from [2-¹⁴C]mevalonic acid is labelled only in the methyl carbon shows that the reduction of the Δ^{24} -double bond of desmosterol to form cholesterol, as well as the oxidation of the terminal carbon atom of the side chain of cholesterol, is stereo-specific. If this were not the case, the C-26 and C-27 would become equivalent, and the [¹⁴C]propionic acid removed enzymically from the side chain would be labelled equally in the methyl and carboxyl carbon atoms.

The results from the two experiments, in both of which the pattern of labelling in the cholesterol side chain was retained in the [¹⁴C]propionate, are difficult to explain if the [¹⁴C]propionate was formed by recombination of C₁ and C₂ fragments derived from the side chain, but would be expected if the carbon skeleton of the isopropyl unit was

removed intact as a C₃ fragment. This could be propionic acid itself or, as suggested by Suld *et al.* (1962), the CoA ester of propionic acid.

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A Mechanism for the Biosynthesis of α -Carotene

By T. W. GOODWIN and R. J. H. WILLIAMS

Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth

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In the biosynthesis of terpenoids from the basic C₅ precursor isopentenyl pyrophosphate, the first step is the isomerization of isopentenyl pyrophosphate to dimethylallyl pyrophosphate and this involves the expulsion of a proton from what was originally C-4 of mevalonic acid (eqn. 1). Chain elongation begins by the condensation of a molecule of dimethylallyl pyrophosphate with a second molecule of isopentenyl pyrophosphate to form geranyl pyrophosphate (C₁₀); this also involves the loss of a proton from the carbon atom originating from C-4 of mevalonic acid (eqn. 2); further chain

elongation to farnesyl pyrophosphate (C₁₅) and geranylgeranyl pyrophosphate (C₂₀) involves the same mechanism. In an elegant series of investigations Cornforth and Popják and their colleagues (Cornforth, Cornforth, Donninger & Popják, 1965a; Cornforth, Cornforth, Donninger, Popják & Ryback, 1965b) have synthesized [2-¹⁴C,4*R*-³H]mevalonic acid and [2-¹⁴C,4*S*-³H]mevalonic acid and shown that, in the biosynthesis of squalene (the C₃₀ steroid precursor formed by the dimerization of farnesyl pyrophosphate) in liver, the elimination of each proton arising from C-4 of mevalonic acid is

stereospecific and that all the eliminations have the same specificity; the 4*R*-hydrogen is retained and the 4*S*-hydrogen is eliminated. Dr J. W. Cornforth and Dr G. Popják kindly offered us samples of these substrates to examine the same problem that would arise during the biosynthesis of carotenoids. We demonstrated that the stereospecificity of hydrogen elimination during the biosynthesis of phytoene (the C₄₀ precursor of carotenoids formed by the dimerization of geranylgeranyl pyrophosphate) by carrot-root slices was the same as that found by Cornforth *et al.* (1965*b*) for squalene formation (Goodwin & Williams, 1965*a*).

The availability of [2-¹⁴C,4*R*-³H]mevalonic acid

also made it possible to propose a mechanism for the formation of β-carotene from an acyclic precursor. The acyclic phytoene (I) is stepwise dehydrogenated through phytofluene and ζ-carotene to neurosporene (II), which is then converted into β-zeacarotene (III); this, in turn, probably undergoes further dehydrogenation to γ-carotene and cyclization to β-carotene (IV) (see Goodwin, 1965). It is thus the end of the molecule directly connected to the long chain of conjugated double bonds that cyclizes. The mechanism of cyclization is outlined in eqn. (3). This mechanism requires two tritium atoms (at C-6 and C-6') to be ejected from an acyclic precursor in the formation of the two ring

systems of β -carotene in the presence of $[2-^{14}\text{C}, 4R-^3\text{H}]$ mevalonic acid as substrate; thus if the $^{14}\text{C}/^3\text{H}$ ratio in phytoene is taken as 8:8 (eight isoprenoid residues) then the ratio in β -carotene should be 8:6. This was experimentally demonstrated in β -carotene formed by *Phycomyces blakesleeanus* cultured in the presence of DL- $[2-^{14}\text{C}, 4R-^3\text{H}]$ mevalonic acid (Goodwin & Williams, 1965b).

