TABLE II

REACTIONS CATALYZED BY THIOLASE

110-fold purified heart thiolase (specific activity 37 when prepared). Final concentrations: 0.01 *M* TRIS–HCl buffer *p*H 8.1, 1.0×10^{-4} *M* AcAc–SR, 2.0×10^{-4} *M* R–SH, 5×10^{-3} *M* $MgCl_2$, 1.5×10^{-3} *M* Ac–S-pantetheine.

Reaction	Specific activity
AcAc–S–CoA + CoA–SH	4.05
AcAc–S–Pantetheine + CoASH	3.02
AcAc–S–Pantetheine + pantetheine	0.80
AcAc–S–CoA + pantetheine	.79
2-Ac–S–pantetheine	.09[c]
Ac–S–Pantetheine[a] + CoA–SH	.15
AcAc–S–Ac–N–βMEA[b] + HS–AcN–βMEA[b]	0

[a] 1.0×10^{-3} *M*; *p*H 7.5; spontaneous rate was 0.2 times the enzymatic one. [b] 3.3×10^{-3} *M*. [c] Indirect assay[11] *p*H 7.1. By direct assay (*p*H 9.2) 0.035.

In liver extracts pantetheine did not substitute for CoA in the aceto-CoA-kinase reaction[13] nor in the butyro-CoA-kinase reaction.[14] However, the synthesis of Ac-S-pantetheine[15] from Ac, ATP and pantetheine occurs in pigeon liver extract provided catalytic amounts of CoA-SH are added. This indicated the coupling of the aceto-CoA-kinase (reaction 7) with reaction 8 (the reverse of reaction 5):

$$\text{Ac} + \text{CoA-SH} + \text{ATP} \rightleftharpoons \text{Ac-S-CoA} + 5\text{-AMP} + \text{PP} \quad (7)$$

$$\text{Ac-S-CoA} + \text{pantetheine-SH} \rightleftharpoons \text{Ac-S-pantetheine} + \text{CoA-SH} \quad (8)$$

The reversibility of reaction 8 was shown by coupling it with the crystalline citrate condensing enzyme and purified malic dehydrogenase[16] and observing a CoA-SH dependent reduction of DPN in the presence of Ac-S-pantetheine, L-malate, and pigeon liver extract. The specific activity was 0.014. The enzyme catalyzing reaction 8 belongs to the class of thioltransacetylases recently described in pigeon liver by Brady and Stadtman[17] who did not test pantetheine. Pigeon liver extracts were also found to catalyze a CoA-SH dependent hydration of CROT-S-pantetheine to *d*-BOH-S-CoA (specific activity 0.0056) indicating the presence of a thioltranscrotonylase catalyzing reaction 9

$$\text{CROT-S-pantetheine} + \text{CoA-SH} \rightleftharpoons \text{CROT-S-CoA} + \text{pantetheine-SH} \quad (9)$$

Since liver also catalyzes a stepwise conversion of sorbyl-S-CoA through β-ketohexenoyl-S-CoA to CROT-S-CoA and Ac-S-CoA,[18] the exact relation of the thioltransacylases present in liver extract to thiolase-type enzymes remains to be determined.

It would seem that, as with crystalline crotonase, the pantetheine thioesters serve as substrates at physiological concentrations for the enzymes of the CoA fatty acid cycle. The demonstration of enzymes which synthesize pantetheine thioesters is further evidence for the occurrence of a pantetheine fatty acid cycle. Unlike the CoA cycle, the pantetheine cycle is not directly linked to the citric acid cycle; and, since crotonase appears to be the rate-limiting step in this cycle, it may play a role in isoprenoid synthesis. The relative amount of CoA and pantetheine in bacteria and tissues,[19] and hence the quantitative significance of the respective cycles, has still to be assessed.

(13) M. E. Jones and F. Lipmann, in S. P. Colowick and N. O. Kaplan, *Methods in Enzymology*, *New York*, **1**, 585 (1955).

(14) H. R. Mahler, S. J. Wakil and R. M. Bock, *J. Biol. Chem.*, **204**, 453 (1953).

(15) Optical assay (λ240) according to E. R. Stadtman, *ibid.*, **203**, 501 (1953).

(16) J. R. Stern, S. Ochoa and F. Lynen, *ibid.*, **198**, 313 (1952).

(17) R. O. Brady and E. R. Stadtman, *ibid.*, **211**, 621 (1954).

