sufficient for the conversion of 15s particles into ribosomes and neosomes to take place (Turnock & Wild, 1965).

Kurland & Maaløe (1962) suggested that transfer RNA is involved in the regulation of RNA synthesis by amino acids. On this view, each species of transfer RNA can inhibit RNA synthesis; the inhibition is released by reaction with the appropriate amino acid to form the corresponding species of aminoacyl-transfer RNA. Thus withdrawal of methionine from mutant H 239 leads to cessation of RNA synthesis because transfer RNA specific for methionine must exist predominantly in the unesterified form during starvation; in a relaxed mutant such as 58-161 this control mechanism is defective. The effects of thienylalanine that we have observed are consistent with this hypothesis. The analogue has been shown to be activated non-additively with phenylalanine in crude cell-free systems from *E. coli* (Wolfe & Hahn, 1960) and to replace phenylalanine in protein synthesized during inhibition (Munier & Cohen, 1959). In experiments with the relaxed mutant similar to that in Fig. 1, the incorporation into 5%-trichloroacetic acid-insoluble material of [^{14}C]thienylalanine ($10\text{ }\mu\text{g./ml.}$ and $2\text{ m}\mu\text{C/ml.}$) was proportional to the amount of protein synthesized, as measured by the incorporation of [^{35}S]methionine. However, the rapid inhibition of growth brought about by thienylalanine is probably due not to the production of

inactive proteins but to inhibition of 3-deoxy-D-arabino-heptulosonic acid 7-phosphate synthetase, the enzyme that catalyses the first step in the synthesis of phenylalanine, tyrosine and tryptophan (Ezekiel, 1965). The enzyme present in *E. coli* growing exponentially in minimal medium is strongly inhibited by both phenylalanine and thienylalanine. Incubation with thienylalanine will therefore cause a considerable decrease in the rate of synthesis of both tyrosine and tryptophan; operation of the hypothetical control mechanism outlined above would then lead to inhibition of the synthesis of RNA by the stringent mutant but not in the relaxed strain.

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The Incorporation of [$2\text{-}^{14}\text{C}, 4\text{R-}^3\text{H}_1$]Mevalonate and [$2\text{-}^{14}\text{C}, 4\text{S-}^3\text{H}_1$]Mevalonate into Squalene, Ubiquinone and Ergosterol of *Aspergillus fumigatus* Fresenius

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Intermediate steps in the biosynthesis of polyisoprenoid compounds from mevalonate include the conversion of isopentenyl pyrophosphate into either (a) dimethylallyl pyrophosphate or (b) the isoprenyl derivatives of dimethylallyl pyrophosphate (Scheme 1). Step (a) involves the addition of a proton to what was originally C-2 of mevalonic acid followed by the removal of a proton from what was originally C-4 of mevalonic acid. In step (b) an isoprenyl ion is added instead of a proton.

The work reported by Cornforth, Cornforth,

Donninger & Popják (1965a), Cornforth, Cornforth, Donninger, Popják & Ryback (1965b) and Popják (1965) on the biosynthesis of *trans-trans*-farnesyl pyrophosphate and all-*trans*-squalene shows that the elimination of the proton from what was originally C-4 in mevalonate is stereospecific. When either [$2\text{-}^{14}\text{C}, 4\text{R-}^3\text{H}_1$]- or [$2\text{-}^{14}\text{C}, 4\text{S-}^3\text{H}_1$]-mevalonate was incubated with liver preparations, counting of the farnesol and squalene isolated revealed that the hydrogen eliminated was that which, when replaced by a heavy hydrogen isotope, would confer the 4S

Scheme 1.

configuration on the original mevalonate. The 4*R*-³H was not removed. It is clear that the presence of ³H in an isoprenoid unit formed from 4*R*-³H-labelled mevalonate together with the absence of ³H from the same unit when 4*S*-³H-labelled mevalonate is the substrate is evidence that the unit is biogenetically *trans*. Alternatively, loss of 4*R*-³H and retention of 4*S*-³H of the tritiated mevalonate, such as occurs in the biosynthesis of the poly-*cis*-isoprene rubber (Archer & Barnard, 1965), is characteristic of biogenetically *cis*-isoprene units.

At the suggestion of Dr Popják we have extended the studies with doubly labelled mevalonate to the biogenesis of the isoprenoid compounds of *Aspergillus fumigatus* Fresenius (LSHTMA46).