A pigment that frequently accompanies β -carotene in carotenogenic systems is α -carotene (V), and lutein (3,3'-dihydroxy- α -carotene) is the main xanthophyll of all green leaves. The important question that arises is that of the mechanism of the formation of the α -ionone ring in these compounds; more specifically, is α -carotene formed from β -carotene by the isomerization of one β -ionone ring of β -carotene (eqn. 4) or, according to Anderson & Porter (1962), by the isomerization of the β -ionone ring of β -zeacarotene to α -zeacarotene (eqn. 5), or, alternatively, is the α -ionone ring formed independently, i.e. via α -zeacarotene formed from neurosporene, and not via β -zeacarotene

(Goodwin, 1965). If the first possibility, the isomerization of the β -ionone ring, were correct then with $[2-^{14}\text{C}, 4R-^3\text{H}]$ mevalonic acid as substrate the $^{14}\text{C}/^3\text{H}$ ratio in α -carotene would be the same as that in β -carotene, i.e. 8:6. If the α -ionone ring were formed independently, a probable mechanism would be that indicated in eqn. (6), in which case the tritium at C-6 would be preserved, and the $^{14}\text{C}/^3\text{H}$ ratio in α -carotene would be 8:7. We have investigated this problem by using root slices of a strain of carrot that synthesized relatively large amounts of α -carotene as well as β -carotene.

The carrots were obtained from a local green-grocer and were of Texas (G.P. grand prize) origin. Thin slices (1–2 mm. thick) of the root were placed in a medium consisting of 14 ml. of 0.1 M-tris-HCl buffer, pH 7.25, and DL- $[2-^{14}\text{C}, 4R-^3\text{H}]$ mevalonic acid (a total of $0.55\mu\text{C}$ of ^{14}C and $5.76\mu\text{C}$ of ^3H). Incubation was carried out in a Petri dish for $69\frac{1}{2}$ hr. at $23-25^\circ$. The carrot-root slices were washed free from mevalonic acid and the unsaponifiable matter was extracted by a standard method (Goodwin, 1955). The percentage incorporation of

the substrate into the total unsaponifiable matter was 19.4. The unsaponifiable material was chromatographed on a column (10g.) of alumina (grade III; Woelm), and the squalene, colourless polyenes (phytoene and phytofluene), α -carotene and β -carotene were collected in the light-petroleum (free from aromatic hydrocarbons) fraction (100ml.). This fraction was concentrated to a small volume and rechromatographed on a column of magnesium oxide. Four fractions were collected: (1) phytoene (slight blue fluorescence), first cut from elution with light petroleum (b.p. 40–60°, and free from aromatic hydrocarbons) containing 1% (v/v) of acetone; (2) phytofluene (green fluorescence), second cut with the same solvent; (3) α -carotene (yellow) eluted with light petroleum containing 2–3% (v/v) of acetone; (4) β -carotene (orange) eluted with light petroleum containing 5% (v/v) of acetone. The phytoene was further purified by thin-layer chromatography on Kieselgel with light petroleum (b.p. 60–80°). It was removed from the plate and counted for ^{14}C and ^3H in a Packard Tri-Carb scintillation spectrometer. α -Carotene and β -carotene were purified on two thin-layer systems: (a) Kieselgel with light petroleum (b.p. 60–80°) containing 5% (v/v) of benzene as solvent; (b) alumina with the same solvent. The purified zones were removed from the plate, dissolved in 0.5ml. of toluene, decolorized in sunlight and assayed for ^{14}C and ^3H . The results are given in Table 1.

The results demonstrate that the $^{14}\text{C}/^3\text{H}$ ratio for β -carotene synthesized by carrots approaches

8:6, the same as for that synthesized by *P. blakesleeanus* (Goodwin & Williams, 1965b), whereas that for α -carotene approaches 8:7; further, the ratio for phytoene is, as expected, the same as that for the substrate. In a second experiment the proportions 8:6:7 for the relative ^3H content for phytoene, β -carotene and α -carotene were confirmed, the actual proportions being $8:6.0 \pm 0.15:7.4 \pm 0.1$. It can be concluded therefore that α -carotene is not synthesized from β -carotene, but that it arises separately with the β -ionone residue probably formed by the mechanism outlined in eqn. (3) and the α -ionone residue by the mechanism outlined in eqn. (6). This means that, though β -zeacarotene is a possible precursor of α - and β -carotene via γ -carotene, it is ruled out as a precursor of α -zeacarotene. β -Carotene is also ruled out as a precursor of α -carotene, but the possibility that α -carotene is a precursor of β -carotene remains. It is more reasonable, however, to assume that two enzymes are concerned with cyclization of carotenoids; both cause the formation of the same transient carbonium ion, and the disposition of the substrate on the active sites of the two enzymes causes the ejection of a proton from C-6 (β -ionone formation) or C-4 (α -ionone formation) respectively. The probable pathways from neurosporene to the various cyclic carotenes are indicated in Scheme 1.

