oxidized histone f3-DNA, reduced histone f3-DNA and mercurial-blocked histone f3-DNA were compared. When 18.3 μ g. of histone was combined with 10 μ g. of DNA, oxidized histone f3-DNA, reduced histone f3-DNA and mercurial-blocked histone f3-DNA complexes showed 5, 27 and 28% respectively of the priming ability of 10 μ g. of DNA alone.

The fact that monofunctional blocking of the thiol groups of reduced histone f3 does not affect the template activity of the histone f3-DNA complex suggests that any importance of histone thiol groups in relation to the control of synthesis of RNA lies in their ability to form intermolecular disulphide bridges. To obtain some support for this the thiol groups of histone f3 were blocked with the difunctional mercurial bis-3,6-(nitratomercurimethyl)dioxan and the priming ability of the difunctionally blocked histone f3-DNA complex compared with that of the chloromercuribenzenep-sulphonate-blocked histone f3-DNA complex (Table 1). The results are consistent with the thesis that the formation of intermolecular crossbridges between thiol groups modifies the ability of histone f3 to decrease the template activity of DNA.

The present results suggest further ways in which the effectiveness of DNA as a template for RNA synthesis can be modified. Littau, Burdick, Allfrey & Mirsky (1965) showed that the removal of histone f1 from nuclei transformed dense into diffuse chromatin. It has already been pointed out

there is a difference in the thiol content of the two chromatins, and in view of the difference in activity of diffuse and dense chromatin as templates of RNA synthesis it could be that modifications of an already bound histone could also alter its metabolic potential.

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The Stereochemistry of Phytol Biosynthesis in the Leaves of *Ficus elastica* and *Aesculus hippocastanum*

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The biosynthesis of polyisoprenoid compounds involves the isomerization of isopentenyl pyrophosphate to 3,3-dimethylallyl pyrophosphate and the condensation of isopentenyl pyrophosphate with either dimethylallyl pyrophosphate or its higher isoprenologues. In each of these steps the formation of a carbon-to-carbon double bond is preceded by the expulsion of a proton from what was originally C-4 of mevalonic acid.

The stereochemistry of the elimination of this hydrogen atom has been studied with [2-¹⁴C-(4*R*)-

4-³H₁]mevalonate and [2-¹⁴C-(4*S*)-4-³H₁]mevalonate as substrates for polyisoprenoid-synthetase systems. Cornforth, Cornforth, Donninger & Popják (1966) have shown that in the conversion of these substrates into *trans-trans*-farnesyl pyrophosphate and all-*trans*-squalene by a rat-liver preparation all the 4*S*-hydrogen is lost and all the 4*R*-hydrogen is retained. The biosynthesis of squalene and ubiquinone by the mould *Aspergillus fumigatus* (Stone & Hemming, 1965) and of squalene and phytoene by carrot-root preparations (Goodwin

& Williams, 1966) has also been shown to involve the loss of 4*S*-hydrogen and the retention of the 4*R*-hydrogen of mevalonate. By contrast, the biosynthesis of poly-*cis*-isoprene rubber in the latex of *Hevea brasiliensis* involves the removal of the 4*R*-hydrogen and the retention of the 4*S*-hydrogen, showing the rubber to be biogenetically *cis* (Archer *et al.* 1966).

Work involving the use of the doubly labelled mevalonates, synthesized by Cornforth *et al.* (1966), has been extended to study the biosynthesis of phytol. Phytol, the principal acyclic diterpenoid in Nature, was discovered to be the isoprenoid fragment of chlorophyll by Willstätter & Hocheder (1907). It was later characterized by Fischer (1928) and shown to have three saturated isoprene residues at the ω -end of the molecule. Although the chemical configuration of the terminal hydroxyl residue is known to be *trans* (Burrell, Jackman & Weedon, 1959) and may be predicted to be biogenetically *trans*, the biogenesis of the saturated residues is less obvious.

Two batches of four excised very young *Ficus* leaves (28g. wet wt.) were allowed to take up through the stem [2-¹⁴C-(4*R*)-4-³H₁]mevalonate (0.5 μ C) and [2-¹⁴C-(4*S*)-4-³H₁]mevalonate (0.2 μ C) respectively for 6 days. Conditions of high light-intensity and a good current of air were provided to promote transpiration. The leaf tissue in each case was macerated in acetone, an equal volume of water was added and the lipid was extracted with diethyl ether–light petroleum (1:1, v/v). To each whole lipid was added cold unsaponifiable lipid from old *Ficus* leaves, to provide carrier amounts of isoprenoid material.

Each combined lipid was chromatographed on a column of acid-washed alumina (Woelm) weakened with 6% (v/w) of water to Brockmann grade III. Fractions were eluted with diethyl ether–light petroleum (1:24, v/v), diethyl ether–light petroleum (1:4, v/v) and methanol–diethyl ether (1:19, v/v). Squalene was isolated from the diethyl ether–light petroleum (1:24, v/v) fraction by preparative thin-layer chromatography (silica gel G; 600 μ thick) with light petroleum as developing solvent (R_F 0.55). The methanol–ether fraction contained chlorophyll and this was saponified by refluxing with 15% (w/v) KOH in 85% (v/v) ethanol for 30 min. The mixture was cooled, diluted with water and extracted with ether three times. The combined ethereal layers were washed with water, dried over anhydrous Na₂SO₄ and evaporated to dryness under a stream of N₂. Phytol, in this saponified fraction, was purified by preparative thin-layer chromatography (silica gel G, 275 μ thick) with chloroform as developing solvent (R_F 0.48). At this stage, the squalene and phytol samples, each of which chromatographed on thin