The mould was grown at 28° in five horizontal 11. Roux bottles, each containing 200ml. of Raulin-Thom medium. On the fourth day of growth a total of either 2.73 μmoles of [2-¹⁴C, 4*R*-³H₁]mevalonate (0.852 μC of ¹⁴C and 4.961 μC of ³H/μmole) or 2.41 μmoles of [2-¹⁴C, 4*S*-³H₁]mevalonate (0.772 μC of ¹⁴C and 2.805 μC of ³H/μmole) were injected into the medium. The thallus was harvested on the ninth day of growth, by which time the yield of isoprenoid compounds is maximal (K. J. Stone & F. W. Hemming, unpublished work), and macerated in 2 vol. of methanolic 2.5% (w/v) pyrogallol. Then

1 vol. of 60% (w/v) KOH was added and the mixture was boiled under reflux for 45 min., after which the unsaponifiable lipid was recovered by extraction with diethyl ether. Chromatography of this on a column of alumina (for details see Butterworth, Draper, Hemming, Pennock & Morton, 1963) yielded a hydrocarbon fraction (A), a fraction (B) containing ubiquinone and a fraction (C) containing ergosterol.

Squalene was isolated from fraction (A) by chromatography on thin layers of silica gel G with light petroleum (40–60°) as solvent. The squalene derived from [2-¹⁴C, 4*R*-³H₁]mevalonate was further purified by forming the thiourea clathrate and the hexahydrochloride. The latter was recrystallized three times.

Fraction (B) was acetylated and the ubiquinone separated from alcohol acetates by alumina chromatography (see Butterworth *et al.* 1963). Ubiquinone was isolated from the appropriate fraction by preparative thin-layer chromatography with methanol–benzene (1:99, v/v) as solvent. Further purification involved reversed-phase partition paper chromatography with liquid paraffin as stationary phase and water–*NN*-dimethylformamide (3:97, v/v) as mobile phase. The ubiquinone was extracted from the paper with diethyl ether and separated from contaminating paraffin by

alumina column chromatography. Before counting, the ubiquinone was rendered colourless by reduction with NaBH_4 .

The ergosterol in fraction (C) from the $[2\text{-}^{14}\text{C}, 4R\text{-}^3\text{H}_1]\text{mevalonate}$ experiment was purified by four crystallizations from ethanol containing a little water, by which time the $E_{\text{cm}}^{1\%}$ at $282\text{m}\mu$ was close to the theoretical value. Further purification involved preparative thin-layer chromatography with ethyl acetate–benzene (9:41, v/v) as mobile phase. The ergosterol derived from the $[2\text{-}^{14}\text{C}, 4S\text{-}^3\text{H}_1]\text{mevalonate}$ was isolated from fraction (C) by preparative thin-layer chromatography.

All samples were assayed for ^3H and ^{14}C content in a Tri-Carb liquid scintillation spectrometer with toluene containing 2,5-diphenyloxazole (0.5%) and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene (0.03%) as the scintillation fluid. Counting was by the screening method (Stein & Stein, 1962; Okita, Kabara, Richardson & Le Roy, 1957) and efficiencies were 21.29% for ^3H and 29.5% for ^{14}C . Samples were usually counted for 5–10 min., three times. The disintegrations/min. in each sample counted are recorded in Table 1. Each $^3\text{H}/^{14}\text{C}$ disintegrations/min. ratio was divided by the $^3\text{H}/^{14}\text{C}$ ratio of the original mevalonate to give the ratios that would have been obtained if the $^3\text{H}/^{14}\text{C}$ ratio in the mevalonate had 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 of the compound in question. The final ratio thus obtained is the 'corrected ratio' appearing in Table 1.

Squalene derived from $[2\text{-}^{14}\text{C}, 4R\text{-}^3\text{H}_1]\text{mevalonate}$ gave a $^3\text{H}/^{14}\text{C}$ ratio very close to 6:6, showing that each isoprene unit contained a ^3H atom that was originally in the $4R$ configuration in the mevalonate. Essentially no ^3H was incorporated from $[2\text{-}^{14}\text{C}, 4S\text{-}^3\text{H}_1]\text{mevalonate}$. Thus, as expected from work with other organisms (Cornforth *et al.* 1965*a,b*; Popják, 1965; Goodwin & Williams, 1965), all of the isoprene units in squalene are biogenetically *trans*.

Ubiquinone from *Aspergillus fumigatus* contains ten isoprene units in its side chain (Burgos, Butterworth, Hemming & Morton, 1964; Lavate & Bentley, 1964). In ubiquinone from other natural sources the stereochemistry of the isoprene unit next to the quinone nucleus is uncertain; the terminal unit is *cis-trans* and the remaining eight are known to be stereochemically *trans* (Bates & Gale, 1960; Bates, Carnighan, Rakutis & Schauble, 1962). The $^3\text{H}/^{14}\text{C}$ ratios for ubiquinone (Table 1) indicate that all ten isoprene units are biogenetically *trans*. The ratios rule out the possibility that the isoprene units are incorporated in the *cis* configuration followed by isomerization to the *trans* configuration.

