abundant evidence that the heats of donor acceptor type interactions are generally small in value and do not vary widely with changes in component structure.[3]

Further work on the heats of formation of these complexes is under way. The authors are indebted to the National Science Foundation for a grant in support of this research.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA C. E. CASTRO
DAVIS, CALIFORNIA L. J. ANDREWS

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RESOLUTION AND MUTAROTATION OF *cis*-NITRITO-NITRO-BIS-(ETHYLENEDIAMINE)-COBALT(III)

Sir:

It previously has been demonstrated[1] that the nitrite ion can attach itself to the inner coördination sphere of a cobalt ion in two ways: through an oxygen (Nitrito) or through the nitrogen (Nitro). The conversion of the nitrito form to the nitro takes place rapidly and completely in solution or in the solid state with little or no *cis–trans* interconversion. The mechanism of this reaction is of especial interest since it may provide valuable information on the mode of substitution in coordination compounds.

In connection with a study of the mechanism of the formation of nitrito compounds and of the nitrito-nitro conversion, we have recently succeeded in resolving *cis*-$[Co(en)_2(NO_2)(ONO)]^+$ by the use of ammonium *d*-α-bromocamphor-π-sulfonate, (*d*-NH_4BCS), and a partial resolution has been achieved with active powdered quartz. This is believed to be the first reported isolation of a nitrito complex in optically active form.

The resolution was achieved by the addition of a slight excess of solid *d*-NH_4BCS to a saturated solution of *cis*-$[Co(en)_2(NO_2)(ONO)]ClO_4$ at 0°. Reddish-yellow needles were deposited, which were dissolved in water at 0° and recrystallized by the addition of solid *d*-NH_4BCS. The crystals were washed with ice-cold acetone and dried under vacuum: (I) *d-cis*-$[Co(en)_2(NO_2)(ONO)]$*d*-BCS, $[\alpha]^{18}D$ +81°.[2] The resolving agent was removed by triturating the diastereomer with a saturated solution of sodium perchlorate at 0° and washing the precipitated powder with ice-cold ethanol. Recrystallization was accomplished by solution in cold water and addition of solid sodium perchlorate. The orange crystals were freed of sodium perchlorate by washing with ice-cold ethanol and dried under vacuum: (II) *d-cis*-$[Co(en)_2(NO_2)(ONO)]ClO_4$, $[\alpha]^{18}D$ + 62.3°[2]. *Anal.* Calculated for $CoC_4H_{16}N_6O_8Cl$; Co, 15.92; C, 13.02; H, 4.33; N, 22.76. Found: Co, 15.61; C, 13.13; H, 4.42; N, 22.58. Since conversion to the nitro form takes place rapidly, the resolved compounds were used immediately.

Absorption spectra and molecular rotation were used to demonstrate that the resolved products were the nitrito form. The molecular extinction coefficients (extrapolated) of compounds I and II are exactly the same as for the known inactive compounds and quite different from the dinitro compounds. The compounds *d-cis*-$[Co(en)_2(NO_2)(ONO)]$*d*-BCS[2], *d-cis*-$[Co(en)_2(NO_2)(ONO)]ClO_4$[2] and *dl-cis*-$[Co(en)_2(NO_2)(ONO)]ClO_4$[2] had values of $\epsilon_{408\ m\mu} = 82$, $\epsilon_{434} = 106$, $\epsilon_{439} = 113$, $\epsilon_{461} = 130 \pm 2$ while the extinction coefficients for *d-cis*-$[Co(en)_2(NO_2)_2]$*d*-BCS[3], *d-cis*-$[Co(en)_2(NO_2)_2]ClO_4$ and *dl-cis*-$[Co(en)_2(NO_2)_2]ClO_4$[4] were $\epsilon_{408} = 120$, $\epsilon_{434} = 180$, $\epsilon_{439} = 181$, $\epsilon_{461} = 129 \pm 2$.