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Table 1. Comparison of the incorporation of [^{14}C ,4R- ^3H]mevalonic acid into squalene, phytoene, α -carotene and β -carotene in carrot-root slices

Experimental details are given in the text.

Substance	Radioactivity (disintegrations/min.)		Mean $^3\text{H}/^{14}\text{C}$ radioactivity	$^{14}\text{C}/^3\text{H}$ atomic ratio
[^{14}C ,4R- ^3H]Mevalonic acid	187300	36950	5.08:1	1:1
Squalene (from liver)*	30600	5980	5.12:1	6:6.0
Squalene	1000	195	5.13:1	6:6.1
Phytoene	740	144	5.14:1	8:8.1
α -Carotene	470	110	4.27:1	8:6.7 $\pm$ 0.2†
β -Carotene	1470	410	3.59:1	8:5.7 $\pm$ 0.1†

* Kindly provided by Dr Popják as a control.

† Standard error of counting.

Scheme 1.

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The C-Terminal Peptide of the Heavy Chain of the Rabbit Immunoglobulin IgG

By D. GIVOL and R. R. PORTER

Department of Immunology, St Mary's Hospital Medical School, London, W. 2

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Much progress has been reported recently on the determination of the amino acid sequence of the Bence-Jones proteins (Hilschmann & Craig, 1965; Milstein, 1965; Titani, Whitley, Avogardo & Putnam, 1965), which are equivalent to the light chains of human myeloma globulins (Edelman & Gally, 1962). There has also been a report on the separation of the tryptic peptides of rabbit IgG* heavy chains (Nelson, Noelken, Buckley, Tanford & Hill, 1965). We now report the cleavage of these heavy chains with CNBr (Gross & Witkop, 1962) and the isolation and sequence of the C-terminal peptide that was obtained.

After incubation of the heavy chain (500 mg.) with CNBr (1 g.) in 25 ml. of 70% formic acid (Steers, Craven, Anfinsen & Bethune, 1965) for 24 hr. at 25° and 4° the conversion of methionine into homoserine was 95 and 92% respectively. Electrophoresis of the product on starch gel (urea-formate) revealed six bands in both preparations, whereas the control (heavy chain incubated with 70% formic acid under the same conditions) did not show any significant cleavage. The components that appear on the starch gel could be partially separated by gel filtration on a Sephadex G-100 column (200 cm. × 3 cm.) in N-acetic acid. The first peak (C-1), which comprises about 50% of the material, corresponds in its mobility on starch gel to fragment Fd obtained from papain piece I (Fleischman, Porter & Press, 1963), and it is also very similar to it in its amino

acid composition and in the absence of a free N-terminal amino acid. The second fraction (C-2) was obtained in a very low yield and has not been characterized. Fraction C-3 gave three fast-moving bands on the starch gel, and fraction C-4 gave two bands with a mobility between those of fractions C-3 and C-1. All the bands on the starch gel that were obtained from fractions C-3 and C-4 could be similarly observed in a CNBr digest of papain piece Fc and none of them was observed in the CNBr digest of fragment Fd. The last fraction (C-5) did not stain after the electrophoresis in the starch gel. However, ninhydrin staining, after high-voltage electrophoresis on paper at pH 3.5, showed a single component moving quickly towards the cathode. This peptide could be detected on paper from the CNBr digest of the whole heavy chain or of fragment Fc, but not from that of fragment Fd.

Composition and sequence of the peptide C-5. The peptide C-5, as obtained from the Sephadex G-100 column, appeared to be slightly impure, as judged by analysis, and could be further purified on a Sephadex G-50 column. It gave no colour with Ehrlich's reagent and therefore does not contain tryptophan. Its amino acid composition (Table 1) shows that it contains 18 amino acid residues, including 3 of the 5 histidine residues that are present in fragment Fc. The molecular weight of peptide C-5 from its amino composition is 2062 and it was obtained in 80% yield from the CNBr digest of the heavy chain (16 mg. out of 480 mg.). This peptide does not contain homoserine. Its C-terminal amino acid was found by hydrazinolysis to be glycine, the same as was found for the whole heavy chain. We

* Abbreviations: IgG, immunoglobulin; the nomenclature is that recommended by the World Health Organisation (1964); PTH, phenylthiohydantoin.