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Hydroxylation of Fatty Acids by Avocado Supernatant Fraction

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Preparations from the avocado (*Persea americana*) mesocarp rapidly synthesize fatty acids from C₂ precursors. Yang & Stumpf (1965) first showed the incorporation of radioactivity from [¹⁴C]malonate and [¹⁴C]malonyl-CoA into long-chain fatty acids by the 15000g supernatant. This latter activity is due to soluble proteins rather than microsomal particles. However, the 105000g supernatant is unable to elongate medium-chain fatty acids (C₈-C₁₂) in the presence of added malonyl-CoA and appropriate cofactors (NADPH, acyl-carrier protein, GSH).

When fatty acids (C₈-C₁₂) were incubated with the 105000g supernatant under conditions used for fatty acid synthesis (Harwood & Stumpf, 1971) a hydroxy acid was produced. The possibility of the hydroxy fatty acid being α -hydroxy or ω -hydroxy was eliminated by chemical degradation and chromatographic analysis. The product co-chromatographed as the methyl ester or trimethylsilyl ether on g.l.c. and t.l.c. in two separate systems with the β -hydroxy derivative of the substrate. Butyrate, heptanoate, myristate, palmitate and stearate are not hydroxylated.

The reaction requires CoA, ATP, acyl-carrier protein and molecular O₂. The chain-length specificity is not due to the acid-CoA ligase, since myristyl-CoA is not hydroxylated. It does not take place via β -oxidation because (a) no radioactivity was incorporated from ³H₂O under the reaction conditions, (b) no loss of radioactivity from 1-¹⁴C-labelled medium-chain fatty acids took place during the incubation and (c) the observed specificity would not have occurred since β -oxidation utilizes long-chain acids as well.

The hydroxylating enzyme is sensitive to iodoacetamide and, consequently, is protected by GSH. Production of the β -hydroxy acid is diminished in the presence of NADH and/or malonyl-CoA. This is probably due to the production of NAD⁺, since

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addition of the latter (at 0.5mM) completely inhibits the reaction. Slight inhibition of the reaction was achieved with cyanide (0.1mM); 5,5'-bipyridyl (1 μ M) or CO (CO+O₂, 83:17) gave strong inhibition, thus suggesting that a heavy-metal ion (such as Fe²⁺) is involved in the hydroxylation. Because of the soluble nature of the avocado hydroxylating system it should be possible to purify the enzyme and study the exact mechanism of hydroxylation.

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Low-Fat Diets and the Survival of Newborn Rats

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Phospholipids and protein are the major components of cellular and subcellular membranes. In animal tissues the essential fatty acids and their long-chain metabolic derivatives are consistently found as the major fatty acids in phospholipids.

In the rat essential-fatty acid deficiency affects reproduction: deficient females frequently abort or the pups die soon after birth; the male rats are generally sterile and will not mate (Holman, 1968). Deficiency experiments are frequently carried out by using diets completely free from fat; however, this is a situation most unlikely to occur in Nature. We examined rats fed on a low-fat diet (0.6% fat; 10g of diet contained 11mg and 2.2mg of linoleate and linolenate respectively) from weaning to maturity; on this diet the rats did not develop markedly scaly tails and the eicosatrienoate/arachidonate ratio in their liver phospholipids was only 0.1. Female rats on this diet were mated and only about 12% of the pups (13 litters) survived longer than 2 weeks. The same diet with 6% fat added isocalorically (10g of diet contained 211mg and 43mg of linoleate and linolenate respectively; control diet) gave a survival rate greater than 50% (12 litters).

Tissues were analysed within 24h of birth from 11 randomly selected pups on the low-fat diet and nine on the control diet. On the low-fat diet the pups were 27% smaller and there was significantly less lipid in their livers (24.3 $\pm$ 2.3mg/g compared with 45.6 $\pm$ 4.7mg/g). The percentage of *n*-6 and *n*-3 unsaturated fatty acids in the liver lipids was decreased by 50% and the eicosatrienoate/arachidonate ratio was 0.47. The pups of the mothers given this diet had smaller brains (0.20 $\pm$ 0.01g compared with 0.29 $\pm$ 0.01g) and the polyenoic fatty acid content was decreased by 20%. The fatty acid composition had not altered as