The Chemistry and Biochemistry of Polyprenyl Alcohols such as Solanesol and Dolichol

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Rowland's (1956) discovery of solanesol ($C_{45}H_{93}OH$) in tobacco extended the geraniol-farnesol-geranylgeraniol series of unsaturated all-*trans*-polyprenols and gained additional significance by the discovery of quinones such as plastoquinones and ubiquinones with their all-*trans*-polyprenyl side chains.

Work carried out at Liverpool since then has shown that numerous other polyprenols occur naturally. The dolichols, obtained mainly from animal tissues, range from about C_{70} to C_{110} . The α -isoprene unit containing the hydroxyl groups is always saturated and *cis*, whereas the ω -isoprene unit and two internal isoprene units are *trans* with $n-4$ internal *cis*-isoprene units. A typical dolichol is thus $C_{100}H_{163}$ ($n=20$). Pig liver contains about 100mg of mixed dolichols ($n=17-22$)/kg.

Many plants contain significant quantities of deca-, undeca- and dodeca-prenols in which the ω -isoprene residue and three internal isoprene residues are *trans*, whereas the remainder, including the α -isoprene residue, are *cis*. Prenols from silver-birch wood include betulaprenol-7 ($n=7$), in which the ω -isoprene residue and the two other isoprene residues are known to be *trans*. An undecaprenol from *Lactobacillus plantarum* also has three *trans*-isoprene residues including the ω -isoprene residue, and eight *cis*-isoprene residues including the α -isoprene residue. *Aspergillus fumigatus* Fresenius polyprenols ($n=18-24$) have three isoprene residues (α , ψ and ω) saturated, and of the internal isoprene residues two are *trans* and the rest ($n=5$) are *cis*. More polyprenols are likely to be discovered.

Biosynthetic studies with stereospecifically double-labelled radioactive mevalonates are consistent with *cis* addition to all-*trans*-farnesyl pyrophosphate or all-*trans*-geranylgeraniol pyrophosphate, followed in some cases by reduction at one or both ends of the molecule.

In bacteria, the work of groups led by Robbins, Strominger, Lennarz, Baddiley and others indicates that undecaprenyl pyrophosphates act as carriers in the synthesis of complex polysaccharides, particularly in cell walls. This is an important advance, and dolichol metaphosphate glucose has been shown by Behrens & Leloir (1971) to act as an intermediate in glucose transfer in liver.

The study of polyprenols affords a good example of advances made possible by newer techniques of separation, and characterization by means of spectroscopic methods. The biosynthetic work owes much to stereospecific labelling, and the carrier role illustrates the confluence of different types of research.

The Liverpool contributions in this field owe much to the leadership shown by Dr. F. W. Hemming and Dr. J. F. Pennock and to the careful work of research students.

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Metabolism of β -Apocarotenals

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β -Apocarotenals are formed during the chemical oxidation of β -carotene. Although apocarotenals were suggested to be the intermediates during the cleavage of β -carotene in animals, so far there has been no proof to support this hypothesis. In the present study metabolism of apocarotenals and their epoxides was undertaken in order to investigate the mechanism of cleavage of the β -ionone-ring-substituted carotenoids.

Various β -apocarotenals were prepared by oxidation of β -carotene with H_2O_2 in the presence of $KMnO_4$. They were separated and purified by chromatography on deactivated alumina columns. The apocarotenals separated were β -apo-6'-carotenal, β -apo-8'-carotenal, β -apo-10'-carotenal and β -apo-12'-carotenal, with absorption maxima in light petroleum at 414, 437, 457 and 470nm respectively. When tested by the rat-growth assay method, they were found to have biopotencies of 38.5, 31.3, 75.9 and 44.2% respectively of that of β -carotene. They also gave rise to vitamin A in rats, and different amounts of vitamin A were found in the liver.

5,6- and 5,8-Monoepoxides of various β -apocarotenals were also prepared and characterized, but these epoxides did not support the growth of earlier claim (Krishna Mallia *et al.*, 1970) that the double bond in the 5,6-position of the β -ionone ring is essential for exerting vitamin A activity.

Krishna Mallia, A., Jacob, J., John, K. V., Lakshmanan, M. R., Jungalwala, F. B. & Cama, H. R. (1970) *Indian J. Biochem.* **7**, 102

Recent Developments in the Study of the Biosynthesis of Carotenoids

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Many of the recent developments in the study of carotenoid biosynthesis in our laboratory were achieved by using various species of stereospecifically labelled mevalonates, and have been reviewed in

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,5R-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

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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).