Either I or II mutarotates rapidly at room temperature reaching a constant value after several hours owing to the formation of the optically stable *d-cis*-$[Co(en)_2(N_2O)_2]^+$ ion. On warming this with dilute sodium hydroxide solution overnight, only the activity of the resolving agent remains. Table I compares the observed changes in rotation with those calculated. Preliminary kinetic studies indicate that the rate of mutarotation and the rate of conversion to the nitro form are equal, and have a half-time of about 70 minutes (19°). Thus the reaction

TABLE I

MOLECULAR ROTATIONS

	Compound I Obs.	Compound I Calcd.	Compound II Obs.	Compound II Calcd.
Initial[2]	+469	+470	+228	+230
After 24 hours	+394	+384	+146	+144
After heating in dilute NaOH	+240	+241	+ 0	+ 0

$$d\text{-}cis\text{-}[Co(en)_2(NO_2)(ONO)]^+ \longrightarrow d\text{-}cis\text{-}[Co(en)_2(NO_2)_2]^+$$

proceeds with no appreciable racemization and since the *d*-form of the starting material and the product is the least soluble diastereomer with *d*-BCS^-, the generic configuration is probably retained.[5] Tracer experiments now in progress indicate that this reaction proceeds completely by an intramolecular process.

In the near future a more detailed account of this work as well as new results on the kinetics of these reactions will be communicated.

(1) M. Linhard, H. Seibert and M. Weigel, *Z. anorg. Chem.*, **278**, 287 (1955).

(2) Since the rate of reaction is much more rapid in solution, all absorption spectra and optical rotations have been extrapolated to the time of addition to water.

(3) Prepared by allowing *d-cis*-$[Co(en)_2(NO_2)(ONO)]$*d*-BCS to stand in water overnight.

(4) A. Werner, *Ber.*, **44**, 2452 (1911).

(5) A. Werner, *Bull. soc. chim.*, [4] **11**, xix (1912); J. P. Mathiew, *Compt. rend.*, **199**, 278 (1934); **201**, 1183 (1935).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CONNECTICUT R. KENT MURMANN
STORRS, CONNECTICUT

RECEIVED AUGUST 22, 1955

THE REQUIREMENT OF TRIPHOSPHOPYRIDINE NUCLEOTIDE IN FATTY ACID SYNTHESIS[1]

Sir:

The enzymatic reactions involved in the biological oxidation of fatty acids have been investigated extensively. It has been postulated[2] that fatty acids are synthesized from acetyl Coenzyme A (CoA) by a reversal of the oxidative pathway.

(1) This work was supported in part by a grant from the National Heart Institute, U. S. Public Health Service.

(2) F. Lynen and S. Ochoa, *Biochim. et Biophys. Acta*, **12**, 299 (1953).

However, it has not been possible to demonstrate synthesis of fatty acids by use of the purified oxidative enzymes and physiological electron donors, since the reduction of β-unsaturated acyl CoA derivatives has required a leucodye as an electron donor.[3]

It has now been found that the dialyzed soluble fraction of rat liver, when suitably supplemented, is capable of converting 1-C^{14}-acetic acid into higher fatty acids. As shown in Table I, for maximum rates of synthesis the addition of CoA, triphosphopyridine nucleotide (TPN), and diphosphopyridine nucleotide (DPN) is required. The rate of synthesis in such systems compared favorably with the rate observed in unfractionated homogenates. Although it has been clearly established that DPN is specifically required by the β-hydroxyacyl CoA dehydrogenase,[2] a specific role for TPN in the oxidation-reduction steps of the fatty acid cycle has not been reported.

