COMMUNICATIONS TO THE EDITOR

A RACEMASE FOR THREONINE IN *ESCHERICHIA COLI*

Sir:

Growth studies with biochemically deficient mutants of yeast[1] and bacteria[2,3] requiring threonine have suggested that some strains possess a threonine racemase.

I wish to report the demonstration in a cell-free extract from *Escherichia coli* of an enzyme capable of transposing groups on both asymmetric carbon atoms of threonine to convert D-threonine to L-threonine, for which ATP or yeast or muscle adenylic acid serves as a cofactor. In rather limited attempts it has not been possible to demonstrate a vitamin B_6 requirement.

Cells of *Escherichia coli*, strain K-12, grown on the medium of Davis and Mingioli[4] with 0.5% glucose were dried *in vacuo* over phosphorus pentoxide, ground with alumina and extracted with 0.01 *M* phosphate buffer at *p*H 7.1. The activity of the extracts toward threonine was determined after 16 hours dialysis against 0.01 *M* phosphate buffer, *p*H 7.1, at 2–4°. On storage the extracts lost the ability to deaminate L-threonine but retained that of converting L-threonine to the D-form and *vice versa*.

The formation of L-threonine starting from D-threonine was demonstrated by microbiological assay using a fastidious L-threonine-requiring mutant of *Escherichia coli*, ML52, and quantitatively estimated by ammonia liberation with an L-threonine deaminase from *Clostridium welchii*. The welchii enzyme proved to be specific for L-threonine and will not attack the L-allo, D- or D-allo forms. The conversion of L-threonine to D-threonine, followed by the disappearance of L-threonine, appeared to occur at the same rate as the reverse transformation.

TABLE I

CONVERSION OF D-THREONINE TO L-THREONINE BY EXTRACTS OF *Escherichia coli*, STRAIN K-12

The complete reaction system contained K-12 extract (3.5 mg. extract protein), 50 μM. of D-threonine, 10 μg of pyridoxal phosphate, welchii deaminase (2.4 mg. protein), 5.0 μM. of ATP and 1.0 ml. of 0.20 *M* phosphate buffer, *p*H 7.8; incubed at 37° for 3 hours in tubes flushed with nitrogen and stoppered; reaction stopped with 0.2 ml. of 50% trichloroacetic acid and precipitate removed by centrifugation; ammonia determined on supernatant by micro-Kjeldahl procedure.

Reaction system	μM. of NH_3
Complete	10.2
Minus K-12 extract	0.2
Minus D-threonine	1.2
Minus ATP	3.8
Minus pyridoxal phosphate	10.5
Adenosine-5-phosphate for ATP	10.7
Minus welchii deaminase	6.4[a]

[a] At end of 3-hr. incubation an aliquot was removed, from which K-12 enzyme was precipitated with 6 *N* HCl, precipitate removed by centrifugation, the supernatant returned to *p*H 7.8 with NaOH and welchii enzyme added for 90 min. at 37°.

The presence of welchii deaminase during the enzymatic racemization of D-threonine accelerated this conversion by removing L-threonine as it was formed. In such a system the disappearance of threonine and the appearance of α-ketobutyric acid could be followed by colorimetric estimation after paper chromatography[5,6] (Table I).

Adenosine-3-phosphate stimulates racemization as effectively as ATP and muscle adenylic acid. The Christman synthetic coenzyme for aspartic deaminase[7] has no effect. The quantity of enzyme in K-12 is depressed sharply by the presence in the growth medium of D-threonine. Fractionation of the crude extract for more precise characterization of the enzyme is in progress.

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TANTALUM SUBCHLORIDE[1]

Sir:

Among the subhalides mentioned in the literature, tantalum subchloride is one of the few whose existence has neither been confirmed nor denied. According to Ruff and Thomas,[2] thermal decomposition of the dichloride *in vacuo* leads to a pyrophoric compound with the empirical formula Ta_2Cl. We have attempted to prepare this compound by the procedure outlined by these authors.

Aluminum powder and tantalum pentachloride were mixed in stoichiometric proportions according to the equation

$$3TaCl_5 + 2Al = 2AlCl_3 + 3TaCl_3$$

and heated in evacuated Pyrex tubes for 100 hours at 300 to 350°. The aluminum trichloride was removed by sublimation, and the product, in all probability a mixture of $TaCl_4$ and $TaCl_3$, was transferred to small tantalum crucibles for subsequent thermal reduction. All manipulations were made in dry argon.

The X-ray powder diagram of the initial reduction product was extremely complex, but did not indicate the presence of aluminum, aluminum oxide or tantalum.

Thermal decomposition of this product at 600 to 700° produced a compound whose properties were identical to those reported by Ruff and Thomas for

(1) Under research grant from the National Research Corporation, Cambridge, Mass.
(2) O. Ruff and F. Thomas, *Z. anorg. Chem.*, **148**, 1 (1925).

the dichloride. The powder diagram was relatively simple and easily could be recognized in mixtures. The pattern was unchanged when the compound was heated *in vacuo* at any temperature up to 700°, regardless of the time of heating. Above 700°, however, a further disproportionation took place, and the tantalum pattern became evident in the powder diagrams with increasing predominance until only the metal remained. In none of the powder diagrams were there lines other than those of tantalum or those attributed to the dichloride. Moreover, none of these products was pyrophoric, but all appeared stable in dry air.

