THE FERMENTATIVE PRODUCTION OF L-ORNITHINE (Preliminary report)

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In the previous communications^{(1),(2)}, the authors have reported on the accumulation of a large amount of L-glutamic acid from carbohydrate and ammonia source by *Micrococcus glutamicus*. During the course of mutation studies of a strain of this organism, an amino acid other than glutamic acid was found to be accumulated by an arginine or citrulline requiring-mutant strain. The amino acid accumulated was proved to be L-ornithine by isolation and purification of the fermentation product. L-ornithine, which is known as one of the important intermediates of so-called "ornithine cycle" and arginine biosynthesis, has heretofore never been produced in such a large amount by microorganisms.

The fermented broth, which was examined by paper chromatography and confirmed to contain only one amino acid exclusively, was treated with a strong acidic cation exchange resin, Amberlite IR-120, and the amino acid absorbed was eluted with 2N HCl. The eluate was concentrated and adjusted to approximately pH 6. After the addition of absolute ethanol in the cold, a fine crystalline precipitate was obtained. This crystal was purified by recrystallization and active carbon treatment. The amino acid thus obtained gave the same R_f value of an authentic ornithine on a paper chromatogram, and melted at 235–6°C. *Anal.* Calculated for C₅H₁₃O₂N₂Cl: C, 35.56; H, 7.77; N, 16.60. Found: C, 35.49; H, 7.40; N, 16.67. $[\alpha]_D^{20}$; +10.9° (C=5.5, H₂O). These analytical data are in accordance with the literature values of L-ornithine. The expected amount of CO₂ was also evolved from a sample solution by an L-ornithine decarboxylase preparation of *Clostridium septicum* P. III (NCTC No. 547)⁽³⁾.

The fermentative production of L-ornithine was successfully conducted as follows: the medium contained 10% glucose, 0.1% K₂HPO₄, 0.025% MgSO₄·7H₂O, 1.0% NH₄Cl, 0.3% urea, 0.5% corn steep liquor, 1.0% NZ-amine, 0.0015% phenol red. The pH was adjusted to 7. Twenty ml of this medium was dispensed in a 250-ml Erlenmeyer flask and inoculated with 5% of inoculum broth. The organism was cultured at 28°C on a rotary shaker. The pH of the culture medium was maintained at a state of neutrality with the addition of a small amount of 20% urea solution. The course of this fermentation is presented in Table 1. The quantitative determination of ornithine was carried out by the method of Chinard⁽⁴⁾.

As shown above, about 0.36 mole of L-ornithine was produced from 1

Table 1. The course of L-ornithine fermentation.

Incubation time	Glucose	L-Ornithine	L-Glutamic acid	α -Ketoglutaric acid	Lactic acid
hours	%	mg/ml	mg/ml	mg/ml	mg/ml
0	10.0	—	—	—	—
24	5.48	10.0	0	1.2	5.0
48	1.77	24.0	0.23	1.5	1.8
72	0.54	26.2	0.1	2.5	1.1

mole of glucose. Because of this extremely high yield of ornithine production, the investigation of the mechanism of this fermentation is now in progress.

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