PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 493rd Meeting of the Society was held at the University of Liverpool on Tuesday–Friday, 15–18 April 1969, when the following papers were presented:

SYMPOSIUM ON 'NATURAL SUBSTANCES FORMED BIOLOGICALLY FROM MEVALONIC ACID'

Chemistry of Mevalonic Acid

By J. W. CORNFORTH and R. H. CORNFORTH.
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Mevalonic acid (I) has become a key intermediate in isoprenoid biosynthesis since its discovery in 1956. Among its advantages are the following.

(1) It does not seem to be utilized in metabolic processes for anything except isoprenoid synthesis.

(2) It is water-soluble and can penetrate to the site of synthesis in many (though not all) living tissues.

(3) It is relatively easy to synthesize, and ^{14}C -labelled preparations were available very soon after its discovery.

In the course of investigating the biosynthesis of isoprenoids, and the mechanism of individual enzymic steps in this natural process, our group has had occasion to synthesize fourteen distinct varieties of labelled mevalonic acid and to use all of them to answer specific questions presented by the research. In addition, both the 'natural' and the 'unnatural' enantiomers of mevalonic acid have been synthesized. Several of the labelled mevalonic acids made available by these chemical syntheses have proved useful beyond the range of the experiments for which they were initially devised.

The biosynthetic experiments have usually dictated the nature of the label to be used, as well as its position in the precursor. The interplay of chemistry with biochemical requirements is illustrated in several of the examples discussed.

As a dihydroxyisovaleric acid, mevalonic acid would not be expected to exhibit any unusual chemical properties, and it has never been studied for its chemical behaviour alone. Nevertheless, there is at least one unsolved mystery surrounding the chemistry of mevalonic acid derivatives: the

observed acid-catalysed exchange of oxygen at position 3 with the oxygen of aqueous media. In addition, one synthesis of the acid has revealed an unsuspected exchange of hydrogen in borohydride anions.

Conversion of Mevalonic Acid into Terpene Hydrocarbons

By G. POPJÁK. (*Department of Biological Chemistry, University of California School of Medicine, Los Angeles, California, U.S.A.*)

On the Enzymes Catalysing the Transformation of Squalene to Lanosterol

By S. YAMAMOTO and K. BLOCH. (*The James Bryant Conant Laboratory, Departments of Chemistry and of Biochemistry and Molecular Biology, Harvard University, Cambridge, Mass. 02138, U.S.A.*)

The oxidative cyclization of squalene to lanosterol has been the subject of both chemical and enzymic studies by a number of investigators. Recently the intermediacy of chemically synthesized 2,3-oxidosqualene (Van Tamelen & Curphey, 1962) in the squalene–lanosterol conversion was established in two laboratories (Corey, Russey & Ortiz de Montellano, 1966; Van Tamelen, Willet, Clayton & Lord, 1966). These findings have received further support by the isolation and partial purification of a microsomal enzyme that catalyses the cyclization of 2,3-oxidosqualene to lanosterol (Dean, Ortiz de Montellano, Bloch & Corey, 1967). This reaction does not require oxygen or NADPH.

2,3-Oxidosqualene sterol cyclase has been solubilized from hog liver microsomes with the aid of sodium deoxycholate (Dean *et al.* 1967). After numerous futile attempts to purify the solubilized enzyme further, it was found that high concentrations of salt are required for maximal enzyme activity. The effect of salt on enzyme activity has been partially clarified by the results of gel filtration on Sephadex G-200. When the Sephadex column was equilibrated and eluted with KCl (0.4M) in

0.01M-phosphate buffer, the cyclase was retained and enzymically active on assay in the same buffer-salt solution. By contrast, enzyme chromatographed in the absence of KCl was eluted with the void volume and showed activity only when deoxycholate and KCl were present in the assay system. Further, it was observed that both KCl and deoxycholate are required to prevent enzyme activity from sedimenting on high-speed centrifugation. In the absence of salt the enzyme appears to aggregate to an insoluble, catalytically inactive species. Under appropriate conditions the aggregation process is reversible. The molecular weight of the dissociated active form of the enzyme is estimated to be about 90 000, whereas the inactive aggregate ranges in molecular weight from 100 000 to 400 000.

A separate enzyme system catalysing the conversion of squalene into 2,3-oxidosqualene was demonstrated as follows. Rat liver microsomes were heated to 50° for 5 min., combined with the soluble 105 000g supernatant, and then incubated in air with squalene and NADPH. A compound accumulated, which was identified as 2,3-oxidosqualene. No lanosterol was formed under these conditions. The soluble fraction of the rat liver homogenate contains one or two unidentified factors necessary for squalene epoxide formation. Carbon monoxide or potassium cyanide failed to inhibit epoxidase activity.