TABLE I

BIOSYNTHESIS OF FATTY ACIDS BY THE SOLUBLE FRACTION OF RAT LIVER

	Fatty acids specific activity[b]
Complete[a]	100
Omit CoA	0
Omit TPN	56
Omit DPN	85
Omit DPN and TPN	19

[a] The complete system contained CoA, 0.3 μmole; TPN, 0.625 μmole; DPN, 1.25 μmoles; glucose-6-phosphate, 10 μmoles; potassium acetate-1-C^{14}, 20 μmoles; cysteine, 10 μmoles; ATP, 20 μmoles; ethylenediaminetetraacetate (EDTA), 1 μmole; $MgCl_2$, 30 μmoles; KCl, 400 μmoles; nicotinamide, 100 μmoles; tris-(hydroxymethyl)-aminomethane (TRIS) buffer (*p*H 7.4), 100 μmoles. Dialyzed rat liver soluble fraction, 1.0 ml.; total volume 3.0 ml. Incubated 30 minutes at 31°. Rat liver was homogenized in 2.5 volumes of 0.13 *M* sucrose, 0.05 *M* potassium phosphate, *p*H 7.4 and centrifuged at 100,000 × *g* for 20 minutes. The clear supernatant was dialyzed at 0° for 5–18 hr. against the following medium: KCl, 0.05 *M*; cysteine, 10^{-3} *M*; EDTA, 10^{-4} *M*; potassium phosphate buffer, *p*H 7.4, 0.05 *M*. [b] Specific activity values are expressed as per cent. of the specific activity of the complete system. The complete system incorporated 1.6 milliμmoles of 1-C^{14}-acetate into higher fatty acids.

These preparations of rat liver also catalyze the oxidation of TPNH[4] in the presence of crotonyl CoA. Results of a typical experiment are shown in Fig. 1. It can be seen that TPNH undergoes no endogenous oxidation. However, the addition of crotonyl CoA[5] is followed by a rapid decline in the optical density at 340 mμ. Approximately 0.5 mole of TPNH disappears per mole of crotonyl CoA added. No evidence was obtained which indicated that DPNH could substitute for TPNH in this reaction. Crotonyl CoA could not be replaced by crotonate. The crotonyl CoA samples used were free of oxidized glutathione.

Butyryl CoA has been tentatively identified as a product of this reaction. After allowing the reaction to proceed to completion, the medium was deproteinized with perchloric acid, neutralized, and the acyl CoA derivatives were converted to

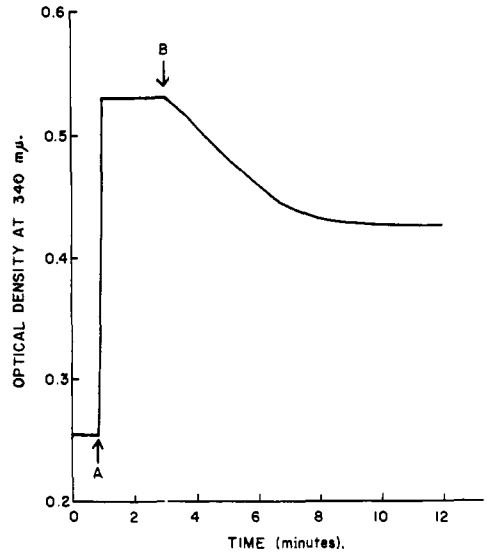

Fig. 1.—The cuvette initially contained 400 μmoles KCl, 30 μmoles $MgCl_2$, 100 μmoles nicotinamide, 1 μmole TRIS, *p*H 7.5, and 0.5 ml. of dialyzed enzyme in a total volume of 3.0 ml. At "A" 0.18 μmole TPNH was added; at "B" 0.1 μmole of crotonyl CoA.

the corresponding hydroxamic acids by reaction with neutral hydroxylamine.[6] Following chromatography of the resulting hydroxamic acids on filter paper in a butanol–NH_3 system,[7] the spots were developed with ferric chloride and compared with the authentic samples of crotono- and butyrohydroxamic acids, which were chromatographed simultaneously. A component which yielded a hydroxamic acid having an R_f identical with that of synthetic butyrohydroxamic acid was formed during the course of the enzymatic reaction.

The results of these experiments suggest that in the biological synthesis of fatty acids, TPNH may be required as a specific electron donor in the reduction of β-unsaturated acyl CoA derivatives. The possibility is also raised that, while fatty acid oxidation may proceed in the mitochondria, the cellular synthesis of fatty acids may occur predominantly in the reducing environment of the soluble portion of the cell.