layers of silica gel G as single components, were assayed for ³H and ¹⁴C (samples 1). After counting, the sample of squalene was extracted from the scintillation fluid and repurified by preparative thin-layer chromatography as before. The scintillation fluid containing the phytol was mixed with acetic anhydride (A.R.; 5 ml.) and pyridine (A.R.; 3 drops) and left overnight. The mixture was poured into ice-cold 2*N*-HCl (20 ml.) and extracted with diethyl ether–light petroleum (1:1, v/v). The extract was washed free of acid with water, dried over Na₂SO₄ and evaporated to dryness, finally under N₂. The sample of phytyl acetate was purified by preparative thin-layer chromatography (silica gel G, 600 μ thick) with diethyl ether–light petroleum (24:1, v/v) as developing solvent. Phytyl acetate in this system chromatographed at R_F 0.70, well clear of any contaminating material. The samples of squalene and the phytyl acetate were again assayed for ³H and ¹⁴C content (samples 2).

The counting procedure was as described by Stone & Hemming (1965) and efficiencies were 25.5% for ³H and 46.7% for ¹⁴C; less than 0.1% of the ³H counts appeared in the ¹⁴C channel, but 11.3% of the ¹⁴C counts were found in the ³H channel. The disintegrations/min. in each sample are recorded in Table 1. Each ³H/¹⁴C disintegrations/min. ratio was divided by the ³H/¹⁴C ratio of the original mevalonate (counted as the benzohydrylamide) to give those ratios that would have been obtained had the ³H/¹⁴C ratio in the original mevalonate been 1:1. Before insertion in Table 1 these ratios were multiplied by the number of original C-2 atoms of mevalonate that are incorporated into each molecule to give the 'corrected ratio'.

The doubly labelled preparations of squalene and phytol from *Aesculus hippocastanum* were obtained by the same procedure as that used for the *Ficus*. The results are in good agreement. In addition, the *Aesculus* results were checked by assaying ³H and ¹⁴C by the discriminator ratio method (Okita, Kabara, Richardson & LeRoy, 1957). This method gave 'corrected ³H/¹⁴C ratios' 0.31:4 and 4.00:4 respectively for phytol derived from 4*S*- and 4*R*-mevalonate. The corresponding ratios for squalene were 0.35:6 and 5.76:6. These ratios are essentially the same as those in Table 1.

The ratios obtained from the experiments with [2-¹⁴C-(4*R*)-4-³H₁]mevalonate are essentially the same as the reference mevalonic acid benzohydrylamide, indicating that all the 4*R*-hydrogen is retained. In experiments where [2-¹⁴C-(4*S*)-4-³H₁]mevalonate was the substrate, insignificant amounts of ³H were found in the squalene and very little in the phytol.

Thus it appears that in the biosynthesis of phytol

Table 1. *Incorporation of [2-¹⁴C-(4R)-4-³H₁]- and [2-¹⁴C-(4S)-4-³H₁]-mevalonate into phytol and squalene in the leaves of Ficus elasticus and Aesculus hippocastanum*

The values for ¹⁴C and ³H are the actual disintegrations/min. recorded for each sample counted. The corrected ³H/¹⁴C ratio is the ratio assuming that ³H/¹⁴C ratio in the original mevalonate was 1:1 (see the text). The actual ³H/¹⁴C ratio of [2-¹⁴C-(4R)-4-³H₁]mevalonate was 5.92:1 and the actual ³H/¹⁴C ratio of [2-¹⁴C-(4S)-4-³H₁]mevalonate was 3.68:1. The specimens of stereospecifically labelled mevalonates were from the same batch as described and used by Cornforth *et al.* (1966). The ³H/¹⁴C ratios given here are those determined in our Laboratory and differ significantly from those given by Cornforth *et al.* (1966). The difference is most likely due to a difference in the ³H-standards used in the two Laboratories. This circumstance does not affect the results in the least.

	From [2- ¹⁴ C-(4R)-4- ³ H]mevalonate			From [2- ¹⁴ C-(4S)-4- ³ H]mevalonate		
	¹⁴ C (disintegrations/min.)	³ H (disintegrations/min.)	Corrected ³ H/ ¹⁴ C ratio	¹⁴ C (disintegrations/min.)	³ H (disintegrations/min.)	Corrected ³ H/ ¹⁴ C ratio
Phytol from <i>Ficus</i>						
(1)	641	3846	4.06:4	3905	2409	0.66:4
(2)	649	3959	4.12:4	3018	1834	0.66:4
Phytol from <i>Aesculus</i>						
(1)	79456	443132	3.77:4	16585	5363	0.35:4
(2)	76240	438908	3.90:4	15248	5011	0.36:4
Squalene from <i>Ficus</i>						
(1)	54081	331720	6.22:6	13310	522	0.06:6
(2)	54065	322651	6.05:6	13298	408	0.05:6
Squalene from <i>Aesculus</i>						
(1)	23465	140031	6.04:6	6530	1621	0.40:6
(2)	23625	138969	5.96:6	6544	458	0.11:6

there is a stereospecific loss of hydrogen from the C-4 position of mevalonate, indicating that all the isoprenoid units are biogenetically *trans*. This suggests also that the enzymic hydrogenation to saturate the three ω -terminal isoprene residues does not involve removal of olefinic hydrogen atoms. The most likely explanation of these results is that phytol is formed by enzymic hydrogenation of all-*trans*-geranylgeranyl pyrophosphate. However, the results do not preclude the possibility of hydrogenation occurring at earlier stages in the biosynthetic pathway.

The biosynthesis of squalene in *Ficus* and *Aesculus* follows the same stereospecific pattern as in rat liver (Cornforth *et al.* 1966), in carrot-root slices (Goodwin & Williams, 1966) and in *Aspergillus fumigatus* (Stone & Hemming, 1965).

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