MAGNESIUM REQUIREMENT OF AEROBACTER AEROGENES FOR ASSIMILATION OF MOLECULAR AND COMBINED NITROGEN

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Many steps in the metabolic pathway of biological nitrogen fixation are still unknown. In bacteria, the nitrogen-fixing sequence is thought to proceed through ammonia (Zelitch, Rosenblum, and Wilson, J. Biol. Chem. 191:295, 1951; Newton, Wilson, and Burris, J. Biol. Chem. 204:445, 1953; Wilson and Burris, Ann. Rev. Microbiol. 7:415, 1953; Virtanen, Angew. Chem. 65:1, 1953). It has been known for some time that minerals in trace amounts play specific roles in the metabolism of microorganisms.

In the work reported here, we have attempted to determine what amount of magnesium is required by Aerobacter aerogenes, and the relationship between the magnesium requirement and the nitrogen source being utilized.

If the organism's need for a trace metal is in its synthesis of amino acids or the precursors of the amino acids, then we would expect to find little or no requirement for the trace metal if the precursor or amino acid is supplied to the organism as a source of nitrogen.

The organism used in this study was from the collection of the Department of Bacteriology, University of Wisconsin. It was selected because of its ability to readily utilize either molecular or combined nitrogen. The growth medium and analytical procedures were essentially those previously described (Pengra and Wilson, J. Bacteriol. 75:21, 1958), altered as indicated for specific studies. The test medium for studies on the magnesium requirement was the same, except that the water used to prepare the medium was prepared by passing deionized water through a weak hydrogen-charged cation-exchange column. The medium contained a slight modification (Patil, M.S. Thesis, South Dakota State University, Brookings, 1963), in that 0.85% sodium chloride was added to prevent clumping of the cells. Our medium also differed from Pengra and Wilson's in that it did not contain calcium chloride. Analytical reagent-grade chemicals were used for the preparation of the medium. Concentrated solutions of each constituent of the medium were passed through an ion-exchange column (Pengra and Wilson, Proc. Soc. Expnl. Biol. Med. 100:436, 1959). A weak cation-exchange resin, Amberlite IRC-50, charged with the appropriate ion was used. Solutions of the phosphate salts, sodium chloride, and sodium nitrate, one of the nitrogen sources used, were passed through a resin column charged with a 5.0% solution of the appropriate salt. The water, sucrose, and glutamic acid were purified by passing them through a hydrogen-ion charged column. The glutamic acid solution had a pH of 3.4 before and after passing through the exchange column.

The concentrated solutions were made up as follows: 20 g of sucrose in 200 ml of water; 12.5 g of NaH2PO4 in 250 ml of water; 1.5 g of KH2PO4 in 250 ml of water; 21.5 g of NaCl in 100 ml of water; 1 ml of the Fe-Mo solution of Wilson and Knight (Experiments in Bacterial Physiology, Burgess Publishing Co., Minneapolis, 1952); 100 μg of magnesium per ml as MgSO4; and 1,000 μg of sulfur per ml as Na2SO4. Sodium sulfate was added to the purified medium to keep the sulfur level at 20 μg/ml. The solutions of the combined nitrogen sources were made up to contain 5,000 μg of nitrogen per ml as ammonium acetate, sodium nitrate, or glutamic acid.

The solutions were combined in 250-ml Erlenmeyer flasks in the following quantities to make up the test media: sucrose, 10 ml; Na2HPO4, 6.25 ml; KH2PO4, 6 ml; NaCl, 2 ml; Fe-Mo solution, 1.0 ml; Na2SO4, 1.0 ml; and MgSO4 to give 0.0, 0.1, 0.2, 0.5, 0.8, 1.0, 1.5, or 2.0 ppm of magnesium in a series of flasks. A 1-ml portion of the combined nitrogen concentrate was added to give 100 μg of nitrogen per ml in the medium. Water was added to bring the volume of the medium to 50 ml. The technique and

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methods used for the preparation of the flasks and inoculum, and the gassing of the cultures, were those of Pengra and Wilson (Proc. Soc. Exptl. Biol. Med. 100:435, 1959). Helium served as the atmosphere in the cultures utilizing ammoniacal, nitrate, and glutamic acid nitrogen.

*Aerobacter aerogenes* strain M5al definitely requires magnesium in trace quantities to attain maximal growth on all four nitrogen sources (Fig. 1). The effect of magnesium was studied over a range from 0.0 to 2.0 ppm of magnesium added. There were always definite increases in growth with an increased magnesium level until a plateau was reached. On all three of the combined nitrogen sources, ammoniacal, nitrate, and glutamic acid, the optimal level of magnesium required for maximal growth was approximately 1.0 ppm. The amount of total nitrogen assimilated for the three different combined nitrogen sources was not the same. This may be caused by factors other than the magnesium level. The cultures utilizing the ammonium salt and the nitrate showed a significantly higher growth rate and total growth than did the cultures fixing molecular nitrogen.

With the use of the same levels of magnesium in the medium as with the combined nitrogen, N\textsubscript{2} was used as the nitrogen source. Maximal growth, with N\textsubscript{2} gas used as the nitrogen source, was obtained when 1.5 ppm of magnesium had been added to the medium (Fig. 1). The various amounts of total nitrogen utilized were plotted against parts per million of Mg\textsuperscript{++} added (Fig. 1) after correction for the nitrogen in the inoculum.

We have shown that the magnesium requirement for the anaerobic nitrogen fixer, *A. aerogenes*, was nearly the same, whether the organism was assimilating ammoniacal, nitrate, or glutamic acid nitrogen. When the organism was fixing molecular nitrogen, our experiments showed a need of 1.5 \( \mu \)g of magnesium per ml, as opposed to 1.0 \( \mu \)g for the other nitrogen sources. This difference could be due to experimental error or it could mean, as it suggests, a higher requirement for magnesium when fixing molecular nitrogen. We are inclined to think that the organism's requirement for magnesium is independent of nitrogen sources supplied, and that the need for magnesium is in another area of the organism's metabolism.

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**LEAKAGE OF CELL COMPONENTS OF BORDETELLA PERTUSSIS**

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Mager (Biochim. Biophys. Acta 43:529, 1959), Higuchi and Uemura (Nogei Kagaku 33:304, 1959), and Herbst and Doetor (J. Biol. Chem. 234:1497, 1959) found leakage of substance(s) showing a maximum at 260 m\( \mu \) from cells of *Neisseria perflava*, *Pasteurella tularensis*, *Saccha-