studies were then undertaken to explore the role of dietary vitamin A in regulating the production and secretion of retinol-binding protein by the liver. Weanling rats were divided into four groups, each fed on a vitamin A-deficient diet with or without supplements as follows: (1) control, supplemented with an adequate amount of vitamin A; (2) pair-fed control, supplemented with vitamin A but with food intake matched to that of the deficient rats; (3) deficient, no vitamin A supplementation; (4) supplemented, after the initial depletion period, with a modest daily dose (14–28 μg) of retinoic acid. The food intake and growth of each rat was monitored for 75 days. In the deficient group, plasma vitamin A concentrations declined from 45 ± 6 (mean $\pm$ s.e.m.) $\mu\text{g}/\text{dl}$ to $6 \pm 1 \mu\text{g}/\text{dl}$ after 27 days, and then declined more slowly to $2 \pm 1 \mu\text{g}/\text{dl}$ after 75 days. Plasma concentrations of retinol-binding protein declined during the induction of deficiency, with a time-course similar to that seen with vitamin A (from $50 \pm 4 \mu\text{g}/\text{ml}$ to $20 \pm 2 \mu\text{g}/\text{ml}$ at day 27, and then more slowly to $13 \pm 2 \mu\text{g}/\text{ml}$ at day 75). After 3 weeks most of the circulating retinol-binding protein was present as the apoprotein, not containing a molecule of bound retinol. The results obtained with the retinoic acid-fed rats, who continued to grow normally, were identical with those of the deficient rats. Liver homogenates were immunoreactive in the radioimmunoassay for retinol-binding protein, with almost all of the immunoreactivity associated with the particulate fractions. The content of immunoreactive retinol-binding protein in the livers of deficient rats was four times ($P < 0.001$) that in the livers of control rats. When vitamin A was administered orally to deficient rats on day 53, a very rapid increase in the plasma concentration of retinol-binding protein, from a mean of 14 to 56 $\mu\text{g}/\text{ml}$, was seen within 5 h (the first time-interval sampled). These findings suggest that vitamin A deficiency primarily interferes in some way with the secretion, rather than with the synthesis, of retinol-binding protein by the liver, and that the deficient liver contains a pool of preformed apo-(retinol-binding protein) that can be rapidly released into the plasma, as holo-(retinol-binding protein), when vitamin A becomes available.

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Cholesterol Regulation of Hepatic 3-Hydroxy-3-methylglutaryl-Coenzyme A Reductase

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3 - Hydroxy - 3 - methylglutaryl - CoA reductase [mevalonate-NADP oxidoreductase (acylating CoA), EC 1.1.1.34] undergoes striking protein-synthesis-dependent variations with distinct activity peaks at 12:00 midnight and 1:45 a.m. Cycloheximide prevents both the initial (6:00 p.m. to 12:00 midnight) rise in the rate of 3-hydroxy-3-methylglutaryl-CoA reduction and the second smaller rise from 12:30 a.m. to 1:45 a.m. A rapid 35–40% decrease in the rate of 3-hydroxy-3-methylglutaryl-CoA reduction occurs from 12:00 midnight to 12:30 a.m. After 1:45 a.m. the rate of 3-hydroxy-3-methylglutaryl-CoA reduction declines with a half-time of 2–3 h, reaching basal values in the morning.

The rate of 3-hydroxy-3-methylglutaryl-CoA reduction decreases 20–25-fold in rats starved for 36 h. However, the cyclic rhythm with both peaks in activity persists in rats starved for 12–18 h or 36 h. This indicates that cyclic variations in food intake and hepatic tryptophan concentrations are not responsible for the cyclic rise in 3-hydroxy-3-methylglutaryl-CoA reduction. By contrast, short-duration cholesterol feeding suppresses the cyclic rise. Cholesterol and starvation may therefore regulate 3-hydroxy-3-methylglutaryl-CoA reductase via different mechanisms.

