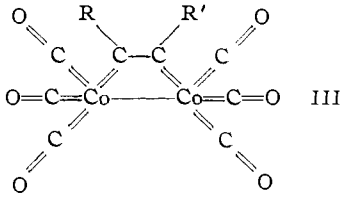
II (R = R′ = CH_2OH), light-orange-red needles from ethanol–water, m.p. *ca.* 135° with decomposition. Calcd. for $C_{10}H_6O_8Co_2$: C, 32.28; H, 1.63; Co, 31.69. Found: C, 32.24; H, 1.67; Co, 31.67.

II (R = R′ = H), a dark-red oil at room temperature; m.p. 13.0–13.6°; b.p. 64–66° (3.5–4 mm.) Calcd. for $C_8H_2O_6Co_2$: C, 30.80; H, 0.65. Found: C, 30.76; H, 0.62.

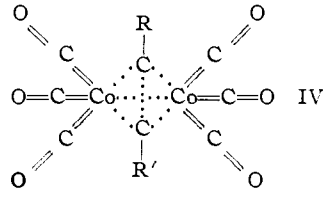
The infrared spectra of these compounds contain a characteristic group of three sharp bands at 2090, 2050 and 2025 cm.$^{-1}$, which are similar to the bands of the terminal carbonyl groups in dicobalt octacarbonyl. The 1859 cm.$^{-1}$ band corresponding to the bridge carbonyl group in I as well as the bands of the $-C\equiv C-$ group, are absent. The spectra of II (R = R′ = H), II (R = $CH_3(CH_2)_4$, R′ = H) and II (R = CH_2OH, R′ = H) contain a band at 3096 cm.$^{-1}$, characteristic of an ethylenic (or aromatic) carbon–hydrogen bond.

It is reasonable to assume that the C–C bond is either parallel to the Co–Co bond, such as in III



III

or perpendicular to the Co–Co bond, such as in IV.



IV

The analytical, spectroscopic, magnetic and dipole moment data, in conjunction with the high volatility and the solubility in organic solvents of II, are compatible with a structure such as III, where cobalt possesses a noble-gas configuration.

In IV the C–C Co–Co bonds may be either in the same plane (IVa) or in different planes (IVb). Structure IVa is very unlikely[3] in view of the dipole moment of II (R = R′ = C_6H_5). Neither the structure of IVa nor that of IVb can be represented readily in terms of localized bonds.

(3) Compound II (R = R′ = C_6H_5) has a molar polarization of 204 ± 4 at 30° and a calculated molecular fraction for the D sodium line of 121. For II (R = R′ = C_6H_5) to be symmetrical it would have to have an extremely high atomic polarization (*cf.* I. E. Coop and L. E. Sutton, *J. Chem. Soc.*, 1269 (1938)).

SYNTHETIC FUELS RESEARCH BRANCH
BUREAU OF MINES
BRUCETON, PA.
HEINZ W. STERNBERG
HAROLD GREENFIELD
ROBERT A. FRIEDEL

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF PITTSBURGH
PITTSBURGH, PA.
JOHN WOTIZ
RAYMOND MARKBY
IRVING WENDER

RECEIVED FEBRUARY 6, 1954

A FORMYLATION COFACTOR[1]

Sir:

This paper reports the biosynthesis of a formylation cofactor and the transfer of its one-carbon group to a purine precursor. Formylation of IRMP-5[2,3,4] in pigeon liver extract may occur as

$$RH_4X + HCOOH + ATP \longrightarrow FRH_4X + ? \quad (1)$$
$$FRH_4X + IRMP\text{-}5 \longrightarrow RH_4X + IMP\text{-}5 \quad (2)$$

The over-all reaction exhibits almost an absolute requirement for IRMP-5, HCOOH, ATP, Mg^{++} and a cofactor. FRH_4X is a heat-stable cofactor obtained enzymatically from FRH_4 or from RH_4[5] and formate by ATP-dependent reactions. The following three experiments provide evidence for the above mechanism: (1) C^{14}-(formyl)-FRH_4X transformylated directly to IRMP-5 (Reaction 2) in a large pool of unlabeled formate to yield IMP-5 with a specific activity more than 10 times that of the formate. The C^{14}-activity of the FRH_4X was displaced by the unlabeled formate in the presence of ATP (Reaction 1) while without acceptor IRMP-5, FRH_4X lost no activity. (2) Purified FRH_4X formylated IRMP-5 in the absence of ATP, but FRH_4 alone was inactive. (3) An excess of FRH_4X obtained from FRH_4 plus ATP converted IRMP-5 completely to IMP-5 in the absence of formate. The resulting RH_4X accepted added C^{14}-formate to yield C^{14}-FRH_4X (paper chromatography) equivalent in quantity to 80% of the initial IRMP-5 (Reaction 1). Exchange between $HC^{14}OOH$ and IMP-5[6] was insignificant. Loss of X during transformylation (Reaction 2) is not excluded.

Table I shows that the synthesis of FRH_4X, which is measured by its cofactor effect on IMP-5 synthesis, depends on the presence of both ATP and FRH_4 during preincubation.

TABLE I

INTERACTION OF LEUCOVORIN AND ATP

Preincubation additions: 20 mg. lyophilized extract (acetone powder extracted with 0.05 *M* $KHCO_3$ and treated with Dowex-1 chloride), 2.5 μM·ATP, 7 μM, phosphoglycerate, 3 μM·$MgCl_2$, 0.5 mg. lyophilized muscle extract fraction,[7] 7.5 μM·$KHCO_3$, 0.2 μM·FRH_4, 8 μH·DL-homocysteine; vol., 0.6 ml.; time, 10 min.; temp., 38°; in air. After preincubation 0.36 μM·IRMP-5, 2 μM. C^{14}-formate and the omitted compounds were added and the reaction continued 5 min.; total vol. 1.0 ml.

Omitted in preincubation	None	FRH_4	ATP	FRH_4[a]	ATP[a]
Δ Amine, μM	0.262	0.084	0.057	0.024	0.002
C^{14} fixed, μM	.294	.116	.093	.010	.0

[a] Also omitted after preincubation.

IMP-5 synthesis is greatest when FRH_4X is preformed. One mole of diazotizable amine[8] disappears per mole of C^{14} formate fixed into IMP-5. On paper chromatograms FRH_4X appears as a blue-fluorescing compound.

(1) Aided by grants from the U. S. Public Health Service and Elisabeth Severance Prentiss Foundation.

(2) Abbreviations: IRMP-5, 5-amino-4-imidazolecarboxamide-5′-phosphoribotide; ATP, adenosine triphosphate; IMP-5, inosine-5′-phosphate; RH_2, 7,8-dihydrofolic acid; RH_4, 5,6,7,8-tetrahydrofolic acid; FRH_4, N-5-formyl-5,6,7,8-tetrahydrofolic acid (leucovorin); X, product of the interaction of a folic acid compound and ATP DPNH, reduced diphosphopyridine nucleotide.

(3) G. R. Greenberg, *Federation Proc.*, **12**, 211 (1953).

(4) G. R. Greenberg, *ibid.*, **12**, 651 (1953).

(5) B. L. O'Dell, *et al.*, THIS JOURNAL, **69**, 250 (1947).

(6) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).

(7) S. Ratner and A. Pappas, *ibid.*, **179**, 1183 (1949).

(8) J. M. Ravel, *et al.*, *ibid.*, **172**, 67 (1948). Diazotization was preceded by acetic anhydride treatment.

The conversion of RH_4 to FRH_4X occurs under conditions such as shown in Table I at a rate comparable to IMP-5 synthesis. Preincubation studies without formate suggest that RH_4 and ATP first react to form RH_4X. RH_2[5] and DPNH replace RH_4 in FRH_4X synthesis. Folic acid did not react under these conditions. These experiments implicate RH_2 and RH_4 as intermediates in the synthesis of FRH_4 from folic acid.

$C^{14}FRH_4X$ is converted to N-10-C^{14}-formylfolic acid during isolation and by dilute acid.[9]

Leucovorin is known to catalyze the exchange between $HC^{14}OOH$ and IMP-5.[6] RH_4,[10] ATP and DPN[11] have been involved in FRH_4 synthesis. Rauen and Jaenicke[12] have reported a cofactor derived from folic acid derivatives. The expected interrelationship of FRH_4X with other 1-carbon acceptor systems[13] has been discussed previously.[14]

(9) Compare M. Silverman and J. C. Keresztesy, *Federation Proc.*, **12**, 268 (1953). The author gratefully acknowledges the aid of Drs. Silverman and Keresztesy in identifying N-10-formylfolic acid.

(10) H. P. Broquist, *et al.*, *J. Biol. Chem.*, **202**, 59 (1953).

(11) C. A. Nichol, *J. Pharmacol. and Exper. Therap.*, **110**, 40 (1954).

(12) H. M. Rauen and L. Jaenicke, *Z. physiol. Chem.*, **293**, 46 (1953).

(13) Tetrahydrofolic acid catalyzes an exchange reaction between glycine and serine (R. Kisliuk and W. Sakami (private communication)).

(14) Dr. E. L. R. Stokstad, Lederle Laboratories, kindly supplied the calcium leucovorin and part of the dihydrofolic acid employed.

DEPARTMENT OF BIOCHEMISTRY
SCHOOL OF MEDICINE G. ROBERT GREENBERG
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO

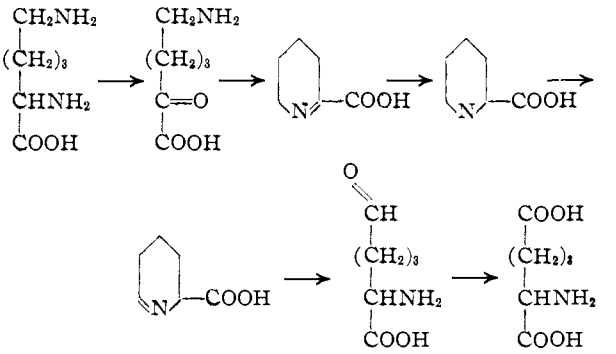
RECEIVED JANUARY 21, 1954

LOSS OF THE α-AMINO GROUP IN LYSINE METABOLISM TO FORM PIPECOLIC ACID

Sir:

The early steps of the metabolism of lysine have been the subject of speculation for many years. It has recently been found in this laboratory that pipecolic acid is a metabolite of L-lysine-ε-C^{14} in the rat.[1] Pipecolic acid must be formed at an early stage of lysine metabolism since it still contains six carbon atoms but has only one amino group. This fact, along with the *in vivo* "metabolic overloading" technique devised to isolate specific metabolites of isotopic precursors[1,2] has afforded a means of ascertaining which amino group of lysine is removed first. This was determined by injecting intraperitoneally into a 24-hour fasted rat a solution containing 74 mg. of DL-lysine-ε-N^{15}·HCl (25.3 atom % excess N^{15}) and 360 mg. of non-isotopic L-pipecolic acid.[3] The effective dose of lysine-ε-N^{15}·HCl is 37 mg. since D-lysine, under similar conditions, does not contribute significantly to the formation of pipecolic acid.[4] After isolation from the urine as previously reported[1], 140 mg. of pure pipecolic acid was isolated and found to contain 2.0 atom % excess N^{15}.[5] This large enrichment of N^{15} in the pipecolic acid is remarkable in view of the large dilution of biologically formed material with non-enriched pipecolic acid. This is in accord with the concept established with C^{14}, namely, that the formation of pipecolic acid is both a major and a primary step in lysine metabolism. Furthermore, this is conclusive evidence that the ε-amino group of lysine remains in large measure intact until after the loss of the α-amino group, and lends support to the hypothesis that lysine forms an α-keto analog. Work with *Neurospora crassa* is in accord with this.[6] The possibility of concomitant formation of pipecolic acid by loss of the ε-amino group of lysine and subsequent cyclization is at present being investigated with lysine-α-N^{15}.

If, as seems probable, the conversion of lysine to pipecolic acid is part of the pathway between lysine and α-aminoadipic acid, the most likely mechanism is



This is a pathway whereby the ε-amino group could be oxidized by what amounts to an intramolecular transamination reaction. It is of interest to note that α-aminoadipic acid-ε-C^{14} does not lead to radioactive pipecolic acid under conditions where lysine-ε-C^{14} with a radioactive count of similar magnitude leads to pipecolic acid containing 1.2 × 10^5 disintegrations/min./mmole, indicating the irreversibility of the pathway.

DEPARTMENT OF RADIATION BIOLOGY
SCHOOL OF MEDICINE AND DENTISTRY MORTON ROTHSTEIN
UNIVERSITY OF ROCHESTER LEON L. MILLER
ROCHESTER, NEW YORK

RECEIVED JANUARY 4, 1954

(1) M. Rothstein and L. L. Miller, THIS JOURNAL, **75**, 4371 (1953).

(2) M. Rothstein and L. L. Miller, *J. Biol. Chem.*, January, 1954.

(3) The authors wish to thank Dr. F. C. Steward of Cornell University for supplying the L-pipecolic acid used in this research.

(4) M. Rothstein, C. G. Bly and L. L. Miller, *Arch. Biochem. and Biophys.*, in press.

(5) We are indebted to Glenn Happ of the Department of Analytical Chemistry, Eastman Kodak Co., Rochester, N. Y., for performing the N^{15} assays.

(6) P. H. Lowy, J. T. Holden and R. S. Schweet, Abstracts, Atlantic City Meeting, A. C. S., 1952, p. 44c.