In all of these experiments considerable care was exercised with respect to purity of the reagents, and in the exclusion of oxygen. Nevertheless we were unable to produce the pyrophoric material described by the previous investigators. We have concluded that tantalum subchloride, if it does exist, cannot be prepared by the thermal decomposition of the higher chlorides.

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UTILIZATION OF BRANCHED CHAIN ACIDS IN CHOLESTEROL SYNTHESIS

Sirs:

It has been suggested[1] that in steroid biogenesis isoprenoid intermediates are formed from three molecules of acetate and that a likely product of this condensation is β-methyl β-hydroxyglutarate which could furnish five carbon intermediates on decarboxylation.[2] In support of these views it has previously been found that isovaleric acid[3] and the polyisoprenoid hydrocarbon squalene[4] are considerably more effective as carbon sources for cholesterol than acetate. We have now synthesized the following branched chain acids: 3-C^{14}-β-methyl-β-hydroxyglutaric acid, 3-C^{14}-*cis*-β-methylglutaconic acid, 3-C^{14}-β-hydroxyisovaleric acid and 3-C^{14}-β-dimethylacrylic acid (DMA) and tested their utilization in cholesterol synthesis. The sodium salts of the isotopically labeled acids were mixed with a stock diet and fed to rats at a level of 0.25 mmole. per 100 g. of rat per day for two days. Cholesterol was isolated from the livers and analyzed for C^{14}. All of these acids were found to furnish carbon for cholesterol synthesis, but with the exception of DMA, were less efficient than acetate. On the other hand, after the feeding of DMA the specific activity (S.A.) of liver cholesterol was 4–6 times greater than after the feeding of equimolar quantities of acetate. This result has prompted us to investigate the distribution of DMA carbon in the steroid molecule. If six isoprene units condense to form squalene (*cf.* Fig. 1) which cyclizes to the steroid structure,[5] then

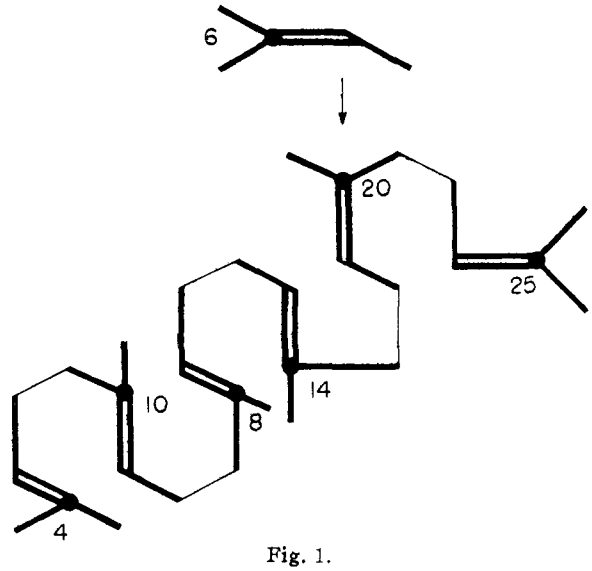

Fig. 1.

cholesterol derived from 3-C^{14}-DMA should contain six labeled carbon atoms: C_4, C_8, C_{10}, C_{14}, C_{20} and C_{25}. Each of these should have a S.A. 27/6 or 4.5 times that of the total molecule. If on the other hand DMA had first been degraded to acetate, then twelve sterol carbons should be labeled and have a S.A. 2.25 times that of cholesterol.[6] Degradation of cholesterol formed from DMA showed (see Table) that C_{10} and C_{25} had specific

TABLE I

DISTRIBUTION OF C^{14} IN CHOLESTEROL FORMED FROM 3-C^{14}-β-DIMETHYLACRYLIC ACID, C.P.M.* AS INFINITELY THICK SAMPLES OF BARIUM CARBONATE.

	Found	Calculated	
		A[a]	B[b]
Cholesterol	100	..	..
C_{10}	440	450	225
C_{25}	360	450	225

[a] Calcd. for six labeled C atoms per molecule. [b] Calcd. for twelve labeled C atoms per molecule.

activities which are 80 and 95%, respectively, of those which the hypothesis of five carbon intermediates predicts. These results preclude prior transformation of DMA to acetate and also to acetoacetate since Blecher and Gurin[7] have shown that in cholesterol formed from 1-C^{14} or 4-C^{14} acetoacetate the C^{14} distribution is indistinguishable from that given by acetate. The biosynthesis of DMA first detected in guayule leaves,[8] has now been demonstrated in liver homogenates by Rudney[9] and a similar observation has been made in this laboratory. The distribution of acetate carbon in DMA as observed by Rudney is identical with that in the terminal five carbon atoms of the cholesterol side chain[10] and hence in full accord with the rule assigned here to DMA. Since in the

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