Addendum.—Popjak and Tietz[8] have reported that preparations derived from the soluble portion of lactating mammary gland are also capable of fatty acid synthesis. After this manuscript had been submitted for publication, Hele and Popjak[9] reported that a partially purified soluble enzyme system from mammary gland oxidized DPNH in the presence of acetate, CoA, and ATP, and these authors suggested that DPNH may serve as the electron donor in both reductive steps of fatty acid synthesis. These findings may be interpreted to mean that mammary gland and liver differ in their nucleotide requirements for fatty acid synthesis. On the other hand, it seems possible that the mammary gland preparations may contain trans-

(3) P. G. Stansly and H. Beinert, *Biochim. et Biophys. Acta*, **11**, 600 (1953).
(4) S. Gutcho and E. D. Stewart, *Anal. Chem.*, **20**, 1185 (1948).
(5) D. S. Goldman, *J. Biol. Chem.*, **208**, 345 (1954).
(6) F. Lippmann and L. C. Tuttle, *ibid.*, **161**, 415 (1945).
(7) E. R. Stadtman and H. A. Barker, *ibid.*, **184**, 769 (1950).
(8) G. Popjak and A. Tietz, *Biochem. J.*, **60**, 147 (1955); A. Tietz and G. Popjak, *ibid.*, **60**, 155 (1955).
(9) B. Hele and G. Popjak, *ibid.*, **60**, xxxii (1955).

hydrogenase[10] activity, which is absent from the rat liver preparations used here.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE
BALTIMORE 5, MARYLAND ROBERT G. LANGDON[11]

(10) N. O. Kaplan, S. P. Colowick and E. F. Neufeld, *J. Biol. Chem.*, **205**, 1 (1953).

(11) This work was conducted during the tenure of a Lederle Medical Faculty Award.

VITAMIN B_{12}. XXVI. DEGRADATION OF FACTOR III TO 5-HYDROXYBENZIMIDAZOLE AND DERIVATIVES AND BIOSYNTHESIS OF FACTOR III

Sir:

Factor III was isolated from fermented sewage.[1] It has been reported to have hematological activity similar to that of vitamin B_{12}, and appeared to differ from vitamin B_{12} by having an unknown moiety in place of 5,6-dimethylbenzimidazole.[2] Friederich and Bernhauer[3] have now reported 5-hydroxybenzimidazole as a degradation product of Factor III.

Last year, through the generosity of Professor Dr. K. Bernhauer, we received samples of Factor III. We have independently identified 5-hydroxybenzimidazole as a part of the molecule. We have also prepared two crystalline cobalt complexes from 5-hydroxybenzimidazole and Factor B[4] by biosynthesis. One of these appears to be Factor III from comparison with the substance isolated from sewage.

Factor III was hydrolyzed with 6 *N* hydrochloric acid for 20 hours at room temperature, and the hydrolysate was subjected to paper electrophoresis in 0.5 *N* acetic acid containing a little cyanide. Material showing bright blue-white fluorescence under ultraviolet light separated from the pigments present. On paper chromatography in a butanol–acetic acid–water system,[5] it separated into two spots (I and II) of unequal intensity with R_f values of 0.16 and 0.22. Further hydrolysis of combined I and II with 6 *N* hydrochloric acid at 95° for 24 hours gave a new substance (IV) with an R_f value of 0.46. Hydrolysis of Factor III, or of the fluorescent materials I and II, with 6 *N* hydrochloric acid at 150° for 21 hours gave another fluorescent substance (V) with an R_f value of 0.65, and phosphate ion was detected in the hydrolysates. These same hydrolysis conditions degrade vitamin B_{12} to isomers of ribazole phosphate, ribazole and 5,6-dimethylbenzimidazole.[6]

In the case of Factor III, it was assumed that the substances obtained were isomers of a riboside phosphate (I and II), the riboside (IV) and the base (V). The base was isolated as a crystalline picrate, m.p. mainly 220–225°, which was converted to a polymorphic crystalline hydrochloride, m.p. mainly 185–190°. There was not sufficient material for elemental analysis, so a detailed study of spectra was made.

