respectively, of that for the control, *cf.* 450% for III. However, spectroscopic and chromatographic evidence indicated a small increase in the absolute amount of lycopene for I and II, compared with III and the control. The fluorescence of I- and II-treated samples was noticeably blue-green, whereas the others were characteristically green.

In summary, production of β-carotene can be markedly influenced in a short period (5 to 20 hours) and of lycopene to a minor extent by use of compounds presumably providing terminal groups in the carotenoid molecule. Results are not incompatible with a tentative scheme postulated by Garton, Goodwin and Lijinsky.[4]

(4) G. A. Garton, T. W. Goodwin and W. Lijinsky, *Biochem. J.*, **49**, 154 (1951.).

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RECEIVED MAY 9, 1952

LIPOTHIAMIDE AND ITS RELATION TO A THIAMIN COENZYME REQUIRED FOR OXIDATIVE DECARBOXYLATION OF α-KETO ACIDS

Sir:

We wish to report evidence indicating that lipothiamide, a catalytic agent present in cellular extracts, is the amide of α-lipoic acid and thiamin, and suggesting that this moiety is part of a thiamin coenzyme required for oxidative decarboxylation of α-keto acids.

Natural materials contain α-lipoic acid, a catalytic factor required for pyruvate oxidation,[1] in bound condition. Acidic or basic hydrolysis degrades the bound material predominantly to α-lipoic acid, whereas enzymatic hydrolysis releases "complex" forms of α-lipoic acid.[1,2]

Our study of α-lipoic acid complexes was directed toward first obtaining an organism which required such a complex for growth. This requirement was exhibited by a mutant strain of *Escherichia coli*, developed by irradiating wild-type cells and isolating the mutant, using the penicillin technique.

Whereas the mutant did not respond to α-lipoic acid in the presence of all other known vitamins and growth factors, it did respond to the α-lipoic acid complexes present in cellular extracts, or to substances formed by incubating α-lipoic acid and thiamin with wild-type *Escherichia coli* or *Streptococcus lactis* cells.

Bioautographs of the incubation mixture revealed the presence of two active principles, possessing R_f values identical with those of two α-lipoic acid complexes present in cellular extracts. Both biosynthetic "conjugates" gave a negative thiochrome test and a positive azo test. These observations suggested that the two conjugates contain α-lipoic acid and thiamin conjugated through an amide linkage.

Chemical synthesis studies support this conclusion. Reaction mixtures obtained by heating α-lipoic acid and thiamin *in vacuo*, and by treating thiamin with the acid chloride of α-lipoic acid, produced maximum growth of the mutant at a level of 2.5×10^{-6} γ per cc. of culture medium. In a similar manner, preparations active in catalytic amounts for the mutant have been obtained by condensing α-lipoic acid or its acid chloride with 2-methyl-5-ethoxymethyl-6-aminopyrimidine, thiamin monophosphate, and thiamin pyrophosphate, respectively.

The active principles in chemical preparations of lipothiamide and its monophosphate possessed R_f values identical with the two active principles produced biosynthetically and with two of those present in cellular extracts. Furthermore, treatment of lipothiamide monophosphate or pyrophosphate with intestinal phosphatase degrades these two factors to lipothiamide, as revealed with bioautographs.

An investigation of possible coenzymatic functions of lipothiamide and its phosphorylated derivatives has revealed: (1) resting cell suspensions of the mutant will not oxidize either pyruvate or α-ketoglutarate unless one of these conjugates is added; (2) the growth requirement of the organism for lipothiamide can be completely by-passed by supplying the products (acetate, citrate and succinate) of these two blocked reactions. *All* three products, however, must be present. The induced mutation apparently destroys the capacity of the organism to conjugate α-lipoic acid and the pyrimidine moiety of thiamin, resulting in a deficiency of an α-keto acid coöxidase required for the reactions producing the three essential metabolites.

(1) I. C. Gunsalus, *et al.*, *J. Biol. Chem.*, **194**, 859 (1952).
(2) L. J. Reed, *et al.*, *ibid.*, **192**, 851 (1951).

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RECEIVED MAY 5, 1952

LYSINE BIOSYNTHESIS IN TORULOPSIS UTILIS[1]

Sir:

In an isotopic study of biosynthetic mechanisms in *Torulopsis utilis*, data from yeast grown on methyl- and carboxyl-labeled acetate have provided a clue to the pathway of lysine synthesis in this organism. Cultural details and other experimental procedures have already been described.[2] The lysine was isolated from the cell material by standard methods, and degraded by the following steps: decarboxylation with ninhydrin; oxidation to δ-aminovaleric acid; degradation of the latter by the Schmidt reaction[3] to carbon dioxide and 1,4-diaminobutane; oxidation of the latter to succinic acid; isolation of succinate carboxyls by the Schmidt reaction. Another sample of lysine was oxidized to glutaric acid and the glutarate carboxyls obtained by the Schmidt reaction. These procedures yielded individual activities of carbons 1, 2, 3 and 6, and the averages of carbons 4 and 5.

(1) Aided by grants from the U. S. Atomic Energy Commission, Contract No. AT(30-1)777, and by an institutional grant from the American Cancer Society.

(2) M. Strassman and S. Weinhouse, THIS JOURNAL, **74**, 1726 (1952).

(3) E. F. Phares, *Arch. Biochem. Biophys.*, **33**, 173 (1951); we are greatly indebted to Dr. Phares for supplying us with this method prior to publication.