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 ($14\text{--}28\mu\text{g}$) 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.

This work was supported by Grant AM-05968 from the National Institutes of Health, Bethesda, Md., U.S.A.

Kanai, M., Raz, A. & Goodman, DeW. S. (1968) *J. Clin. Invest.* **47**, 2025

Smith, F. R., Raz, A. & Goodman, DeW. S. (1970) *J. Clin. Invest.* **49**, 1754

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

By VICTOR W. RODWELL (*Department of Biochemistry, Purdue University, Lafayette, Ind. 47907, U.S.A.*)

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.

This work was supported by a grant from the Indiana Heart Association.

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

By LINDA L. SLAKEY, MARGARET C. CRAIG, ENRIQUE BEYTIA, ANITA BRIEDIS, DONALD H. FELDBRUEGGE, RICHARD E. DUGAN, RICHARD A. MUESING, ASAF QURESHI, CHALLA SUBBARAYAN and JOHN W. PORTER (*Department of Physiological Chemistry, University of Wisconsin, and Veterans Administration Hospital, Madison, Wis., U.S.A.*)

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-