[4] J. I. HARRIS, *Nature*, 184 (1959) 167.
[5] H. B. F. DIXON, *Biochem. J.*, 62 (1956) 25P.
[6] C. H. CHERVENKA AND P. E. WILCOX, *J. Biol. Chem.*, 222 (1956) 635.
[7] P. J. VITTRAYATHIL AND F. M. RICHARDS, *J. Biol. Chem.*, 235 (1960) 1092.
[8] C. H. LI, *Advan. Protein Chem.*, 11 (1956) 102.
[9] J. P. GREENSTEIN, *J. Biol. Chem.*, 109 (1935) 541.
[10] H. B. F. DIXON, *Biochim. Biophys. Acta*, 34 (1959) 251.
[11] H. FRAENKEL-CONRAT, *J. Am. Chem. Soc.*, 76 (1954) 3606.
[12] W. F. WHITE AND W. A. LANDMANN, *J. Am. Chem. Soc.*, 77 (1955) 771.
[13] C. J. WEBER, *J. Biol. Chem.*, 86 (1930) 217.
[14] J. P. WALLER AND H. B. F. DIXON, *Biochem. J.*, 75 (1960) 320.
[15] W. L. HUGHES JR., H. A. SAROFF AND A. L. CARNEY, *J. Am. Chem. Soc.*, 71 (1949) 2376.
[16] J. ROCHE, M. MOURGUE AND R. BARET, *Bull. Soc. Chim. Biol.*, 36 (1955) 85.
[17] C. H. LI AND A. L. LEVY, *J. Biol. Chem.*, 213 (1955) 487.
[18] H. SCHUTTE, *Z. Physiol. Chem.*, 279 (1943) 59.
[19] S. R. BENEDICT AND J. A. BEHRE, *J. Biol. Chem.*, 114 (1936) 515.

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Acetylated intermediates of arginine synthesis in *Bacillus subtilis*

In *Escherichia coli*, ornithine (a precursor of arginine) is synthesized from glutamate via the following acetylated intermediates: *N*-acetylglutamate[1,2], *N*-acetyl-γ-glutamyl phosphate[3], *N*-acetylglutamic-γ-semialdehyde[1], and N^{α}-acetylornithine[1,4]. The formation of ornithine from N^{α}-acetylornithine is mediated, in this organism, by the hydrolytic enzyme, acetylornithinase[1,4,5]. In *Bacillus subtilis*, this enzyme has thus far not been detected, nor has an acetyltransferase such as reported for *Micrococcus glutamicus*[6]. It, therefore, was of interest to examine the path of arginine synthesis in *B. subtilis*.

Tracer experiments have now indicated that *B. subtilis* has a glutamic family (*cf.* VOGEL AND BONNER[7]) consisting of glutamate and its biosynthetic products, arginine and proline. Thus, Table I shows that [2-^{14}C]acetate labels these 3 amino acids at approximately equal specific activity, which differs from that of alanine and from that of threonine and lysine (both of which are derivatives of aspartate[9]). Qualitatively similar results were obtained in analogous experiments with [1-^{14}C]glutamate, [3-^{14}C]-

TABLE I

INCORPORATION OF TRACERS INTO PROTEIN AMINO ACIDS OF *B. subtilis*, ATCC 6051 (AS RELATIVE SPECIFIC RADIOACTIVITY ON MOLAR BASIS)

The organism was grown in a glucose–salts medium, supplemented with tracers (0.1 mg/ml; approx. 1 mC/mmole), as indicated. The isotope methods used were essentially those previously employed[8].

Tracer	*Glu*	*Arg*	*Pro*	*Ala*	*Thr*	*Lys*
[2-^{14}C]Acetate, sodium salt	100	81	104	9	45	42
DL-[2-^{14}C]Ornithine hydrochloride	100	1005	—	—	—	—

aspartate, and [4-^{14}C]aspartate. Table I also shows that, in the bacillus, ornithine is a precursor of arginine. Additionally, it can be seen that the labeled ornithine, to some extent, contributes to glutamic acid. This degradative formation of glutamic acid presumably proceeds via glutamic γ-semialdehyde, as it does in Neurospora[8].

Evidence for acetylated intermediates between glutamate and ornithine was obtained in enzyme experiments with *B. subtilis*, Strain 8^{a+}, isolated as a revertant of the arginine auxotroph, Strain 8. These 2 strains were selected for study, since Strain 8 (which gives a growth response to ornithine, citrulline, or arginine) was found to produce relatively high levels of ornithine carbamoyltransferase (EC 2.1.3.3) under derepressive conditions (H. LEHRER AND M. E. JONES, personal communication).

