detail (Goodwin, 1971). Currently we are investigating various details of carotenogenesis with a *Flavobacterium* sp. that normally produces zeaxanthin (3,3'-dihydroxy- β -carotene) as its only pigment and that will actively incorporate labelled mevalonate, the first specific biosynthetic precursor of terpenes, including carotenoids, into this pigment.

As C-3 and C-3' of β -carotene arise from C-5 of mevalonate, [2- 14 C,5R-5- 3 H $_1$]- and [2- 14 C,5- 3 H $_2$]-mevalonate were used to study the hydroxylation of β -carotene to zeaxanthin. If a ketone were an intermediate then with [2- 14 C,5- 3 H $_2$]mevalonate as substrate zeaxanthin would be expected to have four tritium atoms fewer than β -carotene; however, if a mono-oxygenase were concerned only two tritium atoms would be lost. With [2- 14 C,5-R-5- 3 H $_1$]-mevalonate two tritium atoms would be lost if a ketone were an intermediate, but if a stereospecific replacement of a hydrogen atom by a hydroxyl group occurred either two or none would be lost according to the stereochemistry involved. Results with the *Flavobacterium* sp. showed that two tritium atoms were lost in each case, thus eliminating a keto intermediate but demonstrating a stereospecific elimination of R-hydrogen from C-5 of mevalonate (J. C. B. McDermott, G. Britton & T. W. Goodwin, unpublished work). If one assumes that the hydroxyl group is inserted at C-3 and C-3' in place of the R-hydrogen without change of configuration, then the absolute configuration arrived at for zeaxanthin is the same as that determined chemically (de Ville *et al.*, 1969). The results were confirmed by converting labelled zeaxanthin into rhodoxanthin and by degrading it to 3-hydroxyretinol and β -citaurin: all the compounds gave the expected labelling pattern. The fact that in these and previous experiments with higher plants (Walton *et al.*, 1969) β -carotene contains two tritium atoms more than does zeaxanthin rules out the view (Costes, 1963) that the hydrocarbon carotenoids are formed from xanthophylls to any significant extent.

Direct cyclization of lycopene to β -carotene has been demonstrated in chloroplasts (Hill & Rogers, 1969) and in purified preparations from green tissues (Kushwaha *et al.*, 1969). Howes & Batra (1970) showed that in a *Mycobacterium* sp. nicotine inhibits the formation of the bicyclic β -carotene and causes the accumulation of the acyclic lycopene. If the inhibitor is washed out of the cells then lycopene is rapidly transformed into β -carotene. J. C. B. McDermott, G. Britton & T. W. Goodwin (unpublished work) have shown that the same inhibiting effect is observed in their *Flavobacterium* sp. They further showed in radioisotope experiments that lycopene is directly converted into β -carotene and that the cyclization proceeds anaerobically. This makes it difficult to understand the need for NADP and FAD in the reaction with the partly purified

isomerase. If nicotine-inhibited cells are washed freed from inhibitor and resuspended aerobically then the reaction runs through to zeaxanthin.

The desaturation of phytoene to lycopene results in the formation of four double bonds, in each case involving carbon atoms arising from C-2 and C-5 of mevalonate. Experiments with mevalonate stereospecifically tritiated at C-5 have shown in a variety of preparations that there is stereospecific removal of a 5R-hydrogen of mevalonate during the formation of each double bond; similar experiments with [2R- 3 H $_1$]- and [2S- 3 H $_1$]-mevalonate gave equivocal results because of the reversibility of the isopentenyl pyrophosphate-dimethylallyl pyrophosphate isomerase (see Goodwin, 1971). Preparation from our *Flavobacterium* sp. of a cell-free system that will very actively convert mevalonate into phytoene and zeaxanthin has allowed us to circumvent this difficulty and to show that the 2-S-hydrogen is removed stereochemically during formation of the four double bonds involved in the desaturation of phytoene (F. Leuenberger, G. Britton & T. W. Goodwin, unpublished work).

