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Biosynthesis of Vitamin B_6 by Bacteria

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The biosynthesis and control of vitamin B_6 in bacteria could be studied more easily if an organism which produced large amounts of this family of compounds could be found. Unfortunately, the measurements of bacterial vitamin B_6 that have been reported have often failed to include the total extracellular vitamin B_6, and thus they do not adequately reflect the total vitamin B_6 in the whole culture. Consequently, the total vitamin B_6 content of cultures of bacteria freshly grown in simple defined media has been measured to allow a comparison of their abilities to synthesize this vitamin.

All identified bacteria were isolated before use from stocks obtained from either the American Type Culture Collection or the Department of Bacteriology, University of Florida. The measurement method for total vitamin B_6 (pyridoxine) in bacterial cultures has been described (W. B Dempsey, J. Bacteriol. **90**:431, 1965). Except for *Thiobacillus thiooxidans*, *Streptomyces griseus*, and *Achromobacter xerosis*, all data reported in Table 1 were obtained with samples withdrawn within one doubling time after the cultures reached stationary phase. The three exceptions had samples withdrawn within 24 hr after growth had stopped. *T. thiooxidans* cultures were filtered through coarse filter paper to remove sulfur particles before either optical density measurements or samples were taken. Dry weights were determined by drying to constant weight, at 110 C, duplicate, washed samples of bacteria containing a minimum of 15 mg each (dry weight).

In addition to the identified organisms, 23 unidentified organisms from soils of widely scattered origin were tested after primary isolation on a glucose plus salts-agar seeded with *Escherichia coli* B-B_6-53. This pyridoxineless mutant and the method of seeding it have been described (W. B. Dempsey and P. F. Pachler, J. Bacteriol. **91**:642, 1966). The organisms used were those which retained the combined abilities to grow on a minimal medium and to cross-feed the seeded mutant after isolation in pure culture. When grown in medium A (Table 1), 19 of the organisms had vitamin B_6 contents in the range 60 to 80 ng/mg. The remaining four organisms had higher amounts of vitamin B_6, and these were retested in a more complete medium (medium C, Table 1) to stimulate rapid and complete growth. None of these four exhibited sufficiently high vitamin B_6 contents to warrant immediate

TABLE 1. *Total vitamin B_6 content in bacterial cultures*

Organism	Medium[a]	Total ng of vitamin B_6	
		Per ml	Per mg of dry cells
Achromobacter xerosis	B	200	100
Aerobacter aerogenes	A	75	70
A. aerogenes[b]	A	75	70
Arthrobacter flavescens	B	70	60
A. globiformis	A	85	80
A. simplex	B	110	40
Bacillus licheniformis	B	65	60
B. polymyxa	B	90	75
B. subtilis	B	100	75
Escherichia coli B	A	60	80
E. coli B[b]	A	55	75
E. coli B	B	60	70
E. coli B	C	65	50
Mycobacterium phlei	B	160	40
Pseudomonas aeruginosa	A	60	70
P. aeruginosa[b]	A	60	70
Serratia marcescens	A	50	60
S. marcescens[b]	A	50	55
Streptomyces griseus	A	50	200
Thiobacillus thiooxidans[c]	D	90	130
Soil organism A	C	120	60
Soil organism B	C	100	170
Soil organism C	C	120	100
Soil organism D	C	130	130

[a] Media: A, glucose minimal (W. Dempsey, J. Bacteriol. **90**:431, 1965). B, 0.5% glucose, 0.05% succinate, 0.1% citrate, 0.1% glutamate, 63 mM potassium phosphate, 7.5 mM $(NH_4)_2SO_4$, 0.8 mM $MgSO_4$, and 10 ml/liter of metal mix (M. Sauter, J. Boyer, and V. Sauter, J. Bacteriol. **78**:197, 1959), pH, 7.0. C, 0.05% succinate, 0.02% glycine, 0.02% serine, 10 mg/liter of calcium pantothenate, 10 mg/liter of nicotinic acid, 1 mg/liter of thiamine-HCl, and 5 μg/liter of biotin in medium A. D, Starkey's medium as used by I. Suzuki (Biochim. Biophys. Acta **104**:359, 1965).

[b] At 48 hr post-log phase.

[c] Mass determined by optical density only.

investigation or extensive identification tests. Organism A was observed microscopically to grow in clusters similar to those seen in *Arthrobacter*. Organisms B, C, and D, on the other hand, were aerobic sporeformers.

The data in Table 1 led to the conclusion that the vitamin B_6 content of bacterial cultures appears to be constant at approximately 70 ng/mg or 0.4 nmoles/mg. Significantly higher amounts than this have been reported for certain mutant yeasts (G. H. Schur and M. E. Rafelson, J. Appl. Bacteriol. **25**:187, 1962).