(18) J. R. Stern, unpublished observations.

(19) E. E. Snell and G. M. Brown, *Advances in Enzymology*, **14**, 49 (1953).

DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO
JOSEPH R. STERN

RECEIVED JULY 12, 1955

SIMILARITIES BETWEEN SO-CALLED CHLOROPHYLL b″ AND OXIDIZED CHLOROPHYLL b, AND BETWEEN SO-CALLED CHLOROPHYLL a″ AND OXIDIZED CHLOROPHYLL a

Sir:

Heat, alkalies and oxygen convert chlorophylls a and b into several chromatographically unique pigments that are spectrally similar to the parent chlorophylls. Some of these pigments, chlorophylls a′ and b′, are isomers or other reconvertible alteration products of the common chlorophylls.[1] Some are oxidation or allomerization products that have not been reconverted to the chlorophylls.[2] Others, chlorophylls a″ and b″, are regarded as chlorophyll isomers although they also resemble the non-reconvertible allomerized chlorophylls.[3] Additional indications that a″ and b″ are oxidation products are presented herein.

In repetition of the experiments of Strain and Manning[1] and of Strain,[2] heat or traces of alkali converted chlorophyll a, in *n*-propyl alcohol solution, into a′ plus a. The less sorbed a′, isolated in a column of powdered sugar and redissolved in propyl alcohol, was reconverted into a plus a′ by heat or alkalies. These preparations of a and a′ were readily interconverted again by heat or alkalies. Consequently, a and a′ are isomers or other readily interconvertible products. In similar experiments, chlorophyll b yielded the reconvertible, less sorbed b′. Neither a nor b yielded more sorbed green pigments.

Freed, *et al.*,[3] found the strongly sorbed b″ (yield *ca.* 14%), in addition to weakly sorbed b′, when very dilute propyl alcohol solutions of b were heated in vacuum. This pigment was regarded as a chlorophyll, because it was prepared in vacuum, its spectral properties resembled those of b, and it was more sorbed than b. Freed's allomerized b (method of preparation not described) was less sorbed than b. Unlike b and b′, b″ did not give the phase test, and it was not reconvertible to b plus b′.

Strain[2] has found, however, that b yields a par-

(1) H. H. Strain and W. M. Manning, *J. Biol. Chem.*, **146**, 275 (1942).

(2) H. H. Sttain, *Agricultural and Food Chem.*, **2**, 1222 (1954).

(3) S. Freed, K. M. Sancier and A. H. Sporer, THIS JOURNAL, **76**, 6006 (1954).

ticular oxidation product that is similar to b″. Allomerized in methanol or in barley leaves, b yields several pigments all more sorbed than b. One of these is spectrally similar to b (λ max. methanol, b, 652 mμ; oxidized b, 653 mμ). This product, therefore, resembles b″ both spectrally and chromatographically. Like b″ it is not isomerized by heat or alkalies; and it does not give the phase test. Except for the method of preparation, this allomerized b is identical with b″.

The a″, for which few properties have been determined,[3] has its counterpart among the oxidation products[2] of a. The increased sorbability of the oxidized chlorophylls[2] and of a″ and b″ relative to a and b may be attributed to additional oxygen (or decrease in hydrogen).[4]

Molecular structures proposed by Freed, *et al.*,[3] for the chlorophylls and their isomers are unsuitable. If a, a′, and oxidized a have the magnesium atom bound to different pairs of pyrrole nitrogen atoms,[3] the three pigments should yield but one magnesium-free pheophytin. Yet, three have been reported.[1,2] Moreover, oxidized chlorophylls, presumably with magnesium bound as in the chlorophylls, do not undergo isomerization.[2]

The strongly sorbed pigments prepared by Freed, *et al.*, and by Strain are oxidized chlorophylls, not isomeric chlorophylls. These products should not be called chlorophylls (unless found as natural constituents of plants).

(4) H. H. Strain, "Chromatographic Adsorption Analysis," Interscience Publishers, Inc., New York, N. Y., 1942, pp. 14–25.

CHEMISTRY DIVISION
ARGONNE NATIONAL LABORATORY HAROLD H. STRAIN
LEMONT, ILLINOIS

RECEIVED JULY 22, 1955

GLUTAMIC DEHYDROGENASE OF BEEF LIVER, A ZINC METALLOENZYME

Sir:

We recently have examined several crystalline preparations of beef liver glutamic dehydrogenase and find that zinc is an integral and functional component of the enzyme molecule.

