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Studies on the Intestinal Lipids in Normal and Vitamin A-Deficient Rats

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The effect of vitamin A deficiency on rat intestinal lipids was studied. Groups of weanling albino rats were maintained on a vitamin A-deficient diet and the intestinal tissues were taken for analyses of their lipid components at the 'weight-plateau' stage and 'acute' stage of deficiency. Comparable controls on vitamin A-supplemented diet were included. The incorporation of ^{35}S from injected $\text{Na}_2^{35}\text{SO}_4$ into the lipid extract and the fraction corresponding to sulphatides was also investigated. Lipids from washed intestines were extracted with chloroform-methanol (2:1, v/v) and partitioned (Folch *et al.*, 1957). The relative amounts of phospholipids, cholesterol and glycolipids were determined by well established procedures.

The salient findings of these studies are: (1) a significant decrease was obtained in the total lipid content, primarily in the fraction corresponding to glycerides+cholesterol, of the intestine even at the 'weight-plateau' stage of deficiency; (2) in the 'acute' stage of deficiency a decrease in the glyceride fraction with relative increases in the proportion of phospholipids, cholesterol and sulphatides was observed; (3) incorporation of ^{35}S into the total lipids of the intestines was essentially unaltered at the 'weight-plateau' stage of deficiency, whereas at the 'acute' stage of deficiency the total ^{35}S and radioactivity/mg of lipid were greater.

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Mechanism of Malonyl-Coenzyme A-Acyl-Carrier Protein Transacylase

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The fatty acid synthetase of pigeon liver, like the yeast fatty acid synthetase, forms stable acyl-enzyme intermediates on incubation with acetyl-CoA or malonyl-CoA (Lynen, 1967; Joshi *et al.*, 1970; Phillips *et al.*, 1970). There are two thiol binding sites and one non-thiol binding site for acyl groups. One of these thiol sites is the thiol group of the 4'-

phosphopantetheine moiety of the synthetase. Both acetyl and malonyl groups form thiol ester bonds with this thiol group. The second thiol binding site is the thiol group of a cysteine residue. This thiol group binds the acetyl group but not the malonyl group and is part of the condensing enzyme of the complex. Studies from this laboratory on the acetyl-CoA transacylase and malonyl-CoA transacylase activities of the pigeon fatty acid synthetase have suggested that the non-thiol binding site of the synthetase is involved in the transacylation of acyl groups to acyl-carrier protein (Plate *et al.*, 1970). In contrast with the tightly bound multienzyme complex of the synthetase from pigeon, the fatty acid synthetase from *Escherichia coli* is readily dissociable into its component enzymes. We have isolated a homogeneous preparation of malonyl-CoA transacylase from *E. coli* and used this preparation to study the mechanism of transacylation.

Malonyl-CoA transacylase was purified 950-fold from extracts of *E. coli*. The purified enzyme was found to be homogeneous by disc gel electrophoresis and had an estimated molecular weight of 35000 when determined by polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate. The enzyme, though insensitive to thiol-group reagents, was strongly inactivated by toluene-*p*-sulphonyl fluoride, suggesting that it possesses an active serine residue. The transacylase accepted the malonyl group from malonyl-CoA or malonyl-(acyl-carrier protein) to form a malonyl-enzyme intermediate. This intermediate was stable towards performic acid oxidation; on hydrolysis with pepsin it yielded peptic peptides also stable towards performic acid.

The malonyl group from the malonyl-enzyme intermediate can be transferred to acyl-carrier protein, CoA, pantetheine, *N*-(*N*-acetyl- β -alanyl)-cysteamine or *N*-acetylcysteamine. The enzyme was specific for the malonyl group and did not transfer the acetyl group from acetyl-CoA. However, acetyl-CoA was a competitive inhibitor of malonyl-CoA.

