[2] Leifer, E., Roth, L. J., and Hempelmann, L. H., *Science*, **108**, 748 (1948).

[3] Hastings, A. B., Langham, W., Carter, R. E., and Roth, L. J., *Nuclear Science Abstracts*, **4**, 806, No. 5372 (1950).

[4] Kornberg, H. L., and Davies, R. E., *Intern. Congr. Biochem., Abstr. Commun.*, 2nd Congr., Paris, France, 1952, p. 144.

[5] Scholander, P. F., Claff, C. L., Andrews, J. R., and Wallach, D. F., *J. Gen. Physiol.*, **35**, 375 (1952).

[6] Robinson, C. V., *Science*, **112**, 198 (1950).

[7] Tauber, H., and Kleiner, I. S., *J. Biol. Chem.*, **92**, 177 (1931).

[8] Barnett, G. D., and Addis, T., *Ibid.*, **30**, 41 (1917).

PATH OF ORNITHINE SYNTHESIS IN ESCHERICHIA COLI

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The mode of ornithine biosynthesis has been investigated in a number of organisms, including fungi[1-3] and mammals;[4-10] however, it has not been possible to establish with certainty a pathway of ornithine formation in any one species. These investigations were complicated by the close relationship which has been found to exist between the metabolism of ornithine and that of proline;[1-10] it has been suggested that the paths of ornithine and proline formation may be linked by glutamic γ-semialdehyde as a common intermediate.[2, 9, 10] In *Escherichia coli*, glutamic γ-semialdehyde has been shown to be a precursor of proline,[11] but does not appear to participate directly in ornithine synthesis. It was therefore of interest to examine the ornithine pathway in this organism; the results of such an investigation form the subject of this report.

The experiments described here have been conducted with the aid of mutant strains of *E. coli* which, characteristically, accumulate metabolites as a result of a block in ornithine synthesis. Evidence that these metabolites are intermediates in ornithine formation is provided not only by their accumulation, but also by a study of enzymes contained in extracts of the parental wild-type strain. The present findings indicate that in *E. coli* ornithine arises from glutamic acid via N-acetylglutamic acid, N-acetyl glutamic γ-semialdehyde, and N^{α}-acetylornithine as intermediates (see Fig. 1).

N^{α}-Acetylornithine.—Culture filtrates of an ornithine-requiring mutant strain (160-37) derived from *E. coli*, ATCC No. 9637, have previously been shown to contain N^{α}-acetylornithine.[12, 13] The accumulation of this compound as a consequence of an impairment of ornithine synthesis indicated that N^{α}-acetylornithine is a precursor of ornithine. This indication was

supported by the finding that an enzyme capable of converting N^{α}-acetylornithine to ornithine could be detected in extracts of wild-type *E. coli*, but not of mutant strain 160-37.[12] It has now been found that the activity of this enzyme is enhanced by the cobaltous ion.† In this respect the enzyme resembles another acylase[14] and some peptidases.[15] Partial purification has not revealed any other cofactor requirements; the cleavage of N^{α}-acetylornithine therefore appears to be a hydrolytic reaction.

N-Acetylglutamic γ-Semialdehyde.—The fact that strain 160-37 has an ornithine requirement indicates that the accumulated N^{α}-acetylornithine does not arise from the acetylation of ornithine. A search for a possible precursor of N^{α}-acetylornithine has revealed that culture filtrates of strain 160-37 also contain a substance identified as N-acetylglutamic γ-semialdehyde, presumably related to the L-series of amino acids. For the isolation of this substance, a culture of strain 160-37 was grown with aeration in "Medium B"[11] at 30°C. for two days and then filtered. The pH of the resulting filtrate was adjusted to 8.5 and inorganic anions were precipitated with a slight excess of aqueous barium acetate. The precipitated barium salts were filtered off and the solution obtained was freed of cations by passage through a column of Amberlite IR-120 (acid form). From the resulting effluent, water, acetic acid, and other volatile substances were removed in vacuo. The residue was dissolved in water and the resulting solution was extracted exhaustively with ether. The material remaining in the aqueous layer was partitioned between *n*-butanol and water. The contents of the butanol phase were chromatographed on silicic acid (Mallinckrodt) and upon elution with water-saturated ethyl acetate, N-acetylglutamic γ-semialdehyde (about 10 mg. per liter of original culture filtrate) was isolated as a colorless, viscous liquid. This aldehyde reacted with 2,4-dinitrophenylhydrazine to give a yellow, crystalline derivative melting, after recrystallization from absolute methanol, at 208°C. (*Analysis.*[16] Calculated for the dinitrophenylhydrazone, $C_{13}H_{15}O_7N_5$: C, 44.2; H, 4.3; N, 19.8. Found: C, 44.0; H, 4.3; N, 20.1.) The original aldehyde could be regenerated by treatment of this derivative with benzaldehyde in acidified ethanol. An acid hydrolysate of N-acetylglutamic γ-semialdehyde was found to contain acetic acid; after neutralization, the hydrolysate produced a yellow color with *o*-aminobenzaldehyde and satisfied the growth requirement of mutant strain 55-25, but not of mutant strain 55-1 of *E. coli* (see Fig. 1 for the site of the blocks in these strains). This color reaction and growth response are characteristic of Δ^1-pyrroline-5-carboxylic acid, the cyclized form of glutamic γ-semialdehyde.[11] The reaction with *o*-aminobenzaldehyde after acid hydrolysis was used to follow the progress of purification of the N-acetylglutamic γ-semialdehyde.