The protein was crystallized from fresh beef liver.[1]

Quantitative emission spectrography and microchemical determination of zinc were performed in duplicate according to methods previously described.[2] Protein weights were determined by trichloroacetic acid precipitation.[2]

Table I presents quantitative spectrochemical and microchemical data on three times crystallized preparations of glutamic dehydrogenase having high specific activity. Preparation 1 contained 333 μg. Zn/g. of protein, preparation 2 contained 322 μg. Zn/g. of protein, and a third preparation contained 260 μg./g. The zinc content of these crystalline preparations was not lowered by prolonged dialysis. The molecular weight of glutamic dehydrogenase has been determined to be about one million.[1] Based on this molecular weight, the number of gram atoms of zinc per mole of enzyme protein vary between 4 and 5 for these preparations.

(1) J. A. Olson and C. B. Anfinsen, *J. Biol. Chem.*, **197**, 67 (1952).
(2) B. L. Vallee and F. L. Hoch, *Proc. Natl. Acad. Sci.*, **41**, 327 (1955).

TABLE I

ANALYSES OF THREE TIMES RECRYSTALLIZED GLUTAMIC DEHYDROGENASE OF BEEF LIVER

Element	Preparation 1 (μg./g. GDH)	Preparation 2 (μg./g. GDH)
Zinc	333	322
Copper	[a]	[b]
Aluminum	63.1	[b]
Barium	27.0	39.9
Calcium	1060	[b]
Magnesium	149	43.0

[a] Not determined. [b] Not detected, and also beryllium, cadmium, cobalt, chromium, iron, lithium, molybdenum, manganese, nickel, potassium, silver, strontium, tin and lead.

Fractionation of beef liver shows an *aggregation* of *zinc* with purification of the enzyme, and as the activity:protein ratio reaches its maximum value. The metal:protein ratio of all *other elements* studied *decreases* with progressive enzyme purification. The activity:zinc ratio increases progressively with purification, reaching a high value in the third crystals.

The rate of conversion of DPN to DPNH at *p*H 7.7 in the presence of glutamate[1] is inhibited significantly when the enzyme is preincubated with a number of metal binding agents, including sodium sulfide, sodium diethyldithiocarbamate and 1,10-phenanthroline.

These data establish glutamic dehydrogenase as a zinc metalloenzyme according to the criteria published elsewhere.[3]

The oxidation of glutamic acid is the second DPN dependent dehydrogenation reaction known to be catalyzed by a zinc metalloenzyme.[2]

Acknowledgments.—The valuable suggestions of Dr. Eric G. Ball are gratefully acknowledged. This work was supported by grants-in-aid from the Office of Naval Research, Contract No. NR 119-277, to Harvard University, and by the Rockefeller Foundation, New York, N. Y.

BIOPHYSICS RESEARCH LABORATORY BERT L. VALLEE
OF THE DEPARTMENT OF MEDICINE S. JAMES ADELSTEIN[4]
HARVARD MEDICAL SCHOOL AND PETER
BENT BRIGHAM HOSPITAL, BOSTON, MASS.
DEPARTMENT OF CHEMISTRY JAMES A. OLSON
HARVARD UNIVERSITY
CAMBRIDGE, MASS.

(3) B. L. Vallee, "Zinc and Metalloenzymes" in "Advances in Protein Chemistry," Vol. X, Academic Press, New York, N. Y., 1955, in press.
(4) Fellow of the National Foundation for Infantile Paralysis.

STEROIDAL SAPOGENINS. XXXI. GENTROGENIN AND CORRELLOGENIN, NEW SAPOGENINS FROM *Dioscorea spiculiflora*[1]

Sir:

Some years ago Marker announced the isolation of botogenin and neobotogenin from *D. mexicana*.[2a,b] The structures assigned had both a 12-keto and a 5,6 double bond. Since substances with these groupings would be particularly desirable as cortisone precursors, there was an extensive but fruitless search made for them. We have now found in several collections of *D. spiculiflora*

(1) Paper XXX: M. E. Wall, submitted in *Experientia*.
(2) (a) R. E. Marker and J. Lopez, THIS JOURNAL, **69**, 2397 (1947); (b) R. E. Marker, *ibid.*, **71**, 2656 (1949).