TABLE II

SPECIFIC ACTIVITY OF ENZYMES OF ORNITHINE SYNTHESIS IN *B. subtilis*, STRAIN 8^{a+} (AS μmoles REACTION PRODUCT FORMED PER mg PROTEIN PER h)

The organism was cultivated, at 37° with shaking, in dilute Difco Arginine Assay Medium (35 mg dehydrated medium per ml). Actively growing cells were collected, suspended in 0.1 M phosphate (pH 7.0) containing 1.0 mM glutathione, disrupted sonically, and assayed as indicated.

Enzyme	*Reaction product*	*Specific activity*	*Assay method*
N-Acetyl-γ-glutamokinase	*N*-Acetyl-γ-glutamyl hydroxamate	0.2	Ref. 3
N-Acetylglutamic γ-semialdehyde dehydrogenase	TPNH	0.1	Ref. 3
Acetylornithine δ-transaminase*	*N*-Acetylglutamic γ-semialdehyde	0.8	Ref. 10
Acetylornithinase	Ornithine	1.2	Ref. 5

* α-*N*-Acetyl-L-ornithine:2-oxoglutarate aminotransferase (EC 2.6.1.11).

Table II reveals that extracts of Strain 8^{a+} contain four enzymes that can act on acetylated intermediates of ornithine synthesis; the specific enzyme activities are given and the assay methods indicated. The biosynthetic substrates of the 4 enzymes are considered to be the L isomers of *N*-acetylglutamate, *N*-acetyl-γ-glutamyl phosphate, *N*-acetylglutamic γ-semialdehyde, and N^{α}-acetylornithine.

In the bacillus, acetylornithinase activity is markedly stimulated by Co^{2+} and by glutathione (*cf.* VOGEL AND BONNER[5]). However, addition of glutamate does not stimulate the enzymic cleavage of N^{α}-acetylornithine, either in the presence or in the absence of Co^{2+} and glutathione. These results indicate that the N^{α}-acetylornithine-splitting activity is due to an acylase such as the hydrolytic acetylornithinase of *E. coli*[5], and not to an ornithine–glutamate acetyltransferase, such as reported for *M. glutamicus*. Accordingly, the Gram-positive bacillus would seem to resemble, in this respect, the Gram-negative enterobacterium rather than the Gram-positive micrococcus.

In extracts of the auxotrophic Strain 8, no acetylornithinase activity could be detected. In cross-feeding experiments with an *E. coli* auxotroph as responding organism, Strain 8 was indicated to excrete N^{α}-acetylornithine. The enzymic block and the precursor accumulation of this strain support the conclusion that, in *B. subtilis*, the 4 acetylated biosynthetic substrates mentioned above represent the main functional pathway between glutamate and ornithine. Since Strain 8 contains ornithine carbamoyltransferase and responds to citrulline, *B. subtilis* is concluded to convert or-

nithine to arginine by way of citrulline and, probably, of argininosuccinate (*cf.* VOGEL AND BONNER[7]).

When Strain 8^{a+} was cultivated in the general manner described in Table II, but with the addition of L-arginine hydrochloride (0.2 mg/ml) to the culture medium, all 4 enzymes listed in this table were found to be repressed. It seems likely that such repressibility (by endogenous arginine) contributed to earlier failures to detect the acetylated intermediates in *B. subtilis*.

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[1] H. J. VOGEL, *Proc. Natl. Acad. Sci. U.S.*, 39 (1953) 578.
[2] H. J. VOGEL, P. H. ABELSON AND E. T. BOLTON, *Biochim. Biophys. Acta*, 11 (1953) 584.
[3] A. BAICH AND H. J. VOGEL, *Biochem. Biophys. Research Communs.*, 7 (1962) 491.
[4] H. J. VOGEL, *Abstr., Am. Chem. Soc., Atlantic City Meeting*, (1952) 43C.
[5] H. J. VOGEL AND D. M. BONNER, *J. Biol. Chem.*, 218 (1956) 97.
[6] S. UDAKA AND S. KINOSHITA, *J. Gen. Appl. Microbiol.*, 4 (1958) 272.
[7] H. J. VOGEL AND D. M. BONNER, in W. RUHLAND, *Encyclopedia of Plant Physiology*, Vol. 11, Springer Verlag, Heidelberg 1959, p. 1.
[8] R. H. VOGEL AND M. J. KOPAC, *Biochim. Biophys. Acta*, 36 (1959) 505.
[9] H. J. VOGEL, *Biochim. Biophys. Acta*, 34 (1959) 282.
[10] A. M. ALBRECHT AND H. J. VOGEL, *Federation Proc.*, 19 (1960) 2.

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