Data from kinetic studies of the transacylase reaction are consistent with the formation of a malonyl-enzyme intermediate during the reaction. Lineweaver-Burk plots at different concentrations of malonyl-CoA and at various fixed concentrations of acyl-carrier protein gave a series of parallel lines. Moreover, CoA was found to be a competitive inhibitor of acyl-carrier protein and malonyl-(acyl-carrier protein) a competitive inhibitor of malonyl-CoA. These findings are consistent with a Ping Pong Bi Bi kinetic scheme for the malonyl-CoA transacylase reaction:

where E-OH represents the enzyme with its non-thiol binding site and ACP-SH represents acyl-carrier protein.

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Incorporation of Fatty Acids into Monogalactosyl Diglycerides by Spinach Leaf Cell-Free Preparations

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Mono- and di-galactosyl diglycerides are the major lipid components of chloroplasts and, in contrast with phospholipids, these plant galactolipids contain unsaturated fatty acids, usually α -linolenate, in both the 1- and the 2-position of the diglyceride moiety (Sastry & Kates, 1964). Although it has been shown in spinach that monogalactosyl diglyceride is synthesized from diglyceride and UDP-galactose (Neufeld & Hall, 1964; Ongun & Mudd, 1968), the enzymic mechanisms for the biosynthesis of the dilinolenin moiety in monogalactosyl diglyceride are not clearly understood.

Dialysed spinach (*Tetragonia expansa*) leaf homogenate and chloroplast preparations were found to incorporate 1-¹⁴C-labelled palmitate, stearate, oleate and linolenate into monogalactosyl diglyceride. This incorporation was much higher in the chloroplast fraction. It was enhanced by CoA and ATP. The activity increased with time, showing that it was enzymic, and was optimum at 30°C. A higher rate of incorporation was obtained with unsaturated fatty acids than with saturated fatty acids. Addition of galactosyl glycerol significantly stimulated the incorporation of oleate and linolenate only. These studies indicate the possible existence of deacylation and reacylation with specific fatty acids as a mechanism for attaining the peculiar fatty acid distribution in monogalactosyl diglyceride of higher plants.

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The Fatty Acid Composition of Different Classes of Lipids in Developing Human Brain

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Information on the lipid composition of developing human brain, particularly during its intrauterine growth, is scanty. Whatever data available so far are concerned mainly with the quantitative changes in various lipids. However, it is equally important to consider the qualitative changes in these lipids that would be reflected in the fatty acid constituents of given class of lipids. An attempt has therefore been made to determine the fatty acid composition of various classes of lipids in different regions (namely cerebrum, cerebellum and medulla oblongata) of brains of foetuses aged 13, 22, 26, 30, 34 and 40 weeks. The extracted lipids were fractionated into neutral lipids (cholesteryl esters, glycerides etc.), glycolipids and phospholipids on a silicic acid column. The fatty acid pattern, after methanolysis of these fractions, was determined by g.l.c. in a column packed with diethylene glycol succinate in Chromosorb W. The fatty acid profile of neutral lipids indicated palmitate, stearate and oleate as major components in all the regions of brain studied. This trend remained almost constant with the progress of gestation. Analysis of the glycolipid fraction showed that lignocerate (C_{24:0}) and nervonate (C_{24:1}) were absent at earlier periods of intrauterine life, but made their appearance towards term, though at a much lower concentration than expected. Studies with the phospholipid fraction also pointed out palmitate, stearate and oleate as predominant components and this pattern did not alter with the gestational age. Analysis of fatty acids in the glycolipid fraction of the brain of a child (4 years old) who had died of kwashiorkor revealed a considerably low content of lignocerate and nervonate, indicating the possibility of defective myelination.

The Gross Fatty Acid Composition of the Body Fat from the Termite (*Odontotermes assmuthi*) Queen

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An analysis of the total body fat of the termite (*Odontotermes assmuthi*) queen by reversed-phase partition column chromatography (Badami, 1964, 1965) reveals a total of 13 fatty acids as against nine