From the excretion of N-acetylglutamic γ-semialdehyde by strain 160-37 and from the structural similarity of this compound to N^{α}-acetylornithine

it was inferred that the aldehyde is a precursor of N^{α}-acetylornithine and hence of ornithine. This inference received support through the finding that dialyzed cell-free extracts of strain 160-37 and of the corresponding wild-type strain contain an enzyme which, in the presence of glutamate, converts N-acetylglutamic γ-semialdehyde to N^{α}-acetylornithine. This enzyme thus appears to be a transaminase; its activity could be stimulated by addition of pyridoxal phosphate.† Other instances of transamination involving an aldehyde group have recently been described.[3, 17, 18]

N-Acetylglutamic Acid.—The chemical structure of N-acetylglutamic γ-semialdehyde suggested that this compound might be derived from glutamic acid. This view is consistent with the results of Abelson, Bolton, and Aldous[19, 20] on the incorporation of labeled carbon dioxide into amino acids of *E. coli*, including glutamic acid and arginine, which is known to be derived from ornithine. The recent finding by Maas, Novelli, and Lipmann[21] that extracts of *E. coli* contain an enzyme which catalyzes the N-acetylation of glutamic acid makes it reasonable to suppose that N-acetylglutamic acid is a precursor of N-acetylglutamic γ-semialdehyde. This aldehyde may thus arise from glutamic acid by an acetylation step followed by a reduction step. The reverse of this reaction sequence, i.e., reduction followed by acetylation, has now been shown to be unlikely. This negative finding lends a measure of support to the supposed participation of N-acetylglutamic acid in ornithine synthesis.

The evidence against the possibility that N-acetylglutamic γ-semialdehyde is formed from glutamic acid by reduction to glutamic γ-semialdehyde followed by N-acetylation has been obtained from a comparison of the excretion of N^{α}-acetylornithine by strain 160-37 with that by two double mutant strains derived from strain 160-37. These double mutant strains had, in addition to the block in ornithine synthesis, a block in the proline pathway; one of the strains (160-37D1) was blocked before glutamic γ-semialdehyde, and the other (160-37D2) was blocked between glutamic γ-semialdehyde and proline (see Fig. 1). When grown on minimal medium supplemented with 4μg. per ml. L-proline and 5μg. per ml. L-ornithine monohydrochloride, these double mutant strains both excreted 30μg. per ml. N^{α}-acetylornithine; exactly the same amount of this substance was excreted by strain 160-37 when grown on a supplement of 5 μg. per ml. L-ornithine monohydrochloride without added proline, but under otherwise unchanged cultural conditions. Since one of the double mutant strains was blocked in the synthesis of glutamic γ-semialdehyde, the availability of this compound does not appear to affect the excretion of N^{α}-acetylornithine. Consequently, glutamic γ-semialdehyde probably is not a precursor of N^{α}-acetylornithine in *E. coli*.

Discussion.—The present findings taken together with those of Maas, Novelli, and Lipmann[21] and of Abelson, Bolton, and Aldous[19, 20] support

the path of ornithine synthesis in *E. coli* shown in figure 1. The existence of mutant 160-37 in which the impairment of a presumably single biochemical function[22, 23] has given rise to a requirement for ornithine, suggests that the path of ornithine synthesis described here is the only major one in *E. coli*.

