the total) when compared with those of domestic bovids, which may contain only 1-8% of the total (Crawford, 1968; Crawford, Gale & Woodford, 1969). The bovids are ruminants and we have now studied the muscle tissue from a non-ruminant, the wart-hog (*Phacochoerus aethiopicus*); we find an even higher proportion of polyenoic acids. Linoleate and linolenate alone account for 30-40% of the total, and the higher elongation products (eicosatetraenoate, eicosapentaenoate, docosapentaenoate and docosaheptaenoate) constitute another 10-15%. The C₁₈ saturated and monounsaturated acids, which characterize the fat

of domestic animals, are present in the proportion of only approx. 7-9% each.

Previously it has been considered that animal fats are predominantly of a saturated and monounsaturated nature (Hilditch & Williams, 1964; Hubbard & Pocklington, 1968; Crawford, 1968). The facts from free-living herbivores, both ruminants and non-ruminants, show that, on the contrary, animal 'tissue fats' contain appreciable amounts of polyunsaturated acids.

The higher polyenoic metabolic products such as eicosatetraenoate, eicosapentaenoate, docosapentaenoate and docosaheptaenoate are also present in tissues of domestic animals, but have been largely overlooked as the massive infiltration of saturated and monounsaturated fat makes them difficult to detect.

The higher elongation products are used for construction of phospholipids, prostaglandins and cerebral gangliosides. Their consistent presence in animal products along with the parent C₁₈ polyenoic acids indicates that a re-evaluation of the 'saturated' nature of 'animal fat' is needed. We have now examined 14 different species and the saturated and monounsaturated predominate only in animals living under domestic conditions.

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Brain and Cerebellum Fatty Acid Pattern Changes in the Encephalomalacic Chick and the Presence of a Homeostatic Mechanism

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Several workers have reported changes in the fatty acid composition of various tissues in vitamin E deficiency (e.g. Bieri & Andrews, 1964; Witting & Horwitt, 1967; Lee & Barnes, 1969). Witting (1967), working with rat muscle tissue, postulated a homeostatic mechanism whereby the synthesis of certain polyunsaturated fatty acids is stimulated to make up for deficiencies in others. The tissues of the encephalomalacic chick were studied to discover if this homeostatic effect was observable in another tissue in which gross changes occur in vitamin E deficiency.

The total phospholipid from whole brain of encephalomalacic chicks showed significant rises in the relative percentages (expressed as means