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.).

G. MACKINNEY
T. NAKAYAMA
C. D. BUSS
C. O. CHICHESTER
DIVISION OF FOOD TECHNOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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).

BIOCHEMICAL INSTITUTE AND
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF TEXAS, AND
CLAYTON FOUNDATION FOR RESEARCH
AUSTIN, TEXAS
LESTER J. REED
BETTY G. DEBUSK

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.

The observed distributions are shown in the table. Two features are of great interest; first, the distribution of activities in carbons 1 and 2 immediately suggested the incorporation of an intact acetate molecule in these positions; second, the distribution of activity in carbons 3 to 6, assuming equal activities in carbons 4 and 5, is very close to that expected in the succinate moiety (carbons 2 to 5) of α-ketoglutarate arising from acetate via the citric acid cycle (see table). The theoretical basis for the calculation of acetate activities in citric acid cycle components has been described.[2] Despite certain deviations the activity patterns are remarkably close. The higher-than-calculated activities in the lysine carbons 1 and 2 are probably due to incomplete equilibration of acetate with citric acid cycle components, and the presence of low activity in carbons 1 and 6 of lysine formed from methyl-labeled acetate is probably due to recycling,[4] whereby methyl activity migrates to the carboxyl of acetate.

DISTRIBUTION OF ACETATE CARBONS IN LYSINE

Values are specific activities based on 100 for over-all activity of lysine

Lysine carbon number[a]	Precursor: Acetate methyl Observed	Acetate methyl Calcd.	Acetate carboxyl Observed	Acetate carboxyl Calcd.
1	18	0	360	300
2	206	150	0	0
3	121	150	7	0
4	116	150	−11	0
5	116	150	−11	0
6	7	0	226	300

[a] Numbering begins with carboxyl carbon. The actual specific activities of the lysine hydrochlorides were: from methyl-labeled acetate, 6380 c./m. per standard dish; from COOH-labeled, 5708 c./m.

In speculating on a mechanism by which such a distribution might be achieved it has occurred to us that acetate may condense with α-ketoglutarate to yield a homolog of citric acid, which, by undergoing a series of reactions analogous to citrate in the citric acid cycle, should yield "homonisocitrate," oxaloglutarate, α-ketoadipate, and ultimately α-aminoadipic acid. The formation of lysine from α-aminoadipic acid, presumably by the same sequence of reactions involved in ornithine formation from glutamate, has been suggested by the *Neurospora* studies of Mitchell and Houlahan.[5] The existence of such a "homocitric" acid cycle represents an intriguing possibility which is now being investigated further.

An alternate possibility is that acetate condenses with an unsymmetrical succinate, possibly succinyl-CoA, but this is regarded as less likely since it does not as easily account for the formation of α-aminoacidic acid.

FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE AND THE INSTITUTE FOR CANCER RESEARCH, AND THE DEPT. OF CHEMISTRY
TEMPLE UNIVERSITY, PHILADELPHIA MURRAY STRASSMAN
SIDNEY WEINHOUSE

RECEIVED MAY 29, 1952

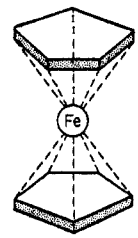
(4) K. F. Lewis and S. Weinhouse, THIS JOURNAL, **73**, 2500 (1951).

(5) H. K. Mitchell and M. B. Houlahan, *J. Biol. Chem.*, **174**, 883 (1948).

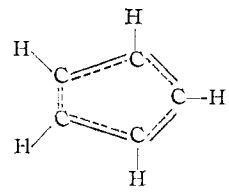
A NEW AROMATIC SYSTEM

Sir:

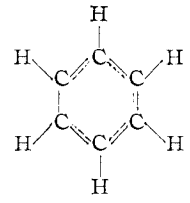
Recently we have assigned to iron biscyclopentadienyl[1] the structure I.[2] It is clear that this molecule contains two rings, each of five equivalent C–H groupings. It seemed likely that the equivalent ring bonds connecting these C–H groups should be of effective order greater than 1. Considering one of the carbocyclic rings alone, then, the situation might be represented as in II. This expression calls to mind the very similar circumstances obtaining in the case of benzene (III).



I



II



III

We were led by these considerations to the view that iron biscyclopentadienyl might behave as an aromatic substance. We now wish to record experiments which demonstrate typically aromatic properties of this unique iron compound, for which we propose the name *ferrocene*.

In spite of its high degree of formal unsaturation, ferrocene does not possess properties typical of polyolefinic substances. It does not react with maleic anhydride in boiling benzene, and is not hydrogenated under normal conditions over reduced platinum oxide. Its resistance to the action of acidic reagents, and its great thermal stability have been mentioned previously.[1,2]

When ferrocene in carbon bisulfide was treated with acetyl chloride in the presence of aluminum chloride, it was very smoothly converted to a beautifully crystalline red **diacetyl derivative,** m.p. 130–131° (calcd. for $C_{14}H_{14}O_2Fe$: C, 62.52; H, 5.22. Found: C, 62.56; H, 5.40), which formed a crystalline **dioxime** (decomposes above 200°; calcd. for $C_{14}H_{16}N_2O_2Fe$: C, 56.02; H, 5.37; N, 9.34; Fe, 18.40. Found: C, 56.13; H, 5.31; N, 9.53; Fe, 18.61). Similarly, β-chloropropionyl chloride gave **bis-β-chloropropionylferrocene,** m.p. 117–121° (calcd. for $C_{16}H_{16}O_2Cl_2Fe$: C, 52.34; H, 4.39; Cl, 19.32; Fe, 15.94. Found: C, 52.84; H, 4.59; Cl, 19.32; Fe, 16.04), and **bisacryloylferrocene,** m.p. 71–71.5° (calcd. for $C_{16}H_{14}O_2Fe$: C, 65.33; H, 4.82. Found: C, 64.92; H, 5.15). With

(1) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951); S. A. Miller, J. A. Tebboth and J. F. Tremaine, *J. Chem. Soc.*, 632 (1952).

(2) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2125 (1952).