The absorption spectrum of V seemed to eliminate purines, pyrimidines, pyridines and alkylbenzimidazoles and indicated an hydroxybenzimidazole structure. The previously undescribed 5-hydroxybenzimidazole was prepared by demethylation of 5-methoxybenzimidazole[7] with hydrobromic acid; m.p. 220–222°; *Anal.* Found: C, 62.26; H, 4.60; N, 20.90. The absorption spectrum ($\lambda_{max.}^{0.1N\ NaOH}$, 2500 (485); 3050 (573): $\lambda_{max.}^{0.1N\ HCl}$, 2400–2500 (shoulder); 2870 (528)) was in good agreement with that of base V. The picrate and hydrochloride of synthetic 5-hydroxybenzimidazole had wide melting point ranges, but mixed melting points with the isolated salts showed no depression. The infrared spectra of the hydrochlorides showed them to be identical.

Factor B and synthetic 5-hydroxybenzimidazole were combined microbiologically using *Escherichia coli* 113-3,[8] and two red crystalline compounds were isolated. One of these did not separate from Factor III on mixed paper chromatograms. This result confirmed the chemical evidence that 5-hydroxybenzimidazole is present in Factor III and is not an artifact. The structure and synthesis of the other degradation products of Factor III are being studied further.

(1) (a) W. Friederich and K. Bernhauer, *Angew. Chem.*, **65**, 627 (1953); (b) K. Bernhauer and W. Friederich, *ibid.*, **66**, 776 (1954).

(2) W. Friederich and K. Bernhauer, *Z. Naturforschung.*, **9b**, 686 (1954).

(3) W. Friederich and K. Bernhauer, *Angew. Chem.*, in press.

(4) J. E. Ford and J. W. G. Porter, *Brit. J. Nutrition*, **1**, 326 (1953).

(5) C. E. Carter, *ibid.*, **72**, 1466 (1950).

(6) (a) N. G. Brink and K. Folkers, THIS JOURNAL, **72**, 442 (1950); (b) N. G. Brink and K. Folkers, *ibid.*, **74**, 2856 (1952); (c) E. A. Kaczka and K. Folkers, *ibid.*, **75**, 6317 (1953).

(7) E. Ochiai and M. Katagu, *J. Pharm. Soc. Japan*, **60**, 543–550 (1940); *Chem. Abs.*, **35**, 1785 (1951).

(8) B. Davis and E. Mengioli, *J. Bact.*, **60**, 17 (1950).

RESEARCH LABORATORIES F. M. ROBINSON
CHEMICAL DIVISION I. M. MILLER
MERCK & CO., INC. J. F. MCPHERSON
RAHWAY, NEW JERSEY KARL FOLKERS

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ON THE ORIGIN OF THE METHYL GROUPS OF PHOSPHOLIPID CHOLINE IN THE RAT[1]

Sir:

We previously reported that the extent of incorporation of the carbon of the methyl group of methionine, labeled with C^{14}, to phospholipid choline is markedly reduced in the folic acid-deficient rat, and as a tentative explanation of this observation we suggested that in the folic acid-deficient rat the synthesis of the acceptor of the methyl group of methionine for choline formation is inhibited[2]. It is generally assumed that aminoethanol is the acceptor of three methyl groups of methionine *in vivo*, the formation of choline taking place via direct transmethylation from methionine. However, existing evidence does not indicate that folic acid or its biological derivative is a co-factor in the enzymatic reactions involving transmethylations from methionine, neither is folic acid or its derivative involved in the *in vivo* decarboxylation of serine to aminoethanol. It occurred to us, therefore, that the acceptor of the methyl group

(1) Aided by grants from the National Cancer Institute, U. S. Public Health Service, from the U. S. Atomic Energy Commission, AT-30-1 (1552), and by an Institutional grant from the American Cancer Society. *The isotopic material was obtained on allocation from the Atomic Energy Commission.*

(2) J. A. Stekol, S. Weiss, P. Smith and K. Weiss, *J. Biol. Chem.*, **201**, 299 (1953).