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Biogenetic Interrelationship between Ubiquinone and Cholesterol

By T. RAMASARMA (*Department of Biochemistry, Indian Institute of Science, Bangalore-12, India*)

Cholesterol and ubiquinone share a common pathway for the synthesis of their constituent isoprene units. Most of the tissues of the rat are capable of synthesizing both of them. Cholesterol of hepatic origin seems to be the source for the serum and for some of the steroidogenic organs (Friedman *et al.*, 1951; Krum *et al.*, 1964). Transport of ubiquinone is dependent on absorption of the dietary ubiquinone through the intestines into the liver via the blood, but it is not mobilized therefrom (Jayaraman *et al.*, 1963).

Biogenesis of hepatic cholesterol is regulated by a variety of agents at a site between acetyl-CoA and mevalonate, most commonly at 3-hydroxy-3-methylglutaryl-CoA reductase (Bucher *et al.*, 1959; Siperstein & Fagan, 1966). Since this step is common for the biogenesis of ubiquinone, the effect of some of these regulatory agents on ubiquinone was tested. Dietary supplements of cholesterol (Krishnaiah *et al.*, 1967) and bile acids (Krishnaiah, 1968) decreased the hepatic ubiquinone concentration marginally, the residual mevalonate being diverted towards ubiquinone as a result of a secondary control point at the squalene stage (Bucher *et al.*, 1959). Starvation decreased the concentration and synthesis of ubiquinone (Inamdar & Ramasarma, 1971), possibly because of a decrease in the 3-hydroxy-3-methylglutaryl-CoA reductase activity (Regen *et al.*, 1966). Deficiency of proteins in the diet caused a decrease in the concentration and biogenesis of ubiquinone (Joshi *et al.*, 1965), but not of cholesterol. Excess of thyroxine (Inamdar & Ramasarma, 1969) and ethyl α -*p*-chlorophenoxyisobutyrate (Krishnaiah & Ramasarma, 1970a) treatments increased synthesis in the intermittent phase, followed by reversion to initial values, with a progressive increase in the content of ubiquinone.

Dietary supplementation of rats with ubiquinone-9 increased its content and decreased the synthesis of cholesterol in the liver, and also lowered serum cholesterol concentrations (Krishnaiah & Ramasarma, 1970b). The effects of the treatment with ethyl α -*p*-chlorophenoxyisobutyrate and with ubiquinone in rats were similar, were lost on exposure of the rats to low environmental temperature and were absent from young rats up to 2 months of age, and were not elicited in mice (Ramasarma, 1969). Decrease in cholesterol genesis, accompanied by increased ubiquinone concentrations in the liver, as observed in a number of cases, appeared to fit the postulate of end-product inhibition of a common enzyme in isoprene synthesis by ubiquinone (Ramasarma, 1968).

Circadian rhythm with a peak of activity at about 12:00 midnight was observed in hepatic cholesterol genesis, as tested by [14 C]acetate incorporation into the sterols *in vitro* (Back *et al.*, 1969) and *in vivo*, and also by the level of 3-hydroxy-3-methylglutaryl-CoA reductase activity (Hamprecht *et al.*, 1969; Shapiro & Rodwell, 1969; White & Rudney, 1970). However, this was not accompanied by corresponding changes in the concentrations of sterols and ubiquinone in the liver, or of serum sterols. When tested at 12:00 midnight, notwithstanding the severalfold increased activity, the regulatory agents showed proportionate inhibition.

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Cell-Free Biosynthesis of Precursors of Polyphenyl Derivatives of *p*-Hydroxybenzoate in Mammalian and Bacterial Systems

By HARRY RUDNEY (*Department of Biological Chemistry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229, U.S.A.*)

Previous studies (Winrow & Rudney, 1969; Rudney, 1970) showed that polyphenyl derivatives of *p*-hydroxybenzoate, potential precursors of ubiquinones, could be formed in mammalian and bacterial cell-free systems. A necessary factor was the supplementation of these systems with polyphenyl pyrophosphate derivatives synthesized from isopentyl pyrophosphate by an extract from *Micrococcus lysodeikticus*. This supplemented system could form polyphenyl-*p*-hydroxybenzoate and polyphenylphenol in bacterial systems; however, in rat tissue homogenates, i.e. of liver, kidneys and brain, only polyphenyl-*p*-hydroxybenzoate could be formed. Subsequent studies have further delineated the requirements and properties of these systems, and these will be reported.