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The Effects of Starvation and Re-feeding on the Rates of Synthesis and Degradation of Enzymes Effecting Intermediate Reactions in the Synthesis of Cholesterol and Fatty Acids in Rat Liver

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Investigations have been carried out on the effects of starvation and of re-feeding after starvation on the activities of acetyl-CoA carboxylase, fatty acid synthetase and each of the enzymes effecting the conversion of 3-hydroxy-3-methylglutaryl-CoA into squalene in rat liver. Studies have also been carried out on the effects of the above factors on the con-

version of acetate and mevalonate into cholesterol by rat liver homogenates. The activities of mevalonate kinase and phosphomevalonate kinase change either only slightly or not at all on starvation for 48 h and re-feeding for 144 h. The activities of all the other enzymes for which assays were performed changed drastically under the above conditions. It appears from determinations of the constants for the rates of synthesis and degradation of mevalonate pyrophosphate decarboxylase and squalene synthetase that these proteins vary in quantity in rat liver because of changes in the rate of their synthesis. In contrast, the changes in the activity of isopentenyl pyrophosphate isomerase appears to be due to a change in the rate of degradation of this protein. The rates of both synthesis and degradation of farnesyl pyrophosphate synthetase change on starvation and re-feeding.

The activity of 3-hydroxy-3-methylglutaryl-CoA reductase changes drastically on starvation and re-feeding. However, when an attempt is made to calculate rate constants for this enzyme by the rate of approach to a steady state, a curved rather than a straight line is obtained. Similarly, although one is able to calculate rate constants during starvation for acetyl-CoA carboxylase and the fatty acid synthetase, this method of analysis fails when applied to the results obtained on re-feeding the animals. Further investigations are being carried out on variations in the activities of these enzymes in liver by methods that utilize labelling of the enzyme *in vivo* or synthesis of the enzyme by isolated liver cells.

The results obtained strongly suggest that the genes coding for the enzymes of cholesterol synthesis are not co-ordinately controlled. In contrast, the activities of acetyl-CoA carboxylase and fatty acid synthetase change together, thereby suggesting that these enzymes are under a single control (and that the genes coding for these enzymes may be located on the same chromosome). There is also a relationship between the enzymes effecting the synthesis of cholesterol and those effecting the synthesis of fatty acids. When animals are transferred from a normal to a fat-free diet, the activities of acetyl-CoA carboxylase and fatty acid synthetase rise to values several times those of controls. At the same time the activities for 3-hydroxy-3-methylglutaryl-CoA reductase and for the conversion of acetate and mevalonate into cholesterol fall markedly. These results suggest that there is a cellular mechanism by which acetyl-CoA can be shunted into the pathway for fatty acid synthesis and away from the pathway for cholesterol synthesis when a fat-free diet is given.

Correlation of Chemical Structure and Biological Activity of Mycobacterial Cell Walls and Derived Lipids

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The mycobacterial cell wall contains a peptidoglycan-glycolipid complex and various more-or-less defined glycolipid fractions. The chemistry has been described in detail (Petit *et al.*, 1969; Adam *et al.*, 1969; Amar-Nacasch & Vilkas, 1970; Azuma *et al.*, 1970; Wietzerbin-Falszpan *et al.*, 1970; for a review see Lederer, 1971).

Mycobacterial cell walls have several interesting biological properties, such as immunological adjuvant activity (White, 1965) and specific and non-specific immunization (Anacker *et al.*, 1969; L. Chedid & A. Lamensans, unpublished work).

Some of these activities are also shown by some glycolipids and other compounds that are biogenetically or functionally more or less closely related to the mycobacterial cell wall, such as wax D (White *et al.*, 1964; Markovits *et al.*, 1971) and cord factor (Bekierkunst *et al.*, 1969).

It is intended to give an up-to-date and critical summary of recent work in this field where the frequent absence of criteria of chemical purity and the variety of biological test systems used are still preventing a general consensus among various workers in the field.

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