It is noteworthy that mycelial extracts of Neurospora have recently been reported to catalyze the transamination of glutamic γ-semialdehyde to

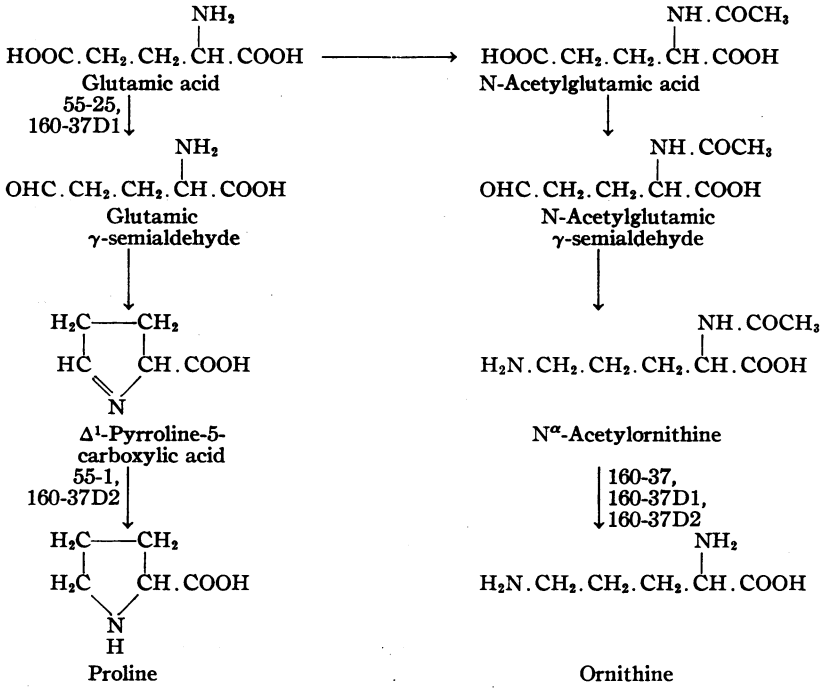

FIGURE 1

Paths of ornithine and proline synthesis in *Escherichia coli*. The sites of blocks in the mutants used, inferred from growth response and precursor accumulation, are indicated by the location of the respective strain numbers. Strains 160-37D1 and 160 37D2 each have two separately induced blocks, as shown.

ornithine.[3] This finding is in harmony with the formulation of the ornithine pathway proposed for this organism by previous authors.[2] On the other hand, the additional finding that ornithine-requiring mutant strains of *N. crassa* have approximately as much transaminase activity as the corresponding wild type led to the suggestion that perhaps another ornithine pathway might be operative in this species.[3] If so, it is nevertheless unlikely that the ornithine path reported here for *E. coli* is a major one in

Neurospora, since N^{α}-acetylornithine does not satisfy the growth requirement of two different mutant strains of *N. crassa*, one of which responds specifically to ornithine, whereas the other responds alternatively to ornithine, Δ^1-pyrroline-5-carboxylic acid, or proline.[24]

The acetylated ornithine precursors encountered in the present studies recall the early suggestion of Knoop[25] that acetylamino acids may be intermediates in the conversion of α-keto acids to the corresponding amino acids. This suggestion has stimulated much fruitful work on the metabolism of acetylamino acids, although it has not been possible to establish the occurrence of the proposed mechanism.[26] It is therefore of interest that the present findings permit the assignment of a definite biosynthetic role to the acetylation of an amino acid and the deacetylation of an acetylamino acid. However, the acetylated intermediates in the ornithine pathway are not formed in the manner contemplated by Knoop.

Summary.—It has been concluded that in *Escherichia coli*, the path of ornithine synthesis is: glutamic acid→N-acetylglutamic acid→N-acetylglutamic γ-semialdehyde→N^{α}-acetylornithine→ornithine.

N^{α}-acetylornithine and N-acetylglutamic γ-semialdehyde have been isolated from culture filtrates of an ornithine-requiring mutant strain of *E. coli*.

Cell-free extracts of wild-type *E. coli* contain a cobaltous ion-stimulated deacetylase capable of converting N^{α}-acetylornithine to ornithine. No such enzymatic activity could be detected in extracts of the ornithine-requiring strain which accumulates N^{α}-acetylornithine.

