

and carnitine biosynthesis in *N. crassa*.

have recently reported the presence of free and protein-bound 6-N-mono-, di- and tri-methylated lysines in *Neurospora crassa*. Horne and Broquist (1973 J. Biol. Chem. 248: 2170) have shown that 6-N-trimethyllysine (I) is a highly efficient precursor of carnitine (IV) via butyrobetaine

(II) in *Neurospora crassa*. Although the same relationship among these metabolites has been found in the rat, (Tanphaichitr and Broquist 1973 J. Biol. Chem. 248: 2176; Cox and Hoppel 1973 Biochem. J. 136: 1083), no intermediates of this metabolic pathway have been isolated nor identified as yet. Neither Lindsted and Lindsted (1965 J. Biol. Chem. 240: 316) nor Cox and Hoppel (1974 Biochem. Biophys. Acta 362: 403) could obtain incorporation of radioactivity from 5-N-($^{14}\text{CH}_3$)trimethylaminopentanoate or 6-N-($^{14}\text{CH}_3$)trimethylaminohexanoate into carnitine. We have now examined the possibility of a lysine decarboxylating pathway for 6-N-trimethyllysine (Me_3Lys). The expected intermediate would be N-trimethylcadaverine (Me_3Cad). Extracts of cultures of *N. crassa* strain lys-1 (33933) (FGSC #74), growing in a medium containing $\text{Me}_3(^{14}\text{CH}_3)\text{Lys}$ or $\text{Me}_3(^{14}\text{CH}_3)\text{Cad}$, were analysed by appropriate automatic ion exchange column chromatography and checked for the eventual conversion of $\text{Me}_3(^{14}\text{CH}_3)\text{Lys}$ into $\text{Me}_3(^{14}\text{CH}_3)\text{Cad}$, as well as for the conversion of the latter into butyrobetaine and carnitine. Neither conversion of $\text{Me}_3(^{14}\text{CH}_3)\text{Lys}$ into $\text{Me}_3(^{14}\text{CH}_3)\text{Cad}$ nor conversion of $\text{Me}_3(^{14}\text{CH}_3)\text{Cad}$ into butyrobetaine and carnitine was observed. Unexpectedly, analysis of the extracts labelled with $\text{Me}_3(^{14}\text{CH}_3)\text{Lys}$ gave rise to three mean radioactive peaks: the first one the unknown was excluded from the column; the second eluted at the position corresponding to that of butyrobetaine-carnitine and the last one, to that of Me_3Lys . The unknown radioactive product gave a positive reaction with 2,4-dinitrophenylhydrazine, indicating the presence of a keto-group. We thought that the unknown compound could be 6-N-trimethylamino, 2-oxohexanoate. In order to check this hypothesis we subsequently transformed the isolated radioactive unknown into its 2,4-dinitrophenylhydrazone derivative and submitted it to hydrogenolysis in a Parr bomb. Analysis of the reaction products by TLC and ion exchange column chromatography (4 different systems) showed a positive ninhydrine-reacting substance at the same R_f and with the same elution time as that of authentic Me_3Lys and containing more than 85% of the radioactivity of the 2,4-dinitrophenylhydrazone before hydrogenolysis. This result strongly suggests that the unknown radioactive product is indeed 6-N-trimethylamino, 2-oxohexanoic acid (II).

Experiments with extracts of *N. crassa* using $\text{Me}_3(^{14}\text{CH}_3)\text{Lys}$ led to the same results as "in vivo" experiments, demonstrating that an enzymate system exists which can convert Me_3Lys (I) into the corresponding ketoacid (II).

Work to verify that this ketoacid is an intermediate in the biosynthesis of carnitine (IV) is in progress.

