UNBALANCED GROWTH INDUCED BY TEMPERATURE SHIFT IN A MUTANT OF *BACILLUS ANTHRACIS*

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Gladstone (1939) first reported that the minimal nutritional requirements of *Bacillus anthracis* at 37°C could be defined by a mixture of amino acids, salts and glucose. An addition of thiamine stimulates considerably the growth of the bacilli in this medium but further metabolites are needed to ensure optimal growth. None of these additional metabolites are, however, essential for this pathogen (Brewer et al., 1946; Puziss and Wright, 1954).

In these studies an acapsulogenic mutant of *Bacillus anthracis*, strain Vollum which has been described earlier was used initially (Ivánovics, 1962). Satisfactory growth of this organism was obtained in a basal medium with the following composition: vitamin free Casamino acids (Difco), 25 g ; L-glutamic acid, 2,5 g ; DL-tryptophan and L-cystine, 0.125 g ; sodium citrate, 2 g ; K_2HPO_4, 10 g ; Na_2SO_4, 0.5 g ; $MgSO_4,7H_2O$, 0.5 g ; ferric ammonium citrate, 25 mg ; $MnSO_4,4H_2O$ 10 mg ; thiamine, 2.5 mg ; glucose, 4 g ; distilled water to 5 l. The pH adjusted to 7.2 with N-NaOH.

Attempts to isolate mutants with a defined growth requirement from the acapsulogenic strain Vollum, yielded a rare mutant which grew well in the above medium at a temperature lower than 34°C. On the other hand, when this mutant was incubated at higher

temperatures it did not grow. At 37°C the mutant thus exhibited a complete dependency on either thymidine or thymidine-5phosphate. Although a concentration as low as 1 µg/ml of the pyrimidine derivates supported the growth of the mutant, the optimal concentration was higher. When limiting concentrations of the above mentioned DNA precursors were included into the medium cell wall formation was markedly affected. Thus long filaments without intersepta, containing empty and distorted cells were seen. Minor deficiencies in cell wall structure were still apparent even in the presence of 20 to 50 µg/ml of thymidine. Nevertheless at 26°C the bacteria grew with normal cell wall formation even in the basal medium.

When a culture of the mutant growing exponentially in basal medium at 22°C was shifted to 37°C, the abnormal appearance of the cells already described was again observed. Associated with this temperature induced morphological change was an unbalanced synthesis of DNA, RNA and protein (Table I) . The synthesis of these cell constituents ceased at 170 minutes by which time a considerable disproportion in their relative amounts was evident. A similar phenomenon exhibited by a thymine auxotroph of Escherichia coli after withdrawal of exogeneus thymine was described by Cohen and Barner (1954).

Temperature shift causing a thymidine dependency in the mutant of Bacillus anthracis however, was not followed by a dramatic loss of viability of the bacteria i.e. no "thyminless death" occured (ct. E.coli)but rather a prolonged and gradual decrease in number of colony formers was observed.

As far as we aware, no mutant of bacteria has been described in which a disproportional synthesis of cell constituents is

caused by a temperature shift. The lag observed in DNA replication is apparently due to some block in thymidine synthesis at the elevated temperature.

TABLE I

Effect of temperature shift on relative amounts of DNA, RNA and protein in the mutant of Bacillus anthracis

	Time of incubation at 37°C (minutes)				
	0	90	130	170	210
DNA	1.0	1.9	2.3	2.4	2.4
RNA	1.0	3.4	4.4	4.2	4.2
Protein	1.0	5.2	5.5	6.5	6.6

Bacteria were grown for 40 hours at 22°C, centrifuged, washed and resuspended in basal medium. A sample taken at this point represents 0 time. The suspension was reincubated at 37°C and moderately aerated. Aliquots removed at intervals were cooled, 5 % trichloracetetic acid (TCA) added and kept in refrigerator overnight. The precipitate was washed with 0.1 % TCA, hydrolysed in 5 % TCA for 30 minutes at 100°C. DNA was measured with diphenylamine, RNA with orcein and protein with Folin reagent.

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