Cell-free extracts of wild-type *E. coli* also contain a pyridoxal phosphate-stimulated transaminase which, in the presence of glutamate, can convert N-acetylglutamic γ-semialdehyde to N^{α}-acetylornithine.

Evidence is presented which points to glutamic acid and N-acetylglutamic acid as precursors of N-acetylglutamic γ-semialdehyde.

Some aspects of the comparative biochemistry of ornithine and proline formation have been discussed.

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† Details of the enzymatic studies will be published elsewhere.

[1] Bonner, D. M., *Cold Spring Harbor Symp. Quant. Biol.*, **11**, 14 (1946).

[2] Srb, A. M., Fincham, J. R. S., and Bonner, D. M., *Am. J. Bot.*, **37**, 533 (1950).

[3] Fincham, J. R. S., *Biochem. J.*, **53**, 313 (1953).

[4] Krebs, H. A., *Enzymologia*, **7**, 53 (1939).

[5] Roloff, M., Ratner, S., and Schoenheimer, R., *J. Biol. Chem.*, **136**, 561 (1940).

[6] Stetten, M. R., and Schoenheimer, R., *Ibid.*, **153**, 113 (1944).

[7] Shemin, D., and Rittenberg, D., *Ibid.*, **158**, 71 (1945).

[8] Womack, M., and Rose, W. C., *Ibid.*, **171**, 37 (1947).

[9] Cedrangolo, di F., Leone, E., and Guerritore, D., *Arch. sci. biol.*, **33**, 503 (1949).

[10] Stetten, M. R., *J. Biol. Chem.*, **189**, 499 (1951).

[11] Vogel, H. J., and Davis, B. D., *J. Am. Chem. Soc.*, **74**, 109 (1952).

[12] Vogel, H. J., Abstracts of Papers, Atlantic City Meeting, Am. Chem. Soc., 43C (1952).

[13] Vogel, H. J., and Davis, B. D.; unpublished data.

[14] Rao, K. R., Birnbaum, S. M., Kingsley, R. B., and Greenstein, J. P., *J. Biol. Chem.*, **198**, 507 (1952).

[15] Smith, E. L., in *Advances in Enzymology*, Vol. XII, Interscience, New York (1951), p. 191.

[16] By the Microchemical Laboratory of New York University.

[17] Meister, A., Sober, H. A., Tice, S. V., and Fraser, P. E., *J. Biol. Chem.*, **197**, 319 (1952).

[18] Bessman, S. P., Rossen, J., and Layne, E. C., *Ibid.*, **201**, 385 (1953).

[19] Abelson, P. H., Bolton, E. T., and Aldous, E., *Ibid.*, **198**, 165 (1952).

[20] Abelson, P. H., Bolton, E. T., and Aldous, E., *Ibid.*, **198**, 173 (1952).

[21] Maas, W. K., Novelli, G. D., and Lipmann, F.; manuscript in preparation.

[22] Cf. Beadle, G. W., in *Genetics in the 20th Century*, Macmillan Co., New York, (1951), p. 221.

[23] Cf. Bonner, D. M., *Cold Spring Harbor Symp. Quant. Biol.*, **16**, 143 (1951).

[24] Unpublished data.

[25] Knoop, F., *Z. physiol. Chem.*, **67**, 489 (1910).

[26] Cf. du Vigneaud, V., Cohn, M., Brown, G. B., Irish, O. J., Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, **131**, 273 (1939).

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*PULCHERRIMIN, THE PIGMENT OF CANDIDA PULCHERRIMA**

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In 1901 Lindner[1] described an, at that time, unknown yeast species to which he gave the name *Torula pulcherrima*, the specific name being connected with the beautiful aspect of its microscopic preparations when the organism had been grown under special conditions: strictly spherical cells of uniform size, each containing a large, highly refractile globule apparently rich in fat. Since then various properties of this yeast, which in present-day taxonomy is known as *Candida pulcherrima* (Lindner) Windisch, have been studied by several authors. In this article we shall restrict ourselves to the property of the organism to produce a red pigment under certain cultural conditions.

The first important observations on the conditions determining pigment production were made by Beijerinck.[2] In 1918 he established that this production was markedly increased by the addition of a certain amount of an iron salt to the media normally used for yeast cultivation. More-