Corey, E. J., Russey, W. E. & Ortiz de Montellano, P. R. (1966). *J. Amer. chem. Soc.* **88**, 4750.

Dean, P. D. G., Ortiz de Montellano, P. R., Bloch, K. & Corey, E. J. (1967). *J. biol. Chem.* **242**, 3014.

Van Tamelen, E. E. & Curphey, T. J. (1962). *Tetrahedron Lett.* p. 121.

Van Tamelen, E. E., Willet, J. D., Clayton, R. B. & Lord, K. E. (1966). *J. Amer. chem. Soc.* **88**, 4752.

Sterol Biosynthesis

By L. J. GOAD. (*Department of Biochemistry, University of Liverpool*)

In the past few years rapid progress has been made in the elucidation of various problems concerned with the final stages of sterol biosynthesis. A number of these investigations have been facilitated by the use of the dual-labelled samples of mevalonic acid developed by J. W. Cornforth and G. Popják. Such studies reveal that, although the biosynthetic route follows a basic pattern, interesting and unexpected divergencies occur in different classes of organism. The most striking example is the nature of the precursor produced by 2,3-oxidosqualene cyclization. In animals lanosterol is firmly established as the triterpene that is converted into cholesterol. Similarly, in yeast lanosterol has

been shown to act as the precursor of ergosterol. By contrast, lanosterol is of very rare occurrence in higher plants or algae and is apparently only found in some members of the Euphorbiaceae (Ponsinet & Ourisson, 1968). However, the related triterpene, cycloartenol, is of widespread distribution and becomes rapidly labelled after administration of radioactive mevalonate or acetate to a number of higher plants (Benveniste, Hirth & Ourisson, 1966; Ehrhardt, Hirth & Ourisson, 1967; Goad & Goodwin, 1966) and to the alga *Fucus spiralis* (Goad & Goodwin, 1969). This led to the suggestion that in plants cycloartenol is produced rather than lanosterol (Ardenne, Osske, Schreiber, Steinfelder & Tümmeler, 1965; Benveniste *et al.* 1966). Evidence supporting this proposal has now been provided by the conversion of labelled cycloartenol and 24-methylenecycloartanol into phytosterol by the phytoflagellate *Ochromonas malhamensis* (Hall, Smith, Goad & Goodwin, 1969) and the accumulation of cycloartenol during anaerobic incubation of 2,3-oxidosqualene with *O. malhamensis* homogenates (H. H. Rees, L. J. Goad & T. W. Goodwin, unpublished work). However, labelled lanosterol is also converted into phytosterol by *O. malhamensis* and also by *Euphorbia peplus* (Baisted, Gardner & McReynolds, 1968), possibly indicating a lack of enzyme specificity in these organisms.

Mevalonate labelled stereospecifically at C-2 has been employed to examine hydrogen elimination during migration of the Δ^8 bond to Δ^7 in various sterols. Formation of cholesterol by liver and poriferasterol by *O. malhamensis* resulted in elimination of the 7β hydrogen atom (Canonica *et al.* 1968a; Caspi, Greig, Ramm & Varma, 1968; Smith, Goad & Goodwin, 1968a; Gibbons, Goad & Goodwin, 1968a), but with a yeast homogenate the 7α hydrogen atom was lost (Caspi & Ramm, 1969). A further difference between a fungus and an alga has been observed in the formation of the sterol Δ^{22} bond. In *Aspergillus fumigatus* the $22proS$ and $23proS$ hydrogen atoms were lost during ergosterol production (Bimpson, Goad & Goodwin, 1969), whereas in *O. malhamensis* the $22proR$ and $23proR$ hydrogen atoms were removed in poriferasterol formation (Smith *et al.* 1968a,b). Finally, the use of $[2-^{14}C, (2S)-2-^3H_1]$ mevalonate has led to the discovery that the 15α hydrogen atom is eliminated during the conversion of lanosterol into cholesterol (Canonica *et al.* 1968b; Gibbons, Goad & Goodwin, 1968b), probably as a result of C-14 demethylation and with the formation of a sterol $\Delta^{8,14}$ -diene intermediate (Canonica *et al.* 1968c; Akhtar, Watkinson, Rahimtula, Wilton & Munday, 1968; Lutsky & Schroepfer, 1968).

Our investigations have been supported by the Science Research Council and by Unilever Ltd.