control and treated animals will be discussed to show the effectiveness of 3-hydroxy-3-methylglutarate in combating atherosclerosis.

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Effects of Cycloheximide and Actinomycin D on Hepatic 3-Hydroxy-3-methylglutaryl-Coenzyme A Hydrolase Activity

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Earlier work in this laboratory showed that starvation or feeding with cholesterol caused a two- to three-fold increase in hepatic 3-hydroxy-3-methylglutaryl-CoA hydrolase activity. This increase is not due to the formation of more enzyme because cycloheximide alone or in combination with actinomycin D did not inhibit, but increased, the enzyme activity. Unlike cycloheximide, actinomycin D is not known to produce fatty liver. To correlate hepatic lipid concentrations and 3-hydroxy-3-methylglutaryl-CoA hydrolase activity, a detailed investigation of liver lipid components was made with animals treated with actinomycin D and with actinomycin D plus cycloheximide.

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Unusual Features of Sterol Excretion and Distribution in the White Carneau Pigeon

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Some features of sterol excretion and distribution were examined in spontaneously atherosclerosis-susceptible white carneau pigeons by using t.l.c., t.l.c. on AgNO₃-impregnated gel, g.l.c. and mass spectrometry. Unlike man and rodents, these pigeons did not excrete any coprostanol or coprostanone derivatives of C₂₇, C₂₈, and C₂₉ sterols. In the chicken, however, the coprostanol derivatives contributed about 20–50% of the total faecal sterols. Unlike human faeces, the faeces of these pigeons cannot convert [¹⁴C]cholesterol into coprostanol.

Bacteriological examination of pigeon faeces indicated that it lacked the anaerobes (such as *Peptostreptococcus* species and *Bacteroides melaninogenicus*, *Bacteroides fragilis* and *Bacteroides incommunis*) found in human faeces, and this might account for the inability to form coprostanol. These pigeons also excreted an unusual amount of 5 α -cholestan-3 β -ol in the faeces (20% of sterols in the cholesterol group and 13% of total 5 α -stanols). Pigeon testes contained 5 α -cholestan-3 β -ol in the highest concentration reported in any normal animal tissue (26% of total sterols; 384 $\pm$ 29.4 μ g/g of tissue). Chicken testes contained only 0.5–2% of total sterols as stanol. Other pigeon tissues contained the stanol to an extent of 1–8% of total sterols. Pigeon faecal cultures containing [¹⁴C]cholesterol indicated that this stanol could not be formed by bacteria, and the diet contained no 5 α -cholestan-3 β -ol, indicating that most of the stanol in the species is of endogenous origin. These unusual features of sterol metabolism are of interest from the standpoint of both spontaneous atherogenesis and comparative biochemistry.

Studies on the Incorporation of [U-¹⁴C]Glucose into Intestinal Lipids in Vitamin A-Deficient Rats

By P. V. BHAT and P. B. RAMA RAO (*Discipline of Biochemistry and Applied Nutrition, Central Food Technological Research Institute, Mysore-2A, India*)

The effect of vitamin A deficiency on the incorporation of ¹⁴C from [U-¹⁴C]glucose into intestinal lipids was studied. Groups of weanling rats were maintained on a vitamin A-deficient diet along with controls receiving vitamin A supplement. Rats at the 'weight-plateau' stage of deficiency with comparable normal controls were used in this study. [U-¹⁴C]Glucose was injected intraperitoneally and the rats were killed 19 h later. Intestinal tissue was removed and the lipids were extracted with chloroform-methanol (2:1, v/v) and processed further (Folch *et al.*, 1957). Fractionation of the pooled lipid samples from normal and vitamin A-deficient animals on a silicic acid column was also carried out. The total lipid, phospholipids, cerebrosides, sulphatides and cholesterol were determined by standard procedures.

These studies confirm our observation that the glycerides are significantly decreased in the vitamin A-deficient state. It was also observed that ¹⁴C incorporation into the glyceride+cholesterol fraction is increased over fivefold with a concomitant increase in specific radioactivity (c.p.m./mg of glyceride). No significant change was observed in the incorporation of label into the glycolipid fraction as indicated by the specific radioactivity (c.p.m./mg of galactose). However, a higher specific radioactivity (about 30% more) for the phospholipid fraction was apparent.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497

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.

Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497

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

By V. C. JOSHI (Departments of Pediatrics and Biochemistry, Duke University Medical Center, Durham, N.C. 27710, U.S.A.)

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: