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.

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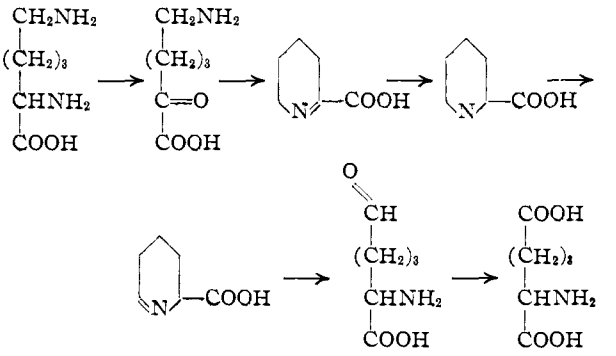
## LOSS OF THE α-AMINO GROUP IN LYSINE METABOLISM TO FORM PIPECOLIC ACID

*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 $1.2 \times 10^5$ disintegrations/min./mmole, indicating the irreversibility of the pathway.

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(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.