The $^3\text{H}/^{14}\text{C}$ ratio for ergosterol has an important bearing on the mode of introduction of the extra methyl group in this sterol. Cholesterol formed from $[2\text{-}^{14}\text{C}, 4R\text{-}^3\text{H}_1]\text{mevalonate}$ by liver preparations retains ^3H in positions 17, 20 and 24, and ^{14}C in positions 1, 7, 15, 22 and 26 (or 27), giving a corrected ratio 3:5 (Popják, 1965). If one assumes that in principle the cyclization of squalene to phytosterols (see, e.g., Battersby & Parry, 1964) is essentially the same as in the formation of cholesterol in animal

Table 1. Incorporation of $[2\text{-}^{14}\text{C}, 4R\text{-}^3\text{H}_1]\text{-}$ and $[2\text{-}^{14}\text{C}, 4S\text{-}^3\text{H}_1]\text{-mevalonate}$ into squalene, ubiquinone and ergosterol of *Aspergillus fumigatus* Fresenius

The values for ^{14}C and ^3H are the actual disintegrations/min. recorded for each sample counted. The corrected $^3\text{H}/^{14}\text{C}$ ratio is the ratio assuming that $^3\text{H}/^{14}\text{C}$ ratio in the original mevalonate was 1:1 (see the text). The actual $^3\text{H}/^{14}\text{C}$ ratio of $[2\text{-}^{14}\text{C}, 4R\text{-}^3\text{H}_1]\text{mevalonate}$ was 5.83:1, and the actual $^3\text{H}/^{14}\text{C}$ ratio of $[2\text{-}^{14}\text{C}, 4S\text{-}^3\text{H}_1]\text{mevalonate}$ was 3.63:1.

	From $[2\text{-}^{14}\text{C}, 4R\text{-}^3\text{H}_1]\text{mevalonate}$			From $[2\text{-}^{14}\text{C}, 4S\text{-}^3\text{H}_1]\text{mevalonate}$		
	^{14}C (disintegrations/min.)	^3H (disintegrations/min.)	Corrected $^3\text{H}/^{14}\text{C}$ ratio	^{14}C (disintegrations/min.)	^3H (disintegrations/min.)	Corrected $^3\text{H}/^{14}\text{C}$ ratio
Squalene after thin-layer chromatography	4525	26100	5.94:6	6510	298	0.08:6
Squalene thiourea clathrate	2290	12746	5.73:6			
Squalene hexahydrochloride	632	3523	5.74:6			
Ubiquinone after thin-layer chromatography	332	1932	9.98:10	997	162	0.45:10
Ubiquinone after paper chromatography	963	5850	10.43:10	1008	157	0.43:10
Ergosterol (crystalline)	2003	6980	2.99:5			
Ergosterol after thin-layer chromatography	3263	11500	3.02:5	5156	408	0.11:5

tissues, it follows that the ratio 3:5 obtained for ergosterol (Table 1) indicates the presence of ^3H on C-24. This same carbon atom carries an extra methyl group in ergosterol, and Lederer (1964) has considered a number of schemes for the alkylation step. These involve intermediates with the following arrangements in the side chain:

which are then converted into ergosterol carrying the methyl group on C-24 thus:

Our results deny any scheme that involves intermediates containing the groups (i) and (ii) in the side chain, for these would require the absence of ^3H on C-24 in ergosterol derived from 4*R*- ^3H -labelled mevalonate. However, intermediates of types (iii)

and (iv) are allowed, as schemes involving these do not necessarily require loss of the ^3H on C-24. This argument holds whether the compound that is alkylated is desmosterol (Lederer, 1964) or, as seems more likely, some compound closely related to lanosterol (Goad & Goodwin, 1965).

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Plastochromanol in the Leaves of *Hevea brasiliensis*

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The latex of the rubber tree, *Hevea brasiliensis*, has been examined and shown to contain α -, γ - and δ -tocotrienol in relatively large amounts (P. J. Dunphy, K. J. Whittle, J. F. Pennock & R. A. Morton, unpublished work). The toco-

trienols were present both free and esterified, γ -tocotrienol being the predominant form in both cases. Since α -tocopherol is normally the main tocopherol-type compound in green leaves, it was decided to investigate leaves of *Hevea*.