work do not show any stimulation upon the addition of AG or related compounds. It appears then logical to assume by analogy that the bacterial enzyme might possess an active center with a structure similar to acetyl glutamate or related compounds. If this is the case two possibilities are open, either that carbamyl phosphate remains attached to the enzyme until reaction with ornithine or other acceptor, or that carbamyl phosphate is split off from the enzyme site before further reaction. As shown in Table II the second possibility seems to be true.

Still to be investigated is whether or not Compound X is split to free carbamyl phosphate before reacting with ornithine or other acceptor and whether this splitting is enzymic or non-enzymic[3].

A further subject for investigation will be to determine the possible existence of a "carbamyl or acetyl active center" in the "native" mammalian enzyme which may be masked or denatured during the isolation of the enzyme[3].

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1 M. E. JONES, L. SPECTOR AND F. LIPMANN, *J. Am. Chem. Soc.*, 77 (1955) 819.
2 S. GRISOLIA AND P. P. COHEN, *J. Biol. Chem.*, 204 (1953) 753.
3 S. GRISOLIA AND P. P. COHEN, *J. Biol. Chem.*, 198 (1952) 561.
4 S. GRISOLIA AND R. O. MARSHALL, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, Johns Hopkins Press, Baltimore, 1955, p. 258.
5 S. GRISOLIA, D. P. WALLACH AND H. J. GRADY, *Biochim. Biophys. Acta*, in press.
6 O. H. LOWRY AND J. A. LOPEZ, *J. Biol. Chem.*, 162 (1946) 421.
7 O. E. HEPLER, *Manual of Clinical Laboratory Methods*, Charles C. Thomas, Springfield, Illinois, 1949, p. 273.
8 J. C. TOWNE AND S. GRISOLIA, unpublished.

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* The following abbreviations are used throughout this paper: AG, acetyl glutamate; CP, carbamyl phosphate[1]; Compound X, the active carbamyl phosphate intermediate formed from AG or related compounds[2]; ATP, adenosinetriphosphate. All analytical methods and procedures have been described in preceding papers[3,4].

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α-L-Formamidinoglutaric acid as a formylating agent*

The isolation of α-L-formamidinoglutaric acid (FAG)[1,2,3,4] from incubation mixtures of histidine or urocanic acid with mammalian liver preparations suggested that FAG might be an intermediate in the pathway of the metabolism of histidine as a donor of the one carbon unit. Since in these enzymic digests FAG accumulated and no further degradation was observed, it was assumed that a suitable formyl acceptor was absent in these extracts[1], although the possibility could not be excluded that activation of histidine as formyl donor followed a pathway other than that through urocanic acid and FAG[5].

We wish to report two lines of evidence which strongly suggest that FAG or a compound in equilibrium with it is an obligatory intermediate in reactions in which histidine acts as a formylating agent. When FAG (5 μM) was incubated with folic acid (0.25 μM) and 0.5 ml of a soluble enzyme extract from rat liver (supernatant of 1:1 liver homogenate with 0.05 M phosphate buffer, pH 7.2, centrifuged at 140,000 × g at 0–4° for 2 hours) in a total volume of 0.8 ml, a sky-blue-fluorescing compound was formed which behaved identically with synthetic 10-formylfolic acid[6] upon paper chromatography with six solvents and ionophoresis. Under these conditions total conversion of folic acid to "formyl-folic acid" was obtained within an incubation time of 3 hours at 37° in the dark. The enzyme extract was inactivated by treatment with Dowex-2-Cl or prolonged dialysis and could be reactivated to a varying degree by the addition of 0.2 ml of boiled (2 min, 100°) enzyme extract. The efficacy of the boiled enzyme was abolished by treatment with Dowex-2-Cl or charcoal. The activity of the inactivated enzyme could also be restored by folic acid which had been reduced either with Adams' catalyst in 0.1 N NaOH[7] or with $NaBH_4$. The enzymic formation of "formylfolic

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acid" was not inhibited by versene or by aerobic conditions and did not require adenosine triphosphate. Formic acid, formyl-*iso*glutamine[1], -glutamine[1] and -glutamic acid[1] were inactive, while urocanic acid was as active as FAG only at high concentrations (5 μM) and histidine was still less active*. The superiority of FAG to urocanic acid or histidine as formyl donor was clearly demonstrated when formic acid-^{14}C (25 μM) was incubated with pigeon liver extracts under the conditions used by GOLDTHWAIT *et al.*[8] for the enzymic synthesis of formylglycinamide ribotide. After rigorous removal of all volatile radioactive material 148 counts/min, corresponding to a utilization of 0.5 μM of formate, were found in the nonvolatile fraction in the presence of formic acid alone. Upon addition of 10 μM each of nonlabeled histidine, urocanic acid, FAG, formyl*iso*glutamine, formylglutamine, formylglutamic acid, formylglycine, formamidinoacetic acid[3], and γ-benzyl-FAG[3], 120, 80, 48, 135, 126, 137, 155 and 125 counts/min, respectively, were found in the nonvolatile fraction. The increasing inhibition of incorporation of formic acid into the nonvolatile fraction as one proceeds from histidine to FAG as well as the lack of inhibition by other formyl derivatives gives strong support to the role of FAG as source of the formyl group. The extent of inhibition shows that FAG does not act as a formylating agent through the production of formate. The same conclusion was reached when labeled histidine-2-^{14}C** (2.5 μM) was incubated under the above conditions in the absence or presence of a 4-fold excess of nonlabeled formate. After deproteinization, histidine was removed on a Dowex-2-formate column and the eluate brought to dryness and counted. In the presence of formate an inhibition of incorporation of ^{14}C of only 40% was observed in the nonvolatile, HCl labile[8], fraction.

The mechanism of the metabolic utilization of FAG as formyl donor as well as the detailed requirements of the soluble enzyme system are under study.

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1 B. A. BOREK AND H. WAELSCH, *J. Biol. Chem.*, 205 (1953) 459.
2 H. TABOR AND A. H. MEHLER, *J. Biol. Chem.*, 210 (1954) 559.
3 A. MILLER AND H. WAELSCH, *J. Am. Chem. Soc.*, 76 (1954) 6195.
4 J. E. SEEGMILLER, M. SILVERMAN, H. TABOR AND A. H. MEHLER, *J. Am. Chem. Soc.*, 76 (1954) 6205.
5 H. WAELSCH AND A. MILLER, *Amino Acid Metabolism*, W. D. MCELROY AND W. B. GLASS (Editors), Johns Hopkins Press, Baltimore, Maryland, 1955, p. 407.
6 Modification of the procedure of M. GORDON, J. M. RAVEL, R. E. EAKIN AND W. SHIVE, *J. Am. Chem. Soc.*, 70 (1948) 878.
7 B. L. O'DELL, J. M. VANDENBELT, E. S. BLOOM AND J. J. PFIFFNER, *J. Am. Chem. Soc.*, 69 (1947) 250.
8 D. A. GOLDTHWAIT, R. A. PEABODY AND G. R. GREENBERG, *J. Am. Chem. Soc.*, 76 (1954) 5258.

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* While this work was in progress K. SLAVIK AND V. MATOULKOVA (*Collection Czechoslov. Chem. Commun.*, 19 (1954) 1032) reported the formation of formylfolic acid, identified by paper chromatography, upon incubation for 22 hours of concentrated liver homogenates with folic acid and histidine or urocanic acid or an ionophoretically separated enzymic degradation product of histidine assumed to be FAG. In stimulating formylfolic acid formation the FAG preparation proved to be superior to histidine or urocanic acid and other formyl compounds were found to be inactive.

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On the nature of the carbamyl group donor in citrulline biosynthesis*

Optimum conditions for the biosynthesis of citrulline from ornithine, ammonia, and carbon dioxide by soluble mammalian liver enzymes require ATP**, magnesium ions and catalytic amounts of certain derivatives of glutamic acid[1]. The catalytic properties of the glutamate derivatives have been ascribed to the formation of an unstable carbamyl intermediate (Compound X) containing the glutamate derivative, ammonia, carbon dioxide and phosphate in a mole-to-mole ratio[1,2,3]. Transfer of the carbamyl group from Compound X to ornithine to form citrulline was considered to result in the regeneration of the glutamate derivative and the release of inorganic phosphate.

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** The following abbreviations are used: ATP, adenosine triphosphate; AG, acetyl glutamate; CG, carbamyl glutamate; AGI and CGI, intermediates formed